INVESTIGATION AND CONTROL OF ANIMAL DISEASES AND INFECTIONS, INCLUDING ZOONOSES

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ABSTRACT OF THESIS

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This thesis brings together my published work in the field of investigation and control of animal diseases and infections, including zoonoses. It was undertaken between 1980 and 2011 whilst employed by the former Veterinary Investigation Service and Veterinary Laboratories Agency (VLA), firstly based in Norwich, Norfolk and subsequently in Bury St Edmunds, Suffolk. Part 1 covers a broad spectrum of predominantly novel and emerging conditions, or unusual manifestations of known diseases, and is subdivided into separate sections for pigs, sheep, cattle, goats and birds. These investigations used clinical field work, diagnostic necropsies and currently available or newly introduced laboratory tests, and relied heavily on initial referrals from local veterinary practitioners and extensive subsequent cooperation from farm managers or owners. Part 2 covers zoonotic diseases and infections, including separate sections on *Escherichia coli* (mainly verocytotoxigenic serogroup O157), cryptosporidiosis and Q fever. Most of the work on zoonoses was undertaken during the period from 2004 onwards when I was VLA project leader for non-statutory zoonoses and it includes investigations into human outbreaks of zoonotic disease initiated at the request of public health professionals. In both parts of the thesis the publications have been grouped within their respective sections to demonstrate progression of ideas and activities, including relevant test development.

In the early 1980s, pig producers in East Anglia and elsewhere experienced devastating piglet losses from transmissible gastroenteritis (TGE). The subsequent history of TGE in Britain until its eventual demise by the end of the 20th century is charted in the pig disease section. Other pig topics include swine influenza, a previously undescribed zinc induced copper deficiency anaemia in swill fed pigs and farm biosecurity Although sheep are a minor species in East Anglia, a number of new and emerging conditions were recognised, often before being seen in other regions. The foremost is maedi-visna which was first identified in indigenous sheep in Norfolk in 1983 and soon became strongly associated with the area. Other conditions recorded include a high morbidity ovine vulvitis outbreak and the Dandy-Walker syndrome. Despite a relatively small cattle industry, the region has exhibited many unusual bovine diseases including probably the most severe outbreak of acute bovine virus diarrhoea (BVD) ever recorded in Britain. The emergence of fascioliasis in East Anglia is also detailed. Close involvement with the former MAFF Cattle Health Scheme in the late 1980s led to an interest in herd biosecurity, abortion and the development of milk antibody tests for *Leptospira* Hardjo, and BVD and IBR virus infections. These tests were subsequently introduced within the VLA and used for assessing and monitoring herd disease status. Regular bulk milk antibody screening of a high health status dairy herd led to the detection of novel high morbidity non-pathogenic IBR virus infection. Publications on several new or emerging diseases of goats and birds are also included in Part 1.

Cattle are the principal reservoir of VTEC O157 and the primary source of most human infection. A number of outbreak investigations, particularly on farms open to the public, revealed the extent of infection in a diverse range of other animal species including sheep, pigs, horses, goats and camelids. An outbreak amongst visitors to a wildlife gardens in Norfolk was linked to contact with wild rabbits co-grazing with infected cattle. *Cryptosporidium parvum* infection is prevalent in young ruminants and has led to zoonotic outbreaks amongst visitors to farms. A series of investigations used a newly adopted sensitive fluorescent antibody test to define the extent of sub-clinical infection in pigs, calves and lambs, including orphan lambs being bottle-fed by visitors to an open farm. Q fever emerged as a zoonosis of increased importance following human outbreaks in Britain and a massive outbreak in the Netherlands. My participation in a multi-disciplinary outbreak control team for a Q fever outbreak in Cheltenham in 2007 led to the development of improved diagnostic tests (PCR and ELISA) which were subsequently used for farm outbreak investigations and surveys in Britain. Other zoonoses featured include *Leptospira* Icterohaemorrhagicae infection acquired from pet rats and zoonotically acquired *Corynebacterium ulcerans* diphtheria.

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DECLARATION

The work presented here spans a period of 30 years and brings together my published output covering the investigation and control of animal diseases and infections, including zoonoses, whilst working within the former Veterinary Investigation Service (VIS), the Veterinary Laboratories Agency (VLA) and briefly the Animal Health and Veterinary Laboratories Agency (AHVLA). The publications cover a diverse range of topics and rely heavily on the considerable opportunities for discussion, collaboration and advice provided by a close network of expert colleagues within these organisations. They are too numerous to mention individually but most are either co-authors or included in the acknowledgements of relevant publications. Where I am first author, the writing was largely or entirely my own responsibility and for the remainder it was shared with others to varying degrees. After I was appointed VLA project leader for Non Statutory Zoonoses in 2004, my role changed to leading a team and increasingly to supervising activities of others, rather than undertaking most of the work myself as was the case with earlier studies. I still instigated and led most of the work during this latter period and contributed to writing the publications. My personal contribution to each publication is outlined in italics after each reference listed.

Several papers and scientific letters on transmissible gastroenteritis (TGE) of pigs were included in the appendix to my 1984 FRCVS thesis (Epidemiological aspects of TGE in pigs in East Anglia) although not part of the actual submission since published work was excluded. A further paper based on a chapter of this thesis was published subsequently. Two papers relate to my 1993 DVM & S thesis (Epidemiology and elimination of bovine herpesvirus 1 infection in cattle in Norfolk). These publications have been included to demonstrate development of areas of work and are clearly identified after the appropriate reference. Apart from these, I hereby declare that none of the publications listed have been submitted by me for any other degree or diploma and that this thesis is entirely my own work.

Date 24/6/2011

ACKNOWLEDGEMENTS

The general support of colleagues and other collaborators is mentioned in the previous section. I further acknowledge the cooperation of my veterinary colleagues at Norwich and Bury St Edmunds who often covered my rota duties for me when I disappeared to undertake investigations at short notice. I owe a debt of gratitude to numerous laboratory colleagues, particularly at Norwich VIC, VLA Bury St Edmunds and at Weybridge for their excellent technical assistance in a number of areas. I thank the office staff at Norwich and Bury St Edmunds for their clerical support and the Library at AHVLA Weybridge for sourcing, photocopying or downloading my publications included here.

The investigations in Part 1 would not have been possible if private veterinary surgeons, farm managers and owners had not brought the conditions to my attention and cooperated in follow-up activities. Some of the publications in Part 2 relate to investigations undertaken at the request of human health professionals, particularly Consultants in Communicable Disease Control (CsCDC) of local health authorities or latterly the Health Protection Agency (HPA) and would not have been possible without their input.

I am grateful to successive VIS, VLA and AHVLA line managers for allowing me the freedom to pursue the various studies leading to this DSc submission and to MAFF and Defra for underpinning the funding required where appropriate.

Finally I would like to thank my family for their support, patience and forbearance.

DEDICATION

This thesis is dedicated to the memory of my father Eric Dudley Pritchard

INTRODUCTION

After graduating in Animal Science from Wye College in 1969, I studied Veterinary Medicine at Edinburgh, qualifying in 1973. I encountered several interesting conditions whilst working in large animal practices in southern England but with limited opportunity for detailed investigation their precise aetiology remained obscure. As a Ministry of Agriculture, Fisheries and Food (MAFF) Veterinary Officer (VO) at Norwich Animal Health Office between 1976 and 1980, I encountered Anthrax, Swine Vesicular disease and Aujeszky's disease, and was also closely involved in the early days of poultry meat inspection in Britain. These experiences encouraged me to transfer to the MAFF Veterinary Investigation Service (VIS) in Norwich in 1980 where I found numerous opportunities to visit farms and investigate unusual diseases with a freedom I had not experienced previously. My first publication was written whilst working as a VO but all subsequent publications were produced after I transferred to the VIS.

I worked as a Veterinary Investigation Officer (VIO) at Norwich Veterinary Investigation Centre (VIC) under Dr Edward Gibson, until it closed in 1993. I then transferred to the newly built regional laboratory in Bury St Edmunds, Suffolk where Dr Graham Jackson was Senior Veterinary Investigation Officer (SVIO), followed later by Ian Griffiths who became Regional Veterinary Manager after yet another reorganisation. In 1995, the VIS combined with the Central Veterinary Laboratory, Weybridge and the Lasswade Laboratory in Scotland to form an extended Veterinary Laboratories Agency (VLA). The latter was an executive agency of MAFF, which became the Department of the Environment, Food and Rural Affairs (Defra) in 2001. In 2004, whilst a VIO at Bury St Edmunds, I was appointed project leader for Non-Statutory Zoonoses and this activity increasingly took up most of my time. I continued in this latter role on a part time basis after semi-retirement in 2008. In April 2011, following the Coalition Government's Major Spending Review of 2010, VLA combined with AH to form a new agency provisionally named the Animal Health and Veterinary Laboratories Agency (AHVLA).

Edward Gibson, the SVIO at Norwich, was my early mentor. He was one of a renowned generation of enthusiasts that fashioned the VIS during the glory days long before budgetary constraints. Edward came from the VIC in Cambridge to set up Norwich VIC in 1958. He encouraged us all to publish and took great delight in keeping a tally of the number of publications coming out of *his* Centre. In the 1980s, private veterinary practitioners, rather than MAFF, were widely regarded as the main customers for our services. Despite many subsequent changes to the organisation, including numerous rationalisations and initiatives, the underlying objectives of the VIS/VLA remained largely unaltered. These were essentially to use diagnostic and disease monitoring laboratory submissions from veterinary practitioners, combined with investigative field work, post mortem examinations, the latest laboratory tests and applied scientific research, to facilitate diagnosis and surveillance for new, emerging and notifiable diseases of (primarily) farmed livestock. Hopefully, the AHVLA will preserve the essence of this organisation with its proud history of excellence and achievement reaching back to a swine fever laboratory in the basement of Whitehall Place, London in 1894.

East Anglia is essentially an arable area, with livestock farming dominated by pigs and poultry and relatively few cattle and sheep. Nevertheless, the region has proved to be a fertile area for new, emerging or unusual diseases of all farmed species as shown by the range of conditions included here. My increased involvement with zoonoses from 2004 onwards coincided with the "one world, one health" concept and realisation that most new diseases are likely to be zoonotic. It heralded a fruitful period of collaborations with the recently formed (and now being disbanded) Health Protection Agency (HPA), including the influential multi-disciplinary Human Animal Infections and Risks (HAIRS) group of which I was a founder member. The HAIRS group (ably chaired by Dr Dilys Morgan from the HPA's Department of Gastrointestinal, Emerging and Zoonotic Infections) was set up to undertake horizon scanning and risk assessment for new and emerging disease hazards in the aftermath of the BSE crisis. Spin-offs from HAIRS included the development of multi-disciplinary Guidelines for the Investigation

of Zoonotic Disease (see www.hpa.org.uk). There was also increased national interest in verocytotoxigenic *Eschericha coli* O157 and other zoonoses on farms open to the public, heightened by events at Godstone Farm, Surrey in 2009; similarly Q fever – fuelled by a huge outbreak in the Netherlands which started in 2007 and continued through to 2011.

Disease investigation, particularly of novel or complex conditions, requires a methodical approach, sound knowledge of differential diagnoses and diagnostic methods, and most importantly a network of able colleagues to bounce ideas off. Much of the work, including investigations into zoonotic diseases, was undertaken under field conditions or within the constraints of a busy veterinary diagnostic laboratory rather than in a controlled research environment. Accordingly, the findings have largely been published in the Veterinary Record, the main journal of the practising profession in Great Britain, In Practice (the CPD journal of the British Veterinary Association), journals of specialist veterinary associations or societies and in diverse conference proceedings or posters in Britain or elsewhere, rather than in high scientific impact factor research journals.

Publications on related topics have been grouped together to facilitate the narrative and demonstrate a coherent body of work, including the range of audiences and readership. The thesis falls into two main parts:

Part 1: Investigation and control of diseases of farm animals (at herd or flock level), with emphasis on new and emerging conditions and unusual manifestations of known diseases. These were mainly undertaken within the geographical area of East Anglia (essentially Norfolk and Suffolk). It is subdivided into sections on diseases of pigs, sheep, cattle, goats and birds.

Part 2: Investigation and control of predominantly emerging zoonotic diseases and infections of domesticated animals. This mainly relates to the period from 2004 onwards and includes investigations into human outbreaks undertaken both in East Anglia and elsewhere in England and Wales. It is subdivided into sections on *Escherichia coli* (mainly verocytotoxigenic *E. coli* O157), cryptosporidiosis, Q fever, *Corynebacterium ulcerans* and *Leptospira* Icterhaemorrhagiae.

PART 1: ANIMAL DISEASE

1.1 Pigs

The pig industry in Britain has been hit by a series of major viral diseases during the last 30 years, including swine fever, Aujeszky's disease, transmissible gastroenteritis (TGE), postweaning multisystemic wasting syndrome (PMWS), porcine respiratory and reproductive syndrome (PRRS) and swine influenza. Of these, TGE occupied much of my time during the 1980s. It is caused by a highly contagious coronavirus infection which produces severe diarrhoea, vomiting and anorexia in pigs of all ages. TGE closely resembles another coronavirus infection of pigs namely porcine epidemic diarrhoea (PED); the diseases can be clinically indistinguishable in finishing herds (hence the term "the other one" or "TOO"). PED can also occasionally cause a similar, although generally much less serious disease in breeding herds. TGE was first reported in Britain in 1958 although it had probably been around since 1952. Epidemics subsequently occurred for two or three consecutive winters every five to seven years until the disease eventually disappeared by the end of the 20th century. During the 1980s, TGE arguably became the disease most feared by pig producers because of the difficulties in control and prevention, the inevitably high piglet mortality and lack of financial compensation in the event of an outbreak.

TGE reappeared soon after I joined the VIC (1.1, 1.2, 1.3, 1.4, 1.5) and as a major pig rearing area East Anglia was hit particularly hard. Although not a statutory listed disease, it was subject to a voluntary notification and early warning system which was introduced by the VIS following the 1970 epidemic. As the epidemic escalated and concerns increased, I was given the opportunity to further investigate the clinical and epidemiological features of this enigmatic disease (1.6, 1.7, 1.8, 1.9) and develop practical control measures for farmers (1.10). TGE was also a major issue for the pig industry in mainland Europe at the time and in 1982 I attended a European Commission workshop in Ghent chaired by the TGE guru Professor Maurice Pensaert. This helped direct my later thinking, particularly on the control of the clinically milder and often

unapparent endemic form of the disease which maintained the infection between epidemics (1.9). In 1984, using experiences gained from field investigations in East Anglia, I advised the State Veterinary Service in Eire on the successful elimination of TGE infection following the country's first and only outbreak (in a large breeding and finishing enterprise in County Leitrim). In the same year I was awarded an FRCVS for my work on the epidemiology of TGE in East Anglia and a chapter from this thesis formed the basis of a subsequent publication about endemically infected herds (1.9).

TGE gradually disappeared from Britain after the 1981-84 epidemic. This was attributed to the emergence in 1984 of a very closely related porcine coronavirus which was first detected by the routine serological monitoring for TGE using the serum neutralisation test (SNT) in Denmark where TGE was a notifiable disease. This non-enteric coronavirus, named porcine respiratory coronavirus (PRCV), was isolated by Maurice Pensaert's group in 1986 and caused only a very mild respiratory disease in pigs but spread rapidly by airborne transmission. It initially led to confusion and concern with screening tests for TGE, especially in breeding companies wishing to export, until a differential ELISA replaced the SNT. There was a recurrence of classical disease in Lincolnshire in 1996 (1.11) and in Humberside in 1999; clinically very mild endemic TGE also persisted in some large breeding herds in East Anglia during the 1990s, being maintained by weaner pools and incoming immunologically naïve gilts. However, it soon became evident that rapidly spreading PRCV had effectively immunised the pig population against TGE, which (with PED) disappeared from Britain by the end of the millennium (1.12, 1.13, 1.14, 1.15).

Unlike TGE, swine influenza, another major pig disease, is now widespread in the GB pig population and is often associated with significant disease problems. It has assumed greater importance in recent years, particularly with zoonotic concerns following the appearance of pandemic HIN1 virus in 2009. The first reported outbreak of clinically severe swine influenza in Britain (H3N2 virus), with associated zoonotic concerns, occurred on a large finishing unit in Suffolk in 1987 (1.16). Despite detecting serological evidence of infection since the late 1960s, classical swine influenza had not previously

been recorded in pigs in Britain although HINI was considered partly responsible for a widespread respiratory condition in pigs during 1986 – for which the newly discovered PRCV (see above) was also partly blamed.

During the 1980s, swill feeding of pigs was widely practised in East Anglia and ensuring compliance with waste food legislation kept a MAFF VO at Norwich Animal Health Office fully occupied for most of his time. Swill feeders tended to keep themselves very much to themselves and rarely sought veterinary assistance. A novel disease outbreak in finishing pigs on a swill plant in Norfolk with the unusual presenting features of aural haematomas and lameness, led to the first recorded incident of naturally occurring zinc toxicity and zinc induced copper deficiency anaemia in farmed livestock (1.17). It also provided a fascinating insight into the world of the swill producer. The source of the zinc was flakes of galvanising material from new metal bins used to store swill before processing. As a corollary, the owner, in common with most other swill plant operators surveyed at the time (1.17), had been unaware of the low cost growth promoting effect of dietary copper in pig rations. If supplementary copper had been included in the swill rations this outbreak would probably not have occurred because of its protective effect of copper on zinc toxicity.

During the 1980s and 1990s, I became increasingly involved with ruminants and less with pigs although I remained as an associate member of the VLA pig group until 2005. My interest in the control and prevention of infectious disease of farmed livestock, combined with expertise gained in Yorkshire during the 2001 Foot and Mouth disease epidemic, led to me being commissioned to write a comprehensive review of biosecurity for pig breeding herds (1.18). This was subsequently adopted as the standard reference document for use by the pig industry (BPEX) and the State Veterinary services in GB and Northern Ireland.

1.2 **Sheep**

East Anglia's wealth in the Middle Ages was founded on the sheep industry and a flourishing wool trade although nowadays the region has one of the smallest concentrations of sheep in Britain. Despite this, a number of novel and emerging sheep diseases have been identified in the area, most notably maedi-visna (MV). This is an important disease of sheep caused by a retrovirus belonging to the lentivirus subfamily. Much as with the history of TGE in pigs, the progress of MV virus infection in Britain, is effectively charted by the series of publications included here.

After a few early cases of MV virus infection (mainly serological evidence only or clinical cases linked to imported exotic breeds) were described in Britain in the late 1970s/early1980s, concerns mounted about the possibility of this infection spreading and causing significant economic losses to the national flock. The first clinical evidence of MV virus infection in indigenous sheep in GB was recorded in Norfolk in 1983 in a commercial crossbred flock (1.19). At the time there was some debate (1.20) about whether the infection was derived from imported sheep. However, subsequent investigations extending through to the mid 1990s revealed more widespread serological evidence of infection (1.21) and overt clinical disease in other flocks in East Anglia (1.22, 1.23, 1.24, 1.25). Visna, the much rarer nervous form of the condition, was first seen in a Norfolk flock at the end of 1989 (1.23) around the same time that it was also described in a Scottish flock by Neill Watt and colleagues at the Edinburgh Veterinary School. Multiple cases of visna were later confirmed in another infected flock in Norfolk in 1995, in which blindness was a novel feature (1.24). Two of the most heavily infected flocks in Norfolk eventually ceased sheep production – a decision which was apparently not influenced by the presence of MV virus infection (which the owners' claimed was not causing significant economic loss).

In 2003, an atypical primarily neurotropic form of MV virus infection appeared in two commercial crossbred flocks: one in southern England and the other in the Midlands (1.26, 1.27). At this time visna had only ever been reported in three flocks in GB, one in

Scotland and two in Norfolk. All these flocks also had co-existent maedi so the recent developments marked an important departure from previous experiences. Several cases of visna in one flock were initially diagnosed clinically as scrapie (a notifiable disease) which was of concern for the regulatory authorities at the time. Clinical MV has since been diagnosed sporadically in GB up to the present day but early concerns expressed in the 1980s have been largely unfounded, and neither the number of reported cases of clinical MV, nor the total number of infected flocks, show signs of escalating. This is despite reports of increasing numbers of serological breakdowns in flocks belonging to the MV accreditation scheme operated by the Scottish Agricultural College (SAC) and the inevitable continuing dissemination of infection present in commercial flocks through sales of live infected cull ewes.

The paucity of reports of clinical MV virus infection is probably mainly attributable to the fact that the disease can exist unrecognised in infected flocks for many years, particularly in sheltered commercial lowland flocks on a good plane of nutrition. Also, disease onset is insidious and not usually apparent until about 60% of sheep in the flock have seroconverted. Furthermore, when dyspnoea and emaciation become obvious, affected sheep are often at the end of their productive life and are culled without veterinary involvement. Even if they are necropsied, the lung lesions of maedi can frequently be masked by concurrent infections such as pasteurellosis or pulmonary adenomatosis. Uncomplicated maedi lesions can also sometimes be missed by inexperienced investigators unless the lungs are carefully evaluated or weighed (maedi lungs invariably weigh >1kg) to differentiate from non-specific pulmonary congestion – an approach I introduced within the VLA as a simple screening test to improve surveillance.

I was asked to contribute a chapter on MV for the 3rd and 4th editions of the authoritative Diseases of Sheep textbook, which I undertook initially with Mike Dawson (1.28) and subsequently with Ian McConnell (1.29). Although these were essentially reviews, they included my own personal field observations and some previously unpublished data. In 2002, I also contributed to the COST Action 834 consensus conference in Lyon on the

European consequences of small ruminant lentivirus infection (1.30). It was apparent from that meeting that established researchers in this field held similar opinions to me about the surprisingly small effect MV can have on economic performance in commercial flocks kept under favourable conditions. This is in marked contrast to the much greater clinical impact of infection observed by the SAC in a Scottish hill flock kept in a harsher environment. In accredited pedigree flocks, serological evidence of MV infection alone has a profoundly serious financial effect on sales of breeding stock due to loss of disease-free status rather than any effect on productivity.

The disease surveillance role of a closely linked national network of veterinary laboratories was well demonstrated by other work concerning new, unusual or emerging conditions of sheep, which were often first reported in East Anglia and subsequently observed elsewhere. These included the Dandy Walker Syndrome, which is a rare congenital brain and skull malformation of unknown aetiology in man. It emerged as a novel inherited condition in the Suffolk breed following the first reported outbreak in a flock in Suffolk (1.31, 1.32, 1.33). Similar examples included rye grass staggers, a bizarre seasonal neurotoxicosis acquired by consuming fungal endophytes of *Acremonium lolii* at the base of closely cropped swards of perennial ryegrass (1.34); kangaroo gait, a bilateral peripheral neuropathy of the radial nerves (1.35) and a (then) unusual generalised form of caseous lymphadenitis – caused by infection with *Corynebacterium pseudotuberculosis* (1.36).

In 2004, an extraordinary outbreak of vulvitis and balanitis with very high morbidity occurred in a flock in Suffolk and was subsequently observed by VLA colleagues in south-west Wales and Worcestershire (1.37). This venereal condition had been described fairly briefly by several previous authors but the aetiology and epidemiology were obscure. The owners of the flock in Suffolk were extremely cooperative and the outbreak provided an ideal opportunity to describe the disease more fully, define the natural course of the infection and further investigate the aetiology. Despite the clinical severity of the acute lesions, complete resolution occurred within a few weeks regardless of treatment and without significant sequelae. This suggested that outbreaks could easily

pass unrecognised, especially in large hill flocks where sheep were not subjected to frequent close inspection. Despite detailed investigations, the precise cause was not confirmed although gammaherpesvirus OHV-2 was tentatively implicated. Further work is clearly needed to define the precise aetiology of this enigmatic condition, which like so many unusual diseases (perhaps more so in sheep) tend to disappear from veterinary consciousness for several years only to reappear and stir up renewed interest.

Cattle

As a predominantly arable region, the cattle population of East Anglia is largely confined to the low lying river valleys and associated traditional seasonal marshland grazing areas which are unsuitable for ploughing. The local dairy industry has declined massively since 1980 when there were at least three full time ADAS/MAFF dairy husbandry advisors covering the area and over 500 herds. By 2011, less than 50 dairy herds remained in Norfolk, with a handful in Suffolk and only two in the adjacent county of Cambridgeshire. The surviving herds have tended to be very large and operate very efficiently to high standards but mostly rely on the arable side of the farm enterprise to maintain economic viability. In view of their sparse distribution, cattle herds in East Anglia usually have much less exposure to common endemic infectious diseases than cattle in other parts of Britain. The resulting immunological naivety can lead to increased susceptibility to common endemic infections and occasionally result in unusually severe disease manifestations. Despite the relatively small local cattle population, a number of novel or unusual conditions were encountered, one of the early examples being a high morbidity alopecia affecting Friesian calves on two unrelated rearing units in Suffolk (1.38). The cause was not convincingly established although the feeding of high fat proprietary milk substitute appeared to be a significant factor. This generated much interest amongst manufacturers of proprietary calf milk powder.

Increased susceptibility to common endemic diseases by virtue of immunological naivety was exemplified by an extremely severe outbreak of acute infection with bovine viral diarrhoea (BVD) virus in a large self-contained dairy herd in Norfolk (1.39). Such was the clinical severity and the very high fever exhibited by affected animals, that the possibility of rinderpest briefly crossed my mind. Shortly before the outbreak, two groups of researchers (Peter Roeder and Trevor Drew at Weybridge, and Joe Brownie and colleagues at Compton) had published ground breaking findings about the pathogenesis of mucosal disease and the relationship with BVD virus infection. However, the potential clinical significance of acute BVD in naïve herds appeared to

have been somewhat underestimated and our publication was resubmitted to the Veterinary Record several times before the reviewers were convinced.

The farm in question was in a fairly remote location and the only significant exposure to other cattle occurred when the heifers were sent away to grazing marshes in Norfolk during the summer months. In November 1984, most recently calved cows experienced very high fever, profuse watery diarrhoea, anorexia and milk drop. These clinical signs persisted regardless of treatment, causing dehydration and dramatic weight loss. Some animals made a protracted recovery but many others died or were culled and over 20% of the herd aborted. Necropsies revealed acute or chronic gastrointestinal ulceration, similar to that seen with mucosal disease. The diagnostic breakthrough finally came when BVD virus was identified in the blood of four acute cases and seroconversion to BVD virus was demonstrated in three of these that survived. There was also evidence of concurrent recent infection with *Leptospira* Hardjo and *Coxiella burnetii* (Q fever), which might have contributed to the abortions. Looking back, this must rank as the earliest, and possibly the most severe outbreak of acute BVD infection ever recorded in Britain. In an unrelated incident, the death of a naïve bull was also similarly attributed to acute BVD infection (1.40) and there were anecdotal reports of similar deaths amongst naïve cattle from Norfolk used to restock herds in Cumbria after the 2001 Foot and Mouth disease epidemic.

In 1987, the State Veterinary Service launched a MAFF Cattle Health Scheme (CHS) to supersede the Enzootic Bovine Leucosis Attested Herd Scheme. This included a voluntary testing and control programme for infectious bovine rhinotracheitis (IBR), and a *Leptospira* Hardjo scheme was added a couple of years later. I operated the CHS in Norfolk from the outset and was the only VIO involved – the others were all VOs from Animal health Offices apart from my friend and colleague Laurence Gibson who led the scheme nationally and had worked at the Moulton (Northampton) VIC before it closed.

The CHS generated a wealth of useful data on the seroprevalence of IBR and *L.* Hardjo infections, both within and between herds. I soon discovered that analysing the

serological status of individual animals in relation to age, time in the herd and source of origin (homebred or purchased), provided an invaluable insight into the epidemiology of these common endemic (and frequently subclinical) infections. IBR was of added interest because of parallels with Aujeszky's Disease (AD) of pigs. This is caused by a herpesvirus similar to IBR virus (ie bovine herpesvirus 1 or BHV1) and had been the subject of a recent control and eradication scheme centred on pig herds in Suffolk, which I had participated in. The ease with which IBR virus was eliminated from cattle herds with a low prevalence of infection using a simple test and cull approach should have been of interest to policy makers dealing with AD eradication. My investigations into IBR in Norfolk, including subclinical infection in CHS herds, led to the award of DVM & S in 1993 and two papers (1.41, 1.42) relating to this thesis are included here for completeness and to link to the development of bulk milk antibody testing for IBR as discussed later.

The CHS was abandoned by MAFF in 1994 and taken over by Peter Bloxham's Axiom Laboratories in Devon and eventually incorporated into what has since become the Biobest Herdcare Cattle Health Scheme. For a short time I represented the VIS (with Graham David) as a steering group member for the Cattle Health Certification Standards (CHeCS) scheme which was set up to ensure consistently high protocols and standards of laboratory testing after the MAFF scheme was privatised. Ironically, VLA have recently re-entered this arena with the introduction of the Herdsure scheme in 2010.

IBR was of particular interest to the cattle industry in the 1980s because disease freedom was essential to gain access to the potentially lucrative international trade in live cattle, semen and embryos to Germany. Unfortunately, farmers who achieved IBR monitored negative status in Norfolk and elsewhere in GB through participation in the CHS were unable to capitalise on this market because of the emergence of Bovine Spongiform Encephalopathy (BSE) which became a compulsorily notifiable disease in 1988. Other than performing some brainstem removals on suspect cases and offering advice on differential diagnosis, VIOs were not particularly involved with BSE once it became

notifiable. However, in 1992, I was asked to investigate an unusual outbreak of BSE-like disease in Norfolk which appeared to have been caused by a neurotoxicosis (1.43).

IBR is the main clinical expression of the broad disease spectrum caused by different molecular subtypes of BHV1 infection. The other main clinical form is infectious pustular vulvovaginitis (IPV)/infectious balanoposthititis (IBP). This has rarely been seen in GB since the early 1970s when it was a major problem for artificial insemination centres. In January 1997, I investigated a severe outbreak of IPV in a dairy herd which, unusually, had concurrent respiratory tract involvement (1.44). I maintained an interest in IBR and in 2003 was rewarded by the opportunity to investigate a novel silent "outbreak" in a high health status dairy herd in Norfolk which was only detected following a routine bulk milk antibody test (see below). The virus had the DNA profile of BHV1 strains normally associated with clinically severe respiratory disease but was remarkably non pathogenic (1.45) and offered potential as a vaccine, although this aspect was never followed up.

Milk antibody testing for common endemic infectious diseases of cattle had been successfully used in compulsory or voluntary health schemes in a number of European countries since the early 1990s but it's development came slightly too late to be included in the CHS. My interest in the subject followed participation in a survey in 1996 to examine the prevalence of antibodies to BVDV and other viruses (including BHV1) in bulk tank milk samples in England and Wales (1.46). Bulk milk antibody testing was being offered by Axiom laboratories at the time but the potential did not appear to have been fully exploited. With excellent technical laboratory support from colleagues at VLA Sutton Bonington (where most VLA viral serology was undertaken at the time), the VLA in-house (serum) ELISA for BHV1 was validated for use with bulk milk; the commercially available Ceditest *L.* Hardjo ELISA (Id-dlo) was also adapted for VLA use (1.47,1.48,1.49). The VLA in-house BVDV and BHV1 serum ELISAs were additionally validated on milk from individual cows to provide a simple farmer-friendly alternative to blood testing (1.50) although this approach never really took off.

Classifying bulk milk results into negative, low positive, mid positive and high positive categories lends itself to prescribing defined courses of action. The categories for IBR were based on findings from my DVM & S studies, particularly the differentiation between high and low prevalence herds. Farmers generally found subdivision into disease status categories very helpful although they unfortunately sometimes agonised unnecessarily over minor decimal point changes in bulk tank optical density readings. These are invariably of no consequence since the test is only aimed at providing a general "ball park" assessment of status (although in practice it has proved to be a very robust and reliable method). The concept of using pooled milk samples from age cohorts and sentinel groups to monitor herds for evidence of active infection at minimal cost (1.49) has similarly proved to be very effective and is now widely adopted.

In addition to categorising disease status, bulk milk testing is also invaluable for monitoring herds for introduction of new infections. It also provides what one local veterinary practitioner aptly described as a "foot in the door" opportunity to discuss the diseases in question with their farmer clients, especially in the context of vaccination. Bulk milk antibody testing has now become a cornerstone of various cattle health schemes currently in operation in GB, including Herdsure and the approach has been extended to other diseases such as fascioliasis (1.51, 1.59).

Herds frequently acquire new infections via added animals (usually purchased breeding replacements) and it is often difficult to convince farmers of the potential risks until after they have experienced avoidable economic loss from this source (1.52). Abortions (1.53, 1.54. 1.55) are a common indicator of newly introduced disease, particularly with infections such *L.* Hardjo. The profile of leptospirosis in cattle was elevated by the introduction of efficacious vaccines during the 1980s and 1990s: the Schering-Plough Bovine Leptospirosis Information Service (BLISS) was introduced on the back of the bulk milk antibody testing introduced by the VLA (1.48). Pfizer was also active in this field through similar marketing initiatives. I advised both companies about *L.* Hardjo in cattle and spoke at several sponsored meetings about the use of bulk milk antibody tests.

Leptospira terminology has been complicated in recent years by the introduction of a classification system based on genomospecies instead of the simpler (although often inaccurate) serological approach using serovars. Serovar Hardjo comprises bovis and prajitno subtypes. Infection with hardjo-bovis has generally been considered as the main endemic form of leptospirosis in cattle in GB but a recent study using PCR positive material from routine submissions to VLA regional laboratories cast doubt on this view by suggesting that hardjo-prajitno predominated (1.56). In this work, all positive clinical field samples (mainly from cattle abortions) examined by D-HPLC were classified as *L. interogans* species (which includes hardjo-prajitno) whereas *L. borgpetersenii* (the species that includes hardjo-bovis) was not detected in any samples (1.56). Acute infection with *L.* hardjo classically causes milk drop/flabby bag syndrome in cattle but is now uncommon. Milk drop can also be caused by other infections, including respiratory syncytial virus (1.57. 1.58).

The emergence of fasciolosis in East Anglia (1.59) took local farmers and veterinary practitioners by surprise since this is traditionally a disease of the wetter western areas of the country and was rarely seen except in stock from endemically infected areas coming into the region for seasonal grazing. A useful collaboration with Andy Forbes (Merial Animal Health) and Diana Williams (Liverpool Veterinary school) using bulked faecal egg counts and bulk milk antibody tests, confirmed the extent of infection in cattle in the region. The change was attributed to increased rainfall and the introduction of sheep from endemically infected areas onto the grazing marshlands. The Environmentally Sensitive Area scheme further conspired to provide an ideal habitat for the essential snail parasite *Limnea truncatula*.

Goats

There was a surge of interest in goats following the formation of the Goat Veterinary Society (GVS) in 1979, and a wealth of knowledge about goat diseases has subsequently been recorded in the GVS journals. During the mid 1980s (including a period when I was the Events Secretary for the society), I encountered several previously unrecorded conditions, including an apparently benign thymic hyperplasia which superficially resembled goitre (1.60, 1.61, 1.62), an unusual fungal mastitis (1.63) and a neoplasm (1.64). I also collaborated with James Wood on describing natural scrapie cases in goats (1.65), presented a review of goat diseases based on the Veterinary Investigation Diagnosis Analysis Database (VIDA) (1.66) and reviewed caseous lymphadenitis in goats (1.67) having been involved in some of the earliest cases following the introduction of this disease into Britain with imported Boer goats in the late 1980s. I also became extensively involved with public health and zoonotic aspects of goats as detailed in Part 2.

Birds

When the Poultry Meat (Hygiene) Regulations 1976 were introduced, individual poultry carcases destined for the domestic market began to be inspected for the first time, although inspection had been undertaken previously for the export trade at some approved poultry slaughterhouses in Britain. The main infectious causes of carcase condemnation seen then were colisepticaemia in chickens, salpingitis in ducks and air sacculitis in turkeys. Whilst working as a VO at Norwich engaged in meat hygiene work, I became interested in the aetiology of air sacculitis in turkeys. I was then on secondment from MAFF and acting as a temporary Official Veterinary Surgeon (OVS) at the large Bernard Matthews turkey processing plant at Great Witchingham, Norfolk. I devised a system for grading the severity of air sacculitis in affected carcases and supported by their veterinarian, Cliff Stuart, the company allowed me to have some carcases (including those of normal healthy birds) to investigate the aetiology. Previous workers (mainly using experimentally inoculated birds) had suggested that *M. meleagridis* was involved, so I enlisted Dr Ron Leach, a mycoplasmologist at the nearby public health laboratory to perform mycoplasma cultures (which were not widely available within the VIS at that time).

Our investigations (1.68) showed that *M. meleagridis* was actually present in the air sacs of both normal and diseased birds and in the aerated wing bones of turkeys with severe disease. Naively, I also sought to compare the general bacterial microflora of normal turkey carcases with those affected with air sac disease in order to justify or refute the rationale behind the then current practice of condemning carcases with severe localised air sac disease. Stan Harrison, the very experienced microbiologist at Norwich VIC who was consigned to assist me, became totally consumed and ultimately overwhelmed by the scale of the project and it was never completed. However, enough work was done to indicate that *Aspergillus fumigatus* played a major role in severe air sacculitis cases. I subsequently managed to largely avoid investigating avian diseases although I published scientific letters on the recurring problem of ionophore toxicity in turkeys (1.69) and novel cases of necrotic enteritis in wild swans (1.70).

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Pigs

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Contributor. Included in the appendix to my FRCVS thesis but was not part of the actual submission

1.2 **Pritchard, G. C** (1981) TGE of pigs warning. Veterinary Record **109**, 367

My initiative. Included in the appendix to my FRCVS thesis but was not part of the actual submission

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My primary work based on my own field studies with support from the Virology Dept at the Central Veterinary Laboratory, Weybridge. This paper was included in the appendix to my FRCVS thesis but was not part of the actual submission

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Commissioned review based on my primary work. Included in the appendix to my FRCVS thesis but was not part of the actual submission

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I provided veterinary input into this collaborative study which was included in the appendix of my FRCVS thesis and was a peripheral part of the work undertaken for the thesis

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My primary work based on a chapter of my FRCVS thesis and other field data

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This was commissioned by MAFF as a total revision of the previous leaflet. It was written entirely by me based on extensive field studies

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Contributor and advisor

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My primary investigation written with help of collaborators at Weybridge (biochemistry and haematology)

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Commissioned CPD review article. Mainly my work with assistance from two local pig practitioners

Sheep

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My primary work with collaborators, particularly Brian Spence, who introduced me to the fascinations of maedi. Mike Arthur contributed the section on flock 3

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Collaboration with Brian Spence

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My initiative and field work, with serology undertaken at Weybridge

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My primary work based on local experiences

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My primary work with histopathology section contributed by Stan Done

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My primary work based on field studies and necropsies with support from co authors for histopathology and serology

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Commissioned article based on my primary work

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Collaborator and advisor, including undertaking joint field visit and assisting with necropsies

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Initiator and primary worker with contributions from other authors and histopathology undertaken by Martin Jeffrey

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Contribution as 1.31 above

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Collaborator and joint contributor

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My initiative and main investigative field work with contributions from colleagues particularly Sandra Scholes (histopathology) and Malcom Banks (virology)

Cattle

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Mainly my work with support from two local veterinary practitioners

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I led this major investigation and was responsible for writing most of the paper with considerable support from colleagues. Edith Borland, my colleague at Norwich VIC, assisted with necropsies (including those undertaken on-farm at the weekend) and shared the burden of a prolonged and difficult investigation

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Written in collaboration with Mike Bardsley, a local veterinary practitioner

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My primary work, which formed the basis of a chapter in my subsequent DVM & S thesis. It provided a good opportunity to present and discuss the data in an appropriate learned environment before I submitted the DVM & S thesis

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Commissioned article. My primary investigative work with details of individual cases contributed by Angela Bowman

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Mainly my own work with Malcolm Banks (Weybridge) providing virology input and Ruth Vernon giving practitioner support on farm

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Commissioned by BCVA. My primary work with technical laboratory assistance

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Initiator and primary worker with Gordon Kirkwood providing technical support

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Commissioned by BCVA. My primary work

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Commisioned by BCVA. My primary work

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Collaborator with Roger Gunning

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Initiator and collaborator with John Fishwick

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Goats

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My primary work

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My primary work in collaboration with Gerald Pepin (mycology)

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Collaborator, including provision of case histories and contributing to text

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Initiator and primary worker with mycoplasma cultures performed by Ron Leach

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Mainly my work with collaborators (particularly Heather Ainsworth)

1.70 **Pritchard, G. C.**, Ainsworth, H. A., Brown, M & Duff, J. P (2004) Suspected necrotic enteritis in wild swans. Veterinary Record **154**, 480

Mainly my work with collaborators (particularly Paul Duff)

PART 2: ZOONOSES

2.1 *Escherichia coli*

Verocytotoxigenic *Escherichia coli* (VTEC) serogroup O157 (predominantly serotype O157: H7) is an important zoonotic pathogen which came to prominence during the 1980s following an outbreak of bloody diarrhoea in the USA linked to consumption of undercooked hamburgers. VTEC O157 has since emerged as a major zoonotic pathogen worldwide and is by far the most commonly encountered serogroup of verocytotoxigenic *E. coli* present in Britain with over 1000 cases reported each year. Cattle are the primary reservoir and they carry the organism in their normal gut flora and shed it intermittently in their faeces. Large outbreaks, such as that in central Scotland in 1996 which prompted the Pennington Report, are mainly attributed to consumption of contaminated food (particularly beef) and most research activity worldwide has focussed on the role of faecal shedding by cattle in relation to controlling food borne infection.

Outbreaks can also occur from direct or indirect faecal-oral contact with infected animals. The full significance of this transmission route, particularly on farms open to the public, has only really been fully appreciated from the findings of numerous outbreak investigations carried out by the VIS/VLA at the request of public health authorities in England and Wales since the mid 1990s. The main purpose of these investigations is to assist outbreak control teams by undertaking animal sampling and microbiological cultures aimed at detecting the case strain of VTEC O157 in animals and thereby confirm the source of infection using molecular techniques. Outbreak investigations have also been used to gather information, particularly in the early days when the epidemiology of VTEC O157 was somewhat obscure. Advice on animal husbandry issues relevant to the control of VTEC O157 is also given by the investigating VIO during outbreak investigation visits. The rationale for VTEC O157 outbreak investigations is conveniently summarised in a poster presented at an international conference on pathogenic *E. coli* held in Sweden in 2008 (2.1).

Farms that open to the public have become firmly established within the rural economy and contribute significantly to the tourism and leisure industries and serve important educational and entertainment roles. The unfortunate downside is that they provide significant opportunities for exposure to common zoonotic hazards such as VTEC O157 and *Cryptosporidium.* Furthermore, visitors often comprise those very groups (such as young children accompanied by elderly grandparents) who are at the greatest risk of developing serious consequences from these infections. Zoonotic outbreaks cause serious public concerns and have significant deleterious effects on the wider farming industry. These and associated issues concerning VTEC O157 infection, cryptosporidiosis and other zoonoses on farms open to the public are discussed at length in a recently commissioned article (2.2) which provides a comprehensive CPD update for veterinary practitioners and others in this important and sensitive area.

My first encounter with VTEC O157 came in 1997 when I was asked by the CCDC of the local Health Authority and the Chief Environmental Health Officer of the Local Authority (who have enforcement responsibility under the Health and Safety at Work etc Act 1974) to assist in the investigation of the first widely publicised outbreak linked to an open farm (2.3). The Health and Safety Executive (HSE) also became involved because they were the enforcing authority for the associated commercial dairy herd to which the public also had access, although not for the open farm itself (such are the inconsistencies of the existing legislation). This outbreak investigation became a high profile saga for several months requiring several farm visits, extensive animal sampling and numerous cultures for O157. It also attracted significant media attention, exacerbated by the fact that a young child developed permanent brain damage as a consequence of the infection.

I subsequently investigated a number of other outbreaks on open and commercial farms in East Anglia, including those featured in publications 2.4 and 2.5. The former describes a longitudinal survey of VTEC O157 infection amongst animals on the premises undertaken after the outbreak whilst the latter paper highlights the risks of occasional visits to infected livestock farms by family members and friends.

The most challenging VTEC O157 investigation occurred in 2001 during the throes of the foot and mouth disease epidemic when I was asked to investigate a serious ongoing outbreak amongst visitors to a well operated wildlife gardens in Norfolk. After extensive animal sampling of the main risk species accessible to the public had failed to indicate the source, we extended the sampling to the adjacent cattle farm. The outbreak strain was found both in the cattle faeces and wild rabbit faeces on this farm, and subsequently in rabbit faeces on picnic areas in the adjoining wildlife gardens. The timing of the "eureka moment" in this complex investigation was memorable because it coincided precisely with news of the burning towers of 9/11. The outbreak came to an end once the rabbits were culled and fencing improved to prevent their movement between the cattle farm and the wildlife gardens; the visiting public were also kept away from areas contaminated with rabbit pellets by installing temporary fencing.

VTEC O157 had not previously been reported in wild rabbits and our findings were potentially very significant. It was therefore decided to publish the findings as soon as possible to alert others to the potential risks from this novel source. Publication of the detailed scientific letter in the Veterinary Record (2.6) caused much interest amongst the press and VTEC researchers; the findings were subsequently presented at the 2003 international VTEC conference in Edinburgh (2.7). Our results were later endorsed by a study commissioned by the HSE and undertaken at the Central Science Laboratory, York, in which VTEC O157 was isolated from rabbits trapped on nearby cattle farms. Surprisingly, wild rabbits have not been implicated in any subsequent outbreaks to date but they may well have contributed to sporadic cases (the majority of all recorded cases) where the source was unknown.

Until the major outbreak at Godstone farm, Surrey in September 2009 (93 cases), most animal associated outbreaks of VTEC O157 had involved only small numbers of cases (mainly less than about 15). A few months before the Godstone outbreak, we published a landmark paper (2.8) which summarised the findings from all VIS/VLA outbreak investigations on animal premises open to the public between 1997 and 2007. This analysis proved invaluable to the Godstone outbreak control team (of which I was a

member and veterinary lead). At the time of writing (June 2011) our extensive multi-author paper on the Godstone outbreak is undergoing peer review.

Concerns generated by Godstone resulted in an independent review commissioned by the HPA and led by Professor George Griffin (to which I gave a presentation). This was published in May 2010 http://www.griffininvestigation.org.uk. It also resulted in a multi-agency reappraisal of the main Health and Safety advice for visits to farms (HSE Agriculture Information Sheet - AIS No 23 rev 2) which was published in March 2011 http://www.hse.gov.uk/pubns/ais23.pdf. An industry code of practice based on AIS 23 is under development at the time of writing and is intended to replace AI 23 in due course. It is now accepted that VTEC O157 is a zoonotic hazard that is always likely to be present in livestock environments and the only realistic way to prevent human infection on farms open to the public is by routine adoption of risk management procedures, particularly strict personal hygiene (2.2).

Unsurprisingly, cattle have the highest prevalence of VTEC O157 infection on open farms (2.8) but the greatest actual risk to visitors appears to come from goats and lambs (2.9). Because of their friendly nature and close contact with people, goats appear to be a particular risk factor (2.10, 2.11, 2.12). The comparatively recent emergence of VTEC O157 as an important zoonotic infection of goats is well illustrated by the fact that this organism did not feature at all in my commissioned review article on Goats and Public Health published back in 1987 (2.13).

In the intensive mixed species environment of most open farms there are significant opportunities for spread of VTEC O157 between the main reservoir hosts (cattle and sheep) and a range of opportunistic hosts such as pigs, goats, equines and camelids (2.8, 2.14, 2.15). This, together with the frequent turnover of young replacement animals to fuel public demand for bottle feeding and similar attractions, may partly explain why the prevalence of VTEC O157 recorded on open farms during outbreak investigations is so much higher than findings from structured farm and abattoir surveys. Whether species other than cattle or sheep can carry and shed infection for long periods is not clear, but

limited follow-up investigations in one outbreak (2.15) suggested that shedding by farm dogs on a cattle farm continued for only a few weeks. It seems likely that infection in species such as wild rabbits and (farm) dogs requires them to have been exposed to infection in cattle or possibly sheep. For example, with the wild rabbit outbreak referred to earlier the infected rabbits had been co-grazing with infected cattle.

Other VLA studies associated with VTEC O157 outbreak investigations included assessing the efficacy of pooled faecal samples instead of individual samples to identify infection (2.16). The control of VTEC O157 infection, and identification of risk factors in cattle herds for VTEC O157 and *Campylobacter* infection, are considered in publications 2.17, 2.18 and 2.19.

Whilst O157 is the most important pathogenic serogroup of *E. coli* in Britain, non-O157 VTEC infections are more prevalent in some countries, such as the Czech Republic and Germany. Such serogroups, including O26 (which causes illness in both man and animals) are also present in Britain (2.20); similarly 103 (2.21). In 2005, in collaboration with colleagues at VLA Weybridge, a method was devised for improving surveillance of pathogenic *E. coli* in England and Wales using isolates from diagnostic submissions to regional laboratories (2.22). The isolates were selected for further testing (serotype and main virulence determinants) using strict criteria (2.23). The survey continued for three years until May 2008 when funding ceased. During this period about 1000 isolates were examined and the survey (2.23) provided a useful insight into the relative prevalence of non-O157 verocytotoxigenic strains in diseased farm livestock in England and Wales – a topic covered only sparsely in the literature. The survey isolates also proved to be very useful for several other VLA studies, including the use of miniaturised microarray to examine virulence profiles and defining the genes in *E. coli* isolates associated with calf diarrhoea and septicaemia (2.24, 2.25, 2.26).

2.2 Cryptosporidiosis

Cryptosporidiosis is caused by a protozoan parasite belonging to the genus *Cryptosporidium*. *C. parvum*, with its very broad host range including man, is by far the main species of veterinary and zoonotic interest and is an important cause of diarrhoea, dehydration and occasionally death in young unweaned mammalian livestock, especially calves and lambs. Human infection with *C. parvum* requires a very low infectious dose and causes unpleasant enteric illness with abdominal cramps. Although exposure to contaminated water is the most common route of transmission for human infection (particularly outbreaks), faecal-oral contact with infected animals on farms open to the public is also an important source (2.2, 2.27). Advances in molecular typing in recent years have enabled outbreak investigators to distinguish between *C. parvum* (principally zoonotically acquired) and *C. hominis* – the other main cause of human cryptosporidiosis, which is acquired directly or indirectly from infected human faeces (but see 2.33).

My first encounter with zoonotic cryptosporidiosis came in 1993 when I assisted the Bedfordshire Public Health Department with the investigation of an (unpublished) outbreak of 66 cases of cryptosporidiosis amongst the 6000 or so visitors to a large sheep farm which opened to the public for three consecutive weekends during the lambing period. There was no recent history of diarrhoea in the lambs and no oocysts were detected in randomly collected faecal samples examined using conventional modified Ziehl-Neelsen (MZN) or phenol auramine (PA) stained smears. Most of the visitors (especially children) had washed their hands in communal buckets of "Savlon" disinfectant which were provided by the farmer who was blissfully unaware that it was totally ineffective against the very resistant oocysts. It was concluded that the lambs had been shedding low undetectable numbers of oocysts which had contaminated the contents of the buckets and caused the outbreak.

Whilst MZN or PA stained smears are perfectly adequate for routine laboratory confirmation of clinical cryptosporidiosis in diarrhoeic animals, they are not sufficiently

sensitive to detect the smaller numbers of oocysts present in faeces of normal consistency from animals with subclinical infection, such as those in the Bedfordshire outbreak. The ability to detect subclinical infection in animals improved considerably after a sensitive fluorescent antibody test (FAT) used by Rachel Chalmers and colleagues at the human Cryptosporidium Reference Unit in Swansea (National Public Health Service, Wales) was validated for veterinary use by colleagues at VLA, Weybridge. The introduction of the FAT, which is at least ten times more sensitive than the conventional smear methods, heralded a series of very productive investigations to measure oocyst outputs by healthy subclinically infected animals. The findings, including the use of a simple scoring system for oocyst counts (developed by Ralph Marshall and colleagues at Weybridge), allowed the potential zoonotic hazards from *Cryptosporidium* species in subclinically (and clinically) affected animals to be readily quantified.

One of the first studies using the FAT was undertaken on an open farm in Norfolk where it readily identified the extent and zoonotic potential of subclinical infection with *Cryptosporidium parvum* in bottle-fed orphan lambs (2.28). These and subsequent investigations utilising lamb, pig and calf submissions to VLA regional laboratories provided further assessments of subclinical infection in these livestock and the range of *Cryptosporidium* species present (2.29, 2.30, 2.31, 2.32, 2.33). Although the sample populations used for these surveys were inevitably biased and based predominantly on diseased animals (but not necessarily with enteric illness), the findings appeared to be fairly representative of the wider livestock population. Much as anticipated, calves presented the greatest zoonotic hazard, followed by lambs and pigs. The combined use of FAT and PCR also led to the novel detection of *Cryptosporidium hominis* (not previously considered to be zoonotic) in a goat and sheep (2.33). The role of FAT, PCR and sequencing was well demonstrated in an investigation in 2009 of a severe outbreak of cryptosporidiosis in large group of children who attended an open day on a dairy farm in Cumbria (2.34). Concluding this fruitful period of work on zoonotic

cryptosporidiosis, a mathematical risk assessment for zoonotic transmission of *C. parvum* from recreational use of farmland was also undertaken (2.35).

2.3 Q fever

Q fever is caused by *Coxiella burnetii*, a zoonotic legionella-like bacterium which is generally of little economic importance to livestock producers other than causing sporadic abortions and occasional abortion outbreaks in sheep, cattle and goats (1.39, 2.12). It is a well documented cause of illness amongst occupationally exposed groups (such as livestock farmers, veterinary surgeons and abattoir workers) from inhaled aerosol exposure to parturition products and discharges from infected animals or to their environment, including contaminated bedding and dust particles. Large scale human outbreaks can also occur, such as a serious one in 2006 at an abattoir and cutting plant at the Bridge of Allan, Stirlingshire. Although most human infection is asymptomatic, Q fever can result in severe flu-like illness and may have serious consequences in high risk groups, particularly pregnant women and people with pre-existing heart disease.

The profile of Q fever increased dramatically in 2007 with the start of the largest community outbreak of Q fever ever recorded worldwide which occurred in the south-eastern part of the Netherlands; by 2010 there had been over 3000 cases. The source of the outbreak was attributed to windborne aerosol spread from an intensive and heavily infected dairy goat farming area with many very large goat herds located close to a large human population. In 2007 there was also an outbreak in Cheltenham, England, which was unusual because the 30 cases were linked only by the fact they lived or worked in the town centre (2.36). Windborne spread from nearby sheep farms was suspected and although the origin was not confirmed, three high risk farms were identified as possible sources (2.36). After much debate, the outbreak control team (of which I was a member) decided not to blood sample livestock on these farms because *C. burnetii* infection was thought to be endemic in Britain and without any recent prevalence survey data it would be difficult to interpret the findings. Also, at that time the only veterinary serological test available was the complement fixation test (CFT), which was somewhat outdated and often difficult to interpret. The farms implicated as potential sources of the Cheltenham outbreak were advised about potential high risk practices that might have contributed to

the outbreak and advisory information on Q fever was circulated to farmers and their veterinary surgeons (2.36).

Suspecting that interest would increase further in the wake of events in the Netherlands, I initiated work to improve diagnosis of Q fever within the VLA. We subsequently compared and evaluated three commercially available ELISAs to improve serological diagnosis (2.37) and used the best performing ELISA (LSI) for a structured seroprevalence survey in sheep and goats using surplus sera from the annual EU sheep and goat survey for *Brucella melitensis*. The low relatively seroprevalence in goats (2.8 %) and sheep (9.7%) identified by this survey, and the different demographics and structure of our goat industry in particular, suggested that an outbreak on the scale seen in the Netherlands was unlikely to occur in Britain. At the time of writing (June 2011) the submitted publication on the survey is undergoing peer review but summary findings from the survey were presented in the 2009 UK Zoonoses report:

http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/zoonoses/documents/reports/zoonoses2009.pdf

We also developed a real-time PCR (2.38) and used it to investigate a major outbreak of Q fever abortions in a large milking goat herd (2.39, 2.40) and a later similar outbreak in Yorkshire. PCR was also used to survey *C. burnetii* infection in abortion material (mainly cotyledons) submitted to VLA regional laboratories in 2010 where this infection had not been suspected from the modified Ziehl-Neelsen (MZN) stained smears (which are used as a screening test in the standard abortion diagnosis package). This survey (short communication currently undergoing peer review) indicated that MZN was a sensitive screening test in sheep, with all samples giving positive MZN smear results being correctly attributed to *Chlamydophila abortus* (enzootic abortion). However, seven percent (95% CI 2.7 - 11.8) of cattle cotyledons were positive by PCR although negative by MZN. This suggested that MZN smears (used mainly in cattle to test for *Brucella abortus*) lack sensitivity when used to screen abortion material from cattle for the possibility of *C. burnetii* infection.

Our efforts to improve diagnostic tests for Q fever within the VLA were vindicated in 2010 when the European Food Standards Agency (EFSA) recommended the uniform adoption of ELISA and PCR to harmonise diagnostic methods and disease surveillance for Q fever within the EU. With the human epidemic in the Netherlands continuing into 2011 despite widespread animal vaccination and various other control measures, Q fever will undoubtedly remain a high profile zoonosis for several years to come.

2.4 *Corynebacterium ulcerans*

During the last 25 years, with the widespread adoption of a vaccination programme in people, there has been a significant decline in the number of human cases of diphtheria due to *Corynebacterium diphtheriae* infection. Conversely an increasingly causative role has been demonstrated for *C. ulcerans.* The latter is a commensal organism found in several animal species including cattle. *C. ulcerans* produces a toxin which is immunologically identical to that of *C. diphtheriae* and it currently accounts for more than two thirds of cases of human diphtheria. Risk factors include foreign travel, consumption of unpasteurised milk and contact with domestic cats and dogs. Interest in possible zoonotic sources for *C. ulcerans* were highlighted by a fatal case of diphtheria investigated in collaboration with the HPA in which the identical case strain was found in a dog which had been in contact with the patient (2.41). A further case occurred in a veterinary student with numerous animal exposures (2.42).

2.5 *Leptospira* Icterohaemorrhagiae

Another emerging zoonotic issue investigated in the last few years which caused concerns amongst public health professionals was *Leptospira* Icterohaemorrhagiae infection (*Weils Disease*) acquired from contact with pet rats (2.43, 2.44, 2.45). The primary source of the infection for the pet rats in these cases appeared to be feral rats. A pet shop was implicated in the first incident. Rats are apparently a popular pet with university students and further similar cases seem inevitable.

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I contributed as project leader and initiator of activities in this field

2.2 **Pritchard, Geoff** (2011) Prevention and control of zoonoses on farms open to the public. In Practice, **33**, 242-251

Commissioned CPD article. My primary work with minor contributions from various colleagues in VLA, Defra, HSE and HPA

2.3 Milne, L. M., Plom, A., Strudley, I, **Pritchard, G. C.**, Crooks, R., Hall, M., Duckworth, G., Susman, M. D., Kerney, J., Wiggins, R. J., Mousedale, M., Cheastey, T & Wilshaw, G. (1999). *Escherichia coli* O157 incident associated with a farm open to members of the public. Communicable Disease and Public Health **2**, 22-26.

I was a member of the outbreak control team, undertook the veterinary field investigations and contributed to writing the paper

2.4 **Pritchard, G. C.**, Willshaw, G. A., Bailey, J. R., Carson, T & Cheasty, T. (2000) Verocytotoxin-producing *Escherichia coli* O157 on a farm open to the public: outbreak investigation and longitudinal bacteriological study. Veterinary Record **147**, 259-264.

I initiated and undertook the follow-up studies having been the veterinary investigator for the initial outbreak and wrote most of the paper in collaboration with Geraldine Smith (Willshaw)

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I undertook the veterinary field investigations and contributed to the writing

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I led the veterinary investigations and collaborated with the CCDC, John Bailey from the public health authority. The text was mainly written with Geraldine Smith (Willshaw)

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Mainly my work based on 2.6

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I led this major work with considerable input from epidemiology (CERA) colleagues at Weybridge, particularly Richard Smith

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I provided veterinary input and contributed to the wording of the poster which was based on previous VTEC O157 outbreak investigations

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Commissioned by GVS. My primary work

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Commisioned by GVS. Based on my primary work and recent investigations

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Commissioned review. My own work

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My initiative. Written in collaboration with Charlotte Featherstone.

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I contributed to this investigation as project leader, collaborated on veterinary aspects with Robert Hogg and assisted with writing the paper

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I initiated the work as project leader and contributed to writing of the non-mathematical part of the paper

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Contributor and member of the group which was led by Joey Ellis-Iversen

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I contributed as project leader and supervisor of the investigation

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I sourced the samples and case histories, and contributed to the writing

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Primarily my *initiative (with collaborators). I supervised the survey and contributed to the poster and abstract*

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Minor contributor (veterinary aspects)

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I provided veterinary input and assistance with writing the paper

Cryptosporidiosis

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Collaboration with Andrew Fleetwood

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My primary work. Written in collaboration with co-authors

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Mainly my initiative and work. Poster designed in collaboration with Julian Cook Photographic Department, VLA, Weybridge (winner of poster prize)

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My initiative and primary worker. Written in collaboration with co-authors.

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I initiated and supervised the survey which was subsequently written up in collaboration with Charlotte Featherstone

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Contributor to writing the paper especially details of the sheep case

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Q fever

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Instigator of the work and veterinary collaborator with Mark Horigan

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Instigator and veterinary lead for development of the PCR by Rebecca Jones

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Instigator and veterinary lead for development of the PCR. Collaborator with Fin Twomey for the abortion outbreak investigation and contributor to paper

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Contributor to the outbreak investigation as project leader and advisor

Corynebacterium ulcerans

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Veterinary collaborator and adviser

***Leptospira* Icterohaemorrhagiae**

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Project leader and veterinary lead for the poster and abstract

2.44 Gaudie, C. M., Featherstone, C. A., Phillips, W. S., McNaught, R., Rhodes, P., Errington, J., Fearnley, C., Fenner, J. S & **Pritchard, G. C**. (2008) *Leptospira*

interrogans *Icterohaemorrhagiae* infection (Weil's disease) acquired from pet rats. Veterinary Record **163**, 599-601

I was veterinary lead for writing - up of the paper, in collaboration with others

2.45 Strugnell, Benjamin W., Featherstone, Charlotte., Gent, Mike., Lister, Patricia., Evans, Gail., Okereke, Ebere., Mawer, Damian., McGann, Hugh., Marchewka, Rebecca., Errington, Jane., Fenner, Jackie & **Pritchard, Geoff**. (2009) Weil's disease associated with the adoption of a feral rat. Veterinary Record **164**, 186

Collaborator and advisor as project leader

PAPERS SUBMITTED

These are referred to the narrative and form an integral part of the activities relating to Part 2 of this thesis. They were still undergoing peer review at the time of writing (June 2011) and are therefore not included in the publications but are listed below for completeness.

Escherichia coli

Ihekweazu, C., Carroll, K., Adak, B., Willshaw, G, **Pritchard, G** and 12 others Large outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection in visitors to a petting farm in South East England in 2009 (Submitted to Epidemiology and Infection)

I was the veterinary lead for the outbreak control team for the Godstone VTEC O157 outbreak and provided most of the veterinary content for the paper

Q Fever

Lambton, S. L., Smith, R., Gillard, K., Horigan, M & **Pritchard, G. C**. Serological survey using ELISA to determine the prevalence of *Coxiella burnetii* infection (Q fever) in sheep and goats in GB (Submitted to Epidemiology and infection)

My primary initiative, including leading validation of the ELISA, and negotiating access to the surplus sera from the Brucella melitensis survey. I also provided veterinary input for the paper. Epidemiological analysis and content was mainly undertaken and written by Sarah Lambton and Richard Smith (Weybridge, CERA)

Pritchard, G. C., Smith, R. S., Errington, J., Hannon, S., Jones, R. M & Mearns, R. Prevalence of *Coxiella burnetii* in livestock abortion material using PCR (Submitted to Veterinary Record)

My primary initiative. Written with contributions from co-authors.

PUBLICATIONS IN SUPPORT OF CANDIDATURE

PART 1 **ANIMAL DISEASES:** publication numbers 1.1 to 1.70 (1980-2008)

PART 2 **ZOONOSES:** publication numbers 2.1 to 2.45 (1987-2011)

The publications are arranged by section within Parts 1 and 2 in the same order as referenced in the narrative and are not necessarily in chronological sequence.

Reprinted from the *Veterinary Record*, 1981, 108 (2) 41 (January 10)

1.1

Transmissible gastroenteritis of pigs

From E. N. Wood and others

SIR, — Transmissible gastroenteritis (TGE) of pigs has reappeared in East Anglia. It was diagnosed in Norfolk in early December 1980 and, at the time of writing (December 31, 1980), we know of another 14 suspected or confirmed cases in Norfolk and Suffolk.

Before the present outbreak we had not diagnosed TGE at the Norwich veterinary investigation centre since the period February to April 1979 when five cases occurred in Norfolk and were confirmed by histopathology using the fluorescent antibody technique (FAT) (Lucas and Napthine 1971).

When epidemic diarrhoea type II (ED II) was first reported (Wood 1977) it was described as usually being less severe than TGE. It was suggested that when mortality in young piglets was less than 30 per cent the disease was more likely to be EDII than TGE. However, clinical differentiation was not clear cut because some recent outbreaks of TGE had tended to be less severe than those described earlier (Goodwin and Jennings 1958, Saunders 1964, State Veterinary Service 1972). This led some practitioners to suggest that TGE had become no more than one component of a viral herd-scour syndrome and no longer warranted special attention.

Our first impression of the present series of outbreaks is that TGE has reappeared as an acute disease that may kill up to 90 or 100 per cent of piglets under a week old. We hope to collect further data but are convinced that TGE is still a distinct clinical entity, the severity of which justifies special measures such as the warning system operated by veterinary investigation centres (State Veterinary Service 1972, Giles 1976, Wood 1979).

TGE may become widespread during the next two or three months and it is self evident that pig farmers throughout the country should reassess and tighten up their disease security arrangements.

We wish to acknowledge the help given us by Miss Sheila Cartwright and her colleagues at the Central Veterinary Laboratory, Weybridge in confirming the present outbreaks by FAT histopathology.

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GOODWIN, R. F. W. & JENNINGS, A. R. (1958) Veterinary Record 70, 271
LUCAS, M. H. & NAPTHINE, P. (1971) Journal of Comparative Pathology 81, 111
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1.2

Reprinted from the *Veterinary Record*, 1981, 109 (16) 367 (October 17) R 3628

Letters

TGE of pigs warning

From Mr G. C. Pritchard

SIR,—May I take this opportunity to warn colleagues of the strong likelihood that transmissible gastro-enteritis (TGE) will return with renewed vigour this coming winter.

This conclusion is based on the history of the disease which shows that it tends to recur in full force during the winter following an initial epidemic. The conclusion is also supported by the following findings from our current investigations into the recent epidemic in East Anglia.

(a) Sporadic new cases in breeding units (about two per month) have been noted throughout the summer, even during the hot spell. These cases have shown all the virulence of the classical disease despite the fact that TGE is traditionally regarded as a disease of the winter months.

(b) There has been a disquietingly high incidence of recrudescence in breeding units, particularly the larger ones, throughout the past months. In part this may be related to the difficulties some owners have experienced when attempting to spread the infection to immunise dry stock. There seems little doubt, however, that the virus has persisted on these premises, probably within carrier sows.

(c) We have obtained good serological evidence, coupled with retrospective clinical histories, to suggest that TGE has been prevalent in fattening units in East Anglia during the past nine months although it would appear to have often passed unrecognised at the time. Moreover, it appears to have persisted on some finishing units throughout this period even on some farms that attempt to follow an 'all-in all-out' system.

In some instances investigations have suggested that weaners derived from breeding units where TGE had occurred, perhaps a few months earlier, introduced infection to susceptible weaners from other sources when mixed together on finishing units.

Regarding the likely means of transmission, most cases appeared to have resulted from 'local spread'. However, considerable circumstantial evidence suggests that wild birds, particularly starlings, have frequently acted as vectors. This is a particularly significant factor on farms where disease security precautions are otherwise very good. As in the past, some cases of disease have also clearly been the result of movement of livestock lorries, infected pigs and personnel.

G. C. PRITCHARD
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Reprinted from the *Veterinary Record*, 1982, 110 (3) 66 (January 16)

1.3

TGE of pigs warning

From Mr. G. C. Pritchard

SIR,—As reported earlier (VR, October 17, 1981, p367) a steady trickle of outbreaks of transmissible gastroenteritis occurred in Norfolk and East Suffolk during the summer, autumn and early winter. Some of these were new outbreaks but we encountered an increasing number of recrudescences as the year progressed.

Recently, however, we have seen a very significant increase in incidence, consisting mainly of new cases. In all, 21 outbreaks have been reported to us between December 29 and January 8. I understand that the Cambridge veterinary investigation centre has also diagnosed four outbreaks in their area.

There seems little doubt that as forecast we are seeing the start of a series of outbreaks which will probably prove as widespread and severe as last winter's epidemic.

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1.4

Reprinted from the *Veterinary Record*, 1982, 111 (22) 512 (November 27) R3747

TGE of pigs

From Mr G. C. Pritchard and Miss S. F. Cartwright

SIR,—A reservoir of transmissible gastroenteritis (TGE) virus has been maintained in East Anglia during the summer months, repeating the pattern seen last year (Pritchard 1981). Because of this, an upsurge in the number of new cases is to be expected this winter, particularly if snow falls during the critical months of December and January. On past experience, however, we would expect there to be fewer outbreaks than last winter.

Of more long term concern are the general epidemiological trends and the behaviour of the virus in this area. Some larger breeding units are continuing to experience clinical recrudescences, indicating that the infection is still present almost two years after the initial outbreak. Diagnosis and control of these recrudescences are becoming increasingly difficult because the clinical features are invariably mild and no longer typical of classical TGE.

Changes in husbandry since the last major epidemic in 1970-73 make it seem far more likely that TGE will now become endemic in East Anglia. Breeding herds are generally much larger, with units of 200 sows more or less commonplace. These larger units usually practise continuous weekly farrowings and often have a regular input of replacement gilts, thus maintaining a supply of susceptible pigs available for infection.

The overall situation has undoubtedly been further exacerbated by the growth of the contract fattening industry during the past decade. Many of the larger finishing units receive weaners from up to 20 or 30 different sources. Mixing these invariably facilitates the perpetuation of the virus (Pritchard 1982). Our epidemiological and serological investigations indicate a significant correlation between size of finishing unit and the occurrence of TGE. Units housing over 1000 pigs, even on an all-in-all-out system, are much more likely to experience TGE in at least one batch of pigs during the year than are smaller units. As expected, this situation is made worse if units are 'topped up' in order to give a continuous throughput.

Since 1957, epidemics of TGE have occurred in England at fairly regular intervals with intervening periods of low incidence or apparent absence. It now seems, however, that, as has happened in some other European countries and the USA, we could expect to see TGE outbreaks occurring each year.

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Reprinted from Veterinary Record, 1982, 111 (22) 512 (November 27) R3739 1.5

Gentamicin resistant salmonella

From W. J. Sojka and others

SIR,—We wish to report the isolation from a pig of a strain of *Salmonella typhimurium* which is resistant to gentamicin (20 µg) and apramycin (15 µg) as well as the following antibacterial drugs: chloramphenicol (10 µg), chlortetracycline (10 µg), ampicillin (10 µg), amoxycillin (25 µg), carbenicillin (10 µg), sulphonamides (500 µg), trimethoprim/sulphamethoxazole (25 µg), streptomycin (25 µg) and neomycin (10 µg). The resistance has been found to be transmissible by conjugation to laboratory strains of *Escherichia coli* K12. Preliminary investigations suggest that the resistant determinants are carried by at least four distinct plasmids.

The strain was isolated from a pulmonary lesion in a four-week-old weaned pig which appeared to have died from transmissible gastroenteritis. During subsequent investigations another three salmonella isolates were obtained from the faeces of recently weaned pigs of similar age. However, only two of the four isolates carry resistant determinants for gentamicin and apramycin. *E coli* and other enterobacteria isolated from the weaners and their environment have not been found to be resistant to either gentamicin or apramycin.

The herd has suffered continuing losses from transmissible gastroenteritis since the end of February 1982. After the initial period of high mortality in piglets, the chief manifestation of the disease has been a post-weaning scour with a mortality of about 5 per cent. More recently, however, there have been further losses in baby piglets in the farrowing house. It seems unlikely that the salmonella is contributing to the post-weaning scour and its recovery appears to be an incidental finding. In-feed medication of the weaners during recent years has included potassium penicillin for the treatment of meningitis and framycetin, neomycin, furazolidone and, more recently, apramycin for the treatment of diarrhoea.

Gentamicin resistance in salmonellas is uncommon but has been demonstrated in strains of human origin isolated in Asia. As far as we know this is the first report of the isolation from animals in the United Kingdom of gentamicin resistant strains of salmonellas. Gentamicin is widely used as a therapeutic agent in man and the appearance of gentamicin resistant salmonellas in animals is a cause for concern because salmonellosis is a zoonotic disease. Consequently we would advise restraint in prescribing antibiotics and the frequent sensitivity testing of enterobacteria to monitor drug resistance.

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Papers and Articles

Observations on clinical aspects of transmissible gastroenteritis of pigs in Norfolk and Suffolk, 1980-81

G. C. PRITCHARD, BVM&S, BSc. MRCVS. *Veterinary Investigation Centre, Jupiter Road, Norwich*

Veterinary Record (1982) **110,** 465-469

Outbreaks of transmissible gastroenteritis in 56 breeding units, six maiden gilt sites and 64 fattening units were investigated during the period December 1980 to October 1981. The disease invariably occurred in classical virulent form, resulting in very high mortality in baby piglets. Reproductive sequelae were sometimes evident. Individual outbreaks continued for longer than in previous years. There was a high rate of recrudescence, particularly in large breeding units, and infection also persisted in many fattening units. The value of control measures and supportive therapy is discussed.

TRANSMISSIBLE gastroenteritis of pigs (TGE) reappeared in East Anglia in the autumn of 1980 and quickly developed into the first major epidemic of the disease since the winters of 1970-73 (Wood and others 1981). Some observations made in Norfolk and Suffolk between December 1980 and October 1981 are reported here, which update previous reports of the clinical aspects of TGE.

Materials and methods

The course of the epidemic was mapped from information obtained at the Norwich veterinary investigation centre under the voluntary warning system (State veterinary service 1972) and from subsequent inquiries. Detailed information was collected from outbreaks on 56 breeding units and six maiden gilt sites in 47 ownerships. These units were selected randomly but almost exclusively from confirmed outbreaks. They showed a wide variation in size and husbandry system. Information was also obtained from outbreaks on 64 fattening units. About half of these were ancillary to affected breeding units; the others were separate premises stocked with purchased weaners.

Diagnosis of TGE was based on the clinical picture and on post mortem examination of piglets submitted to the VI centre. Confirmation was by the direct fluorescent antibody test developed by Lucas and Naphine (1971). Retrospective confirmation was often obtained from the serum neutralisation test carried out at the central veterinary laboratory, Weybridge.

Main findings

The first reports of TGE came from the south west of England in October 1980. The disease had apparently been absent from Great Britain since April 1979, when the Norwich VI centre had confirmed an outbreak in mid Norfolk. The first confirmed outbreak in East Anglia began in Norfolk in late November 1980. However, subsequent investigation suggested that TGE had been active on a Norfolk finishing unit some weeks earlier and on a breeding unit in north east Cambridgeshire in early September.

The incidence of the disease rapidly escalated during December, reaching a peak in January 1981. It subsided during February but sporadic outbreaks continued to occur throughout the spring and summer. Recrudescences were common throughout the summer, autumn and early winter. By the end of September 1981, 199 outbreaks had been reported in England and Wales under the early warning system. Of these, 164 (82 per cent) were in the area served by the Norwich VI centre, namely Norfolk and east Suffolk. Investigation suggested that many other outbreaks, particularly those in fattening units, went unrecognised or unreported by farmers. Often the clinical signs in finishing units were attributed to epidemic diarrhoea. Subsequent serological findings showed, however, that TGE had been quite widespread in fattening units throughout this period. Nearly all the cases reported in Norfolk and east Suffolk were within a broad band about 40 miles wide, extending from the Wash through the lower third of Norfolk to the Suffolk coast.

CLINICAL FEATURES OF THE DISEASE IN BREEDING UNITS

The clinical picture varied greatly but in primary outbreaks was invariably that of the classical disease (Goodwin and Jennings 1958, Cartwright 1969, Wood 1979).

Baby piglets

Morbidity and mortality were very high and at the height of the outbreak approached 100 per cent in piglets under a week of age. Piglets born into an infected environment typically developed signs by about the third or fourth day. The main features were watery, foul smelling, flocculent diarrhoea and occasional vomiting. Death usually ensued within 24 to 48 hours as the piglets became dehydrated, emaciated and moribund. Necropsy usually showed a stomach full of firm clotted milk and thin-walled, translucent intestines distended with watery, greenish-yellow floccular contents. The mesenteric lymph nodes were often enlarged and congested and the kidneys commonly contained urate crystals. The post mortem findings encountered during confirmed recrudescences often failed to show typical features, however.

Older piglets

Mortality was invariably in inverse proportion to age. The average mortality in pigs over two weeks of age was about 5 to 10 per cent, but varied considerably with husbandry and other environmental factors. It was higher in extensive units where losses sometimes reached 15 per cent in four- to five-week-old piglets.

Weaned pigs

These were usually the group least affected, showing only mild clinical signs such as transient inappetance and slight diarrhoea. Morbidity was variable and mortality negligible; any deaths appeared to be mainly associated with secondary infection, usually from coliform organisms. Some pigs remained unthrifty and others showed an upsurge in problems such as meningitis or atrophic rhinitis.

Total piglet losses

The 56 breeding units investigated in detail lost an estimated 16,600 piglets from herds composed of 12,600 sows, an average of 1·3 piglet deaths per sow, caused by TGE. This excludes losses that occurred during recrudescences. The mortality rate was the same as that experienced in the 1970-71 epidemic (State veterinary service 1972) although it would be unwise to attach much significance to this since the criteria used may have been different. Losses recorded on individual farms ranged from 82 piglets in a herd of 10 sows to 70 in a herd of 200 sows where nursing and isolation measures were successful. Elsewhere a typical intensive unit with 500 sows lost 850 piglets over a three month period. Much depended on the breeding programme and the number of susceptible young piglets at risk during the outbreak.

Adult pigs

Morbidity was very variable, ranging from 5 to 100 per cent. On some farms, boars and sows scoured profusely with a watery, foul smelling brown or green 'hosepipe' diarhoea. Less severely affected animals showed only a mild transient looseness of the faeces and sometimes the faeces merely lightened in colour. Anorexia was a fairly constant feature but varied from one missed feed to inappetance persisting for a week or more; occasionally anorexia was not observed and sows continued to feed while scouring. Vomiting occurred infrequently. Agalactia was common; this may have been either a primary effect or caused by the death of sucking piglets. Occasionally adult pigs were severely ill and some were pyrexic. A few sporadic deaths occurred, usually in animals debilitated by concurrent disease. In contrast, many animals showed no clinical signs and some of these remained serologically negative despite several weeks' exposure to infection.

Reproductive sequelae

The State veterinary service (1972) mentioned reproductive failure as a sequel to TGE and Saunders (1964) noted that disruption of the breeding programe could occur. The present observations support these findings since a significant number of farms appeared to suffer some increase in reproductive problems during and after an outbreak.

Some farmers reported an increased incidence of abortions and stillbirths during the period of clinical disease. These were probably associated with maternal failure since there is no evidence for transplacental infection (Wrathall 1975). Some losses may have been caused by the dissemination of other infections such as porcine parvovirus (59e) when dung was spread round the farm as part of the immunisation procedure for TGE.

Most sows that lost their litters because of TGE returned to service within 10 days but over 20 per cent of farmers noted a significantly reduced conception rate to this service. However, low conception rates may follow early weaning (Wrathall 1975). Over 40 per cent of farmers reported reduced litter sizes from services carried out during the height of the outbreak. This was also probably associated with the early weaning since Varley and Cole (1976) reported a decrease of between one-and-a-half and two piglets per litter at the next farrowing when sows were weaned seven to 10 days post partum.

There were no reports of reduced libido in boars, but overworking and service of sows that had not fully recovered possibly played some part in subsequent reproductive failures.

Duration of outbreaks

As the disease progressed on a farm, piglets became protected by colostral and lactogenic antibodies following immunisation of the dams. Sometimes this protection was total; in other litters it persisted only until the piglets were two or three weeks old. A similar delay in onset of clinical signs until 10 to 12 days of age has been noted by other authors including Giles (1976). In some recent cases pigs appeared not to show clinical signs until after weaning.

Of the 56 primary outbreaks in breeding units, 35 (62·5 per cent) lasted for four weeks or more. This contrasts with seven (11·7 per cent) of the 60 outbreaks investigated in 1970-71 (State veterinary service 1972). However, Jackson (1971) commented that outbreaks were tending to last longer than before. The duration of outbreaks was significantly longer on larger units (Table 1).

Recrudescences

It was frequently reported that clinical disease in sucking piglets recommenced after a period of up to 11 months had elapsed, particularly in large units (Table 1). This was also recorded by Giles (1976 and 1977) and the State veterinary service (1972). In all cases of recurrence investigated on breeding units the evidence suggested that recrudescence had occurred rather than reinfection. Generally the mortality was low, with losses restricted to the litters of a few dams. Recrudescences were usually of short duration, but they were sometimes followed by further minor recrudescences. Occasionally recrudescence was limited to the older piglets or those in the post weaning period. Sometimes the recurrence occurred only among purchased gilts brought on to the farm since the initial outbreak.

The explanation of most recrudescences appeared to be that some animals, particularly dry stock, had not been adequately exposed to infection during the main disease episode and that they or their litters had then encountered the virus, perhaps from carrier sows, several months later. This resulted in clinical disease, which was limited to a few susceptible litters sucking from unimmunised dams. This theory is supported by the frequency with which attempts to immunise stock by various feedback methods failed to cause scouring or other signs of infection. Serological investigation of some of these herds indicated, moreover, that frequently

TABLE 1: Relationship between size of breeding herd, duration of initial outbreak and incidence of recrudescences

Size of herd	Number of herds investigated	Average duration of initial outbreak (range) in weeks	Number of herds that showed recrudescence at November 30, 1981
50 sows or less	8	2·8 (1·5-6)	1 (12·5%)
51 to 100 sows	10	5·9 (2-16)	3 (30%)
101 to 250 sows	19	5·6 (2-17)	7 (37%)
Over 250 sows	19	7·1 (2-18)	10 (53%)
All herds	56	5·7 (1·5-18)	21* (37·5%)

*Nine outbreaks confirmed by fluorescent antibody test

up to 20 per cent of sows and, particularly, gilts, had failed to develop positive titres; others produced only a weak antibody response that suggested that their litters would not be protected (Stepanek and others 1971). Serum neutralisation titres are higher in pigs that show diarrhoea than those in which clinical signs are absent (Shimizu and others 1978). The relationship between antigenic stimulation of the intestinal tract and initiation of immune response in the mammary tissue was discussed by Bourne and Newby (1981).

Pathogenicity

There was no real evidence to support the view that the disease was less acute than that seen in previous years (Jackson 1971). In susceptible litters the disease was very pathogenic although occasionally its effects appeared milder when spread in a farm was slower than usual. The low morbidity and mortality described by Giles (1976) appeared to occur only when piglets were sucking from partially immunised dams or in older piglets challenged with infection when passively acquired immunity was waning. Mild TGE also occurred during recrudescences as stated above.

CLINICAL FEATURES OF THE DISEASE IN FATTENING UNITS

The morbidity and severity of disease in fattening units varied considerably, much as described by Wood (1979). The clinical signs noted included a variable degree of inappetance and, or, diarrhoea. The former ranged from one missed feed to a severe anorexia persisting for a week or more. The diarrhoea varied from a grey sludgy looseness to a very watery profuse 'hosepipe' scour. Sometimes the scour was clinically indistinguishable from that of swine dysentery, except that no blood or mucus was present. Vomiting was seldom noted. Affected fatteners were sometimes quiet and lethargic. Usually the infection took one or two weeks to spread through the unit. Differentiation from epidemic diarrhoea was often impossible on clinical findings alone.

Mortality was usually negligible but deaths occasionally occurred when stress from TGE exacerbated concurrent conditions. Considerable mortality did, however, occur on some farms when TGE gave rise to the salt poisoning-water deprivation syndrome in pigs on wet feeding systems (Wood and Borland 1977). Unfortunately farmers were often late in realising that the provision of extra water was essential.

Pigs in a fattening herd investigated by the State veterinary service (1972) took two weeks longer to reach bacon weight. The present investigations suggested that clinically affected fattening pigs often show a significant reduction in daily liveweight gain and feed conversion efficiency (probably about 0·15 to 0·20) attributable to the scour and reduced appetite. Many farmers claimed that one to three weeks' growth was lost, depending on the stage of fattening. Others, however, claimed there was no effect on production performance.

Persistence

In many finishing units, TGE appeared to persist throughout the period of the present investigation, with successive batches of weaners scouring within two to three weeks of arrival. The State veterinary service (1972) also found evidence of continuing disease in fattening units. The situation was worse on units not following an all in-all out system, although some finishing units using that system showed similar recurrences for several batches. It seems possible that this was caused by inadequate cleaning and disinfection. Persistence occurred even throughout the summer months, when the virus is at its most vulnerable. Occasionally, finishing units experienced disease in some weaners which were mixed with apparently healthy weaners derived from breeding units that had suffered from TGE several months earlier. This suggested that a carrier state existed in the latter weaners and that they introduced infection to the other, susceptible, pigs. Underdahl and others (1975) recovered virus from pigs 104 days after experimental inoculation.

Treatment and nursing

No specific chemotherapeutic agents are yet available for the treatment of TGE. However, supportive therapy can be of some benefit in reducing piglet mortality, although success is usually minimal in baby piglets (three to four days) infected at the height of an outbreak.

Most farmers tried a wide range of antibiotics without any detectable success in baby piglets, which supported previous observations (State veterinary service 1972). Broad spectrum antibiotics can be of value in controlling secondary infections, particularly *Escherichia coli* enterotoxaemia (Wood 1979). Growths of typable enteropathogenic *E coli* were obtained occasionally, suggesting that antibiotic therapy should have been of some benefit. However, the consistently high mortality in piglets less than one week old indicated that this was not so in that age group. Some benefit was apparent in slightly older piglets, about three weeks old — an age group sometimes affected by a second wave of clinical TGE as 'lactogenic immunity' (Hooper and Haelterman 1966) waned. In these piglets the viral scour often appeared to be complicated by bacterial infection.

Since dehydration in baby piglets is the main cause of mortality, provision of an adequate supply of electrolyte mixture, preferably with added glucose, is particularly important. Most farmers said that this had been useful and had saved some piglets. Unfortunately the mixture often became grossly fouled and piglets immersed themselves in it and became chilled. The choice of drinker was clearly important and generally the cube type was more useful. There appeared to be little difference in practice between proprietary glucose-electrolyte mixtures and home made preparations.

Early weaning, particularly into piggy box type of accommodation or, to a lesser extent, flat decks, appeared to reduce mortality provided the piglets were at least six or seven days old. Early weaning on to milk substitutes containing glucose has been recommended on the grounds that piglets should be given a sugar that they can assimilate despite their reduced lactase activity (Wood 1979). Provision from an early age of a good quality sow milk replacer and, or, a high protein creep feed also appeared useful, especially when sows were affected with agalactia.

A number of studies have shown that environmental temperature is very important in affecting the ability of newborn piglets to resist infection with TGE virus (Wood 1979). The value of keeping affected piglets warm was demonstrated on one farm where piglet losses were kept to a minimum in a farrowing unit with underfloor heating which was kept at 49°C (120°F). Elsewhere, provision of extra heat lamps was also useful. Keeping piglets dry and clean also appeared to be helpful and the piglets usually did better when provided with some form of litter or bedding rather than when kept on concrete floors.

Noble (1964) claimed that feeding citrated whole blood from recovered sows saved many piglets but no subsequent claims of successful use have been seen and, as noted by Giles (1976), this method is seldom used nowadays. One practice did attempt this form of treatment during the recent outbreak, but without success (J. D. Wilkinson, personal communication).

Piglets obtain immunity from the colostrum of immune

sows and detectable antibody persists in the milk for up to eight weeks (Cartwright 1969). The protective immunoglobulin in the colostrum belongs to the IgA fraction. During the recent epidemic one farmer claimed some success from giving exposed piglets a single oral dose of 2 ml of colostrum collected from recovered sows. However, piglets are only likely to be protected for as long as they continue to be suckled by immune sows (Hooper and Haelterman 1966). The efficacy of administering colostrum from immune sows appears to merit further investigation. The main practical problem is the recognition of animals with a good colostral antibody response. Previous authors, including Stepanek and others (1971), reported considerable variability in the serological and colostral response of adult animals to infection. The present investigations supported their findings.

Cross suckling, to exploit the colostral protection provided by 'immunised' sows, proved to be useful on several farms. This was first noted by chance but later was carried out deliberately. One farm which experienced a series of recrudescences, mainly in gilts' litters, found it useful to exchange these litters with sows' litters at 24 hours of age. In practice, the milk appeared to exercise a protective effect for at least 10 days after farrowing. Cross suckling could therefore be particularly useful when litters are weaned at seven days and replaced by ailing piglets. Successful litter exchanging experiments were carried out by Stepanek and others (1971).

In an attempt to reduce mortality, a few practitioners (I. G. Dick, personal communication) tried levamisole as an immunostimulant (Lowe 1980) in affected piglets. The results, however, were equivocal, mainly because of the lack of untreated controls. In one prolonged outbreak dry sows were injected with levamisole in an attempt to boost the colostral immunity, but this appeared to be unsuccessful.

Although some farmers adopted a fatalistic outlook to treatment, assuming that the outcome was inevitable and that nothing could be done to save baby piglets, most felt it was worth doing something, if only to maintain the morale of the pigmen. Clearly much depends on the individual circumstances of each farm but, in many instances, nursing measures were probably cost effective.

Control

Breeding units

The generally accepted method of control is to attempt to immunise dry stock by feeding back infected material. At the same time an attempt is made to isolate dams due to farrow in less than two weeks and to reduce the amount of virus reaching baby piglets. Pregnant sows infected not less than two weeks before farrowing can rear normal piglets (Saunders and others 1965).

In practice, in nearly all the outbreaks investigated, attempts to isolate imminent farrowings proved unsuccessful. This was also noted by Saunders (1964) who commented that few farmers were able to achieve the total segregation necessary and that infection was usually widespread before its presence was suspected. Attempts at isolation were successful on only a few of the units investigated. On one, sows were moved to makeshift premises away from the main herd. In another, an empty farrowing house, completely isolated from the infected stock, was used and strict segregation and thorough disinfection were maintained. Successful isolation of uninfected farrowing and suckling sows undoubtedly offers the best method of reducing piglet losses and is well worth attempting in outbreaks which are diagnosed or suspected early enough.

For immunisation farmers mainly relied on the collection and 'feeding back' of scour material from affected piglets. This was mixed with food, smeared on troughs or put in the drinking water. Soiled bedding was also used. Some farmers minced or chopped up the viscera of dead piglets and fed this material to the dry stock, although this carries the risk of spreading other infections, including Aujeszky's disease, and would appear to be a contravention of the Diseases of Animals (Waste Food) Order 1973. An alternative method was to put scouring animals with susceptible uninfected pigs.

Many farms experienced difficulties in obtaining a satisfactory response to immunisation procedures (Jackson 1971). The problem was exacerbated when dry stock were on slatted floors and particularly when they were restrained by tethers or stalls. In some outbreaks that occurred during the summer, the difficulty in spreading the infection may have been partly caused by inactivation of the virus by the environmental temperature and sunlight. The virus is considered to be fragile and sensitive to heat and light (Cartwright 1969).

In several prolonged outbreaks, subsequent blood sampling showed a significant proportion of animals to be serologically negative after infective material had been fed to them almost daily for several months. It seems incongruous that whereas some farms experienced great difficulty in deliberately spreading infection, the disease often spread readily to their neighbours' premises. This occurred even during the summer and one outbreak started on the hottest day of the year in the very circumstances described above.

As a general recommendation, it is suggested that fresh material should be fed back daily for sufficiently long to produce as near to 100 per cent morbidity as possible. It is useful to record or mark the dry stock that respond clinically to this procedure, as it is then easier to assess the probable immune status of the herd.

Attempts to limit the duration of prolonged outbreaks on large units by deliberately breaking the farrowing cycle (Wood 1979) were not encountered during these investigations. However, natural breaks on small units often appeared to terminate outbreaks.

Fattening units

TGE persisted on some fattening units throughout the period of this investigation. Some farmers tried to break the cycle of infection by increasing the interval between batches and by carrying out more thorough disinfection, above their usual all in-all out policy. Sometimes this was successful but it seemed that unless it was carried out very conscientiously there was a limited carry-over of virus, even in the absence of pigs. Birds and wild mammals, particularly rats and mice, may be important in this context, and it could be significant that S. Cartwright (personal communication 1981) isolated TGE virus from two of six mice *(Mus musculus)* from a Suffolk finishing unit with continuing disease.

During an epidemic and for several months thereafter it would seem inadvisable to mix weaners from a variety of sources, particularly markets, on the same finishing unit. It appeared that the introduction of symptomless carriers often resulted in clinical disease among the susceptible section of the fattening herd.

Postscript

As expected (Pritchard 1981) the incidence of TGE increased significantly at the end of December 1981. New outbreaks in breeding units are still occurring in March 1982. Initial observations suggest that the clinical features have remained essentially as described above except that on some breeding units the spread of infection has been slower than was noted earlier. This has facilitated the successful isolation of unaffected farrowing houses. A concurrent increased incidence of epidemic diarrhoea in both breeding and

fattening units in East Anglia has complicated differential diagnosis.

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Transmissible gastro-enteritis in pigs

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Introduction

Transmissable gastroenteritis (TGE) ranks amongst the more important pig diseases in many parts of the world, particularly Europe, Japan and the USA. In some pig-producing countries such as Ireland and Denmark the disease has not been diagnosed and has been made compulsorily notifiable. In mainland Britain, although not notifiable, outbreaks have been quite closely monitored since the disease was first reported in 1956. Since 1970 a voluntary notification and early-warning system has been operated by the veterinary investigation centres of the State Veterinary Service.

Although it was first described nearly 40 years ago by Doyle & Hutchings (1946) in the USA, TGE retains a somewhat enigmatic quality and there are still no very satisfactory methods of treatment, control or prevention. In its classical form the disease causes severe outbreaks of diarrhoea, vomiting and high mortality amongst sucking piglets and diarrhoea, vomiting and inappetance of varying severity amongst other pigs of all ages. In some countries, including England, epidemics have occurred at intervals of several years, but this cyclical pattern is less apparent elsewhere. Outbreaks occur mainly during the winter months.

This short review summarises some of the main aspects of TGE with particular reference to recent clinical and epidemiological findings from the current epidemic in the East Anglia region of England.

Aetiology and Pathogenesis

TGE is caused by a coronavirus which although somewhat fragile and sensitive to heat, light and disinfectants, is stable when frozen and at pH 3. The virus infects the mucosal cells of the small intestine resulting in altered function, reduced enzymatic activity (particularly lactase) and villous atrophy. Clinical signs follow a short incubation period, usually of about 18 h.

Clinical features

These depend mainly on the age of pig and the level of herd immunity although other factors, such as herd size and standards of husbandry and management, can influence the picture to a limited extent. In a fully susceptible breeding herd, TGE invariably shows the classical features described by many authors including (Cartwright 1968) and, recently, (Pritchard 1982). The main features are:-

Adults. Typically these show a profuse watery "hosepipe" scour, inappetance and some vomiting. The severity and extent of signs vary considerably in individual pigs, as does the overall morbidity. Lactating sows may show agalactia. Some adult pigs are febrile and appear quite ill but rarely die unless debilitated by concurrent disease.

Growing pigs. Morbidity is again variable but most affected pigs scour and show a loss of appetite for at least 24 h. The scour is usually grey and watery although sometimes it is merely soft and sludge-like in consistency. Vomiting is less commonly observed. Deaths can occur on wet feeding systems due to water deprivation/salt poisoning (Wood & Borland 1977) and the mortality rate may be high if extra water is not provided. Feed conversion efficiency and live weight gain are often reduced although compensatory growth may occur if there is sufficient time before the pigs are marketed.

Weaners. These are usually the least affected but they may scour and show some unthriftiness.

Sucking piglets. This age group is always severely affected. Diarrhoea and vomiting are followed by rapid dehydration and high mortality. At the height of a primary outbreak losses in piglets under a week of age approach 100% but the mortality rate is much less in older sucking piglets. The diarrhoea is usually greenish-yellow, watery, foul smelling and contains undigested milk curd. The typical postmortem findings seen in baby piglets include a stomach full of clotted milk and thin walled, translucent small intestines distended with watery greenish-yellow flocculent contents. The kidneys commonly contain urate crystals.

Diagnosis

In classical outbreaks in breeding units the clinical features of TGE are virtually diagnostic. However, sometimes it may be difficult to distinguish between TGE and Porcine Epidemic Diarrhoea (PED), particularly the clinical form described by Wood (1977). PED is now known to be associated with a corona-like virus that is distinct from that of TGE (Pensaert *et al* 1982). On growing pig units in East Anglia, TGE and PED are usually clinically indistinguishable.

Several methods are available for the laboratory confirmation of TGE and its differentiation from PED. In the UK, the definitive diagnosis of TGE is most commonly carried out by the fluorescent antibody test (FAT) developed by Lucas & Napthine (1971). This detects viral antigen in frozen sections of the small intestine of affected piglets. Retrospective serological diagnosis is often carried out by the serum neutralization test (SNT). New diagnostic methods incorporating the enzyme-linked immunosorbent assay (ELISA) techniques are currently being developed in several countries including the UK.

Course of the outbreak

In a typical outbreak, the herd develops some degree of immunity after about two weeks. This reduces the severity of clinical signs and gives a higher rate of survival amongst baby piglets. Eventually, after a variable period of time, the outbreak terminates. The length of the outbreak depends mainly on the development of immunity in the herd and varies with the size and nature of the herd. The average duration of initial clinical disease in outbreaks investigated in East Anglia in 1980/81 was nearly 6 weeks. However, the duration was directly proportional to herd size (Pritchard 1982).

In England, and probably elsewhere, the average total piglet loss during primary classical outbreaks is about 1.3 piglets per sow (State Veterinary Service 1972, Pritchard 1982). This represents a loss of about 5-6% of the annual pig crop.

Treatment

No specific treatment for affected piglets is available but nursing can sometimes be beneficial. Piglets should, as far as practicable, be kept clean, warm and dry with access to glucose and electrolytes to combat dehydration and reduce lactase activity. Provision of milk replacers is also often helpful. Early weaning at about 6 or 7 days into weaner cages has proved useful in reducing losses amongst older sucking piglets during the recent epidemic in East Anglia: affected piglets usually recover quickly in this favourable warm environment. Little can be done to save piglets affected at 3 or 4 days of age. Antibiotics may be of value in the later stages of the disease particularly amongst older pigs where secondary infection with enteropathogenic strains of *Escherichia coli* is exacerbating the condition. Generally, however, their administration is useless.

In the later stages of the outbreak, and particularly when the disease is endemic, cross suckling and litter switching to exploit the milk and colostrum of immune dams can be very useful.

Control

Immunity. Piglets obtain passive immunity via the colostrum and milk of immune dams. The main protective immunoglobulins belong to the IgA class and are produced in the milk of lactating sows following intestinal infection. The antibodies neutralize TGE virus within the lumen of the gastro-intestinal tract of the piglets, the mechanism being termed "lactogenic immunity" (Hooper & Haeltermann 1966). Dams usually develop protective antibodies within 2 weeks of infection and provided all pregnant sows and gilts acquire infection the outbreak is self-limiting and could finish within 2 weeks.

In an attempt to accelerate the development of immunity in the herd it is common practice to deliberately expose dry sows and gilts to infection by mixing them with other affected pigs, or by "feeding-back" infected faeces or viscera.* Recent experience in East Anglia has indicated a variable response to these procedures. Because of the fragile nature of the virus it is especially important to use fresh infected material. In order to obtain maximum herd immunity, sows or gilts that have scoured should be marked. "Feed-back" can then continue amongst the other stock until all have been clinically affected.

Isolation. Attempts are sometimes made to isolate unaffected piglets and sows and gilts that are due to farrow during the 2 weeks needed for herd immunity to develop. This is rarely successful. However, some successes have been encountered in herds with several small farrowing houses or buildings and with separate personnel for affected and unaffected pigs. In order to protect their subsequent litters, isolated sows should be exposed to infection when their piglets are about 3 weeks old.

Vaccination. Considerable effort has been made in many countries to develop vaccines against TGE. However, a completely satisfactory product is still to be found. Vaccination of pregnant pigs has been attempted using various combinations of virulent, attenuated and inactivated viruses administered by a variety of routes. The main problem is the failure of the vaccines to stimulate adequate IgA production and lactogenic immunity compared with that produced by the virulent field strain of the virus. Vaccines have also been produced for use in sucking piglets, again with only limited success.

ıer consequences of a E outbreak

The effects of a TGE outbreak often extend beyond the initial period of piglet losses and gastro-enteric diseases:-

Reproductive failure. A few abortions may occur during the outbreak. These are probably associated with maternal stress factors since the virus does not cross the placenta. The conception rate is often reduced in sows that are weaned very early and mated at the first oestrus after the loss of the litter; more usually, litter sizes are reduced. These features are probably mainly due to the very early weaning rather than to a direct effect of the TGE virus (Pritchard 1982). Reproductive failure may also be associated with infections such as porcine parvovirus which can be inadvertently spread around the farm during the immunization procedure for TGE. Management problems, including overworking of boars and general upset in the breeding programme, also contribute to reproductive problems.

Clinical recrudescence. Clinical signs sometimes recur on a farm after the disease has been apparently absent for several weeks or months. Further limited milder recurrences may continue over much longer periods. These clinical recrudescences suggest that the infection is still present in the herd. Clinical signs are particularly seen in replacement gilts and/or their litters, in older sucking piglets, or amongst weaners. They are usually milder than in the initial outbreak because there is some level of immunity in the herd. Occasionally, however, classical disease occurs in a few litters. Eventually the infection may become endemic, particularly in large herds (over 200 sows). Diagnosis of endemic TGE is often difficult since clinical features are variabele and atypical of classical disease.

Endemic TGE. In large breeding units with continuous weekly farrowings, TGE virus can be maintained on the farm for long periods (Pensaert 1971, Pritchard 1982) as indicated by continuing recrudescences of clinical disease. This may be associated with the loss of lactogenic immunity at weaning - an effect that is especially marked when early weaning is practised. In larger herds this can present the virus with a continuous series of susceptible weaned animals(Pensaert 1971). This may show as a post-weaning scour, a common recent observation in East Anglia. The problem is further exacerbated if there is a regular input of susceptible gilts into the herd.

The possibility of some sows acting as long-term carriers is debatable although virus has been recovered from pigs 104 days after experimental inoculation (Underdahl *et al* 1975). In East Anglia there has often been clear evidence that clinically unaffected weaners, aged 10-12 weeks, derived from farms which had suffered a clinical outbreak of TGE during the previous few months, introduced infection to susceptible weaners when mixed together on large finishing units. Whether these were true "carriers", or merely animals recently recovered from mild post-weaning TGE but still excreting the virus, is not clear. Gray *et al*(1982) found no evidence for the transmission of the virus by gilts moved off 40 days after the last clinical signs of TGE on two multiplier units in Eat Anglia. The possibility of the virus being maintained by indigenous wild life, such as mice, should not be overlooked.

* In Britain, the use of viscera in a feed-back system contravenes The Disease of Animals (Waste Food) Order, 1973, unless an exemption has been obtained under Article 12 of that Order.

Further epidemiological considerations

In England, TGE epidemics have occurred at fairly regular intervals of 5-7 years with outbreaks occurring for 2 or 3 consecutive winters followed by apparent virtual absence of the disease. TGE was seen again in England in the winter of 1980. Outbreaks continued into the winter of 1981 and through to 1982. During the summer of 1981 the virus survived by virtue of sporadic new outbreaks in breeding units, clinical recrudescences in large breeding units and persistent disease in large (ie over 1000 pigs) finishing units (Pritchard 1981).

Other countries have seen epidemics occurring at shorter intervals than in England. Pensaert (1971) suggested that a 2- or 3-year cycle is a result of a short-lived immunity to TGE in sows and a rapid turnover of the pig population. However, this would not explain why epidemics have occurred in England at intervals of 5-7 years.

Elsewhere, endemic TGE is the commonest form of the disease, with outbreaks occurring every winter. With the increase in herd size and the concentration of the pig industry in specific areas of England, such as East Anglia, it seems possible that future outbreaks of TGE may follow the same pattern. The likelihood is further increased by the development during the past decade of a major contract industry that depends on a supply of weaners from large numbers of sources. Mixing these weaners together on large finishing units inevitably encourages the perpetuation of the virus.

It seems probable that outbreaks occur mainly in the winter because of the better survival of the virus in cold weather and the increased likelihood of its transmission during this time but also because pigs are less susceptible to the infection in warmer weather (Shimizu *et al* 1978).

Transmission of TGE and disease prevention

TGE can, undoubtedly, be spread by vehicles and personnel and also by the movement of infected or recently recovered pigs. It is generally recommended that sales of breeding stock should cease for at least a month after clinical outbreak to avoid transmitting the disease to other farms. There is some evidence to suggest that various other species, including mice, cats, dogs, foxes and starlings, may be involved in transmission of the virus.

Recent findings in East Anglia, supported by previous observations elsewhere, have indicated that outbreaks can often occur when conventional disease security precautions are very good. Follow-up investigations have frequently revealed strong circumstantial evidence to suggest that wild birds, particularly starlings, have been responsible for much of this spread, particularly between farms within a local area of a few miles. Extensive outdoor pig units appear to be especially susceptible. The association between starlings and TGE has been noted many times. In the south of England TGE was termed "Starling disease" (E.A.Gibson, personal communication); farmers in the USA have also blamed starlings for the spread of TGE(Haeltermann *et al* 1976). Pilchard (1965) detected TGE virus in the faeces of starlings 32 h after exposure. Further work carried out in the USA (P.M.Gough, personal communication 1982) confirms that starlings are able to serve as vectors of TGE. In England and the USA epidemics often coincide with heavy snowfalls - times when starlings forage in close contact with pigs.

In attempting to prevent TGE, it is clearly important to reduce the attractiveness of pig farms to starlings in addition to adopting standard disease security precautions. To some extent this can be achieved by management practices such as avoiding feed spillage and also by bird-proofing buildings and feed stores.

Economic aspects

The total cost of an outbreak of TGE on a previously unaffected breeding unit can be considerable. Beside the average loss of 1.3 piglets per sow on the farm (ie 130 piglets per 100-sow unit) there is likely to be a further loss of about 2 piglets per litter and/or conception failure corresponding to at least a 2- or 3-week period of services carried out following the height of the outbreak. Further economic losses are likely to occur as a result of the consequences described earlier. The nucleus breeding herd is particularly severely affected because of loss of gilt and boar sales during and after the outbreak.

The future

There is clearly a need for a satisfactory vaccine to control TGE. Improved methods of treating affected piglets to avoid the otherwise inevitably high losses are also required. Further information regarding disease prevention and other epidemiological factors is still needed.

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THE POSSIBLE ROLE OF STARLINGS IN THE SPREAD OF TGE IN PIGS

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ABSTRACT

Transmissible gastro-enteritis (TGE) is an economically important viral disease of pigs in Britain, particularly in East Anglia. Starlings are often abundant at pig farms where they feed on pig food. The close association between starlings and pigs has led farmers to believe that starlings transmit TGE from one farm to another, but evidence has been circumstantial. The following study was designed to establish whether starlings do move between farms. Starlings which were wing-tagged at a pig farm were relocated at 25 other sites, including eleven other pig farms, so the possibility that starlings can transmit TGE from one farm to another cannot be ruled out.

INTRODUCTION

Transmissible gastro-enteritis (TGE) is an economically important viral disease of pigs that has been reported in many countries of the northern hemisphere. Since the first recognized outbreak in Britain in 1956 (Gibson and Harris, 1963), epidemics have occurred at five-seven year intervals, usually during the winter months between December and April. Throughout the history of TGE in Britain, East Anglia, with its dense pig population, has usually been the area most affected. The main features of the most recent TGE epidemic in East Anglia, in 1980-81, were described by Pritchard (1982).

TGE causes a watery diarrhoea in pigs of all ages. This infectious material is potentially available for transmission by vectors such as starlings (*Sturnus vulgaris*). Starlings are often abundant on pig farms, feeding on pig food in troughs and creep feeders, on spilt food by storage bins, or in surface soil turned over by pigs. The close association between starlings and pigs has led farmers and veterinarians in both Britain and the USA to believe that starlings transmit TGE from one farm to another (Ferris, 1973; Giles, 1976; Pritchard, 1981; 1983; Bohl, 1981). The term "starling disease" has been used by farmers in some areas of Britain to describe TGE (E.A. Gibson, Personal Communication). However, before starlings can be regarded as vectors of TGE, several conditions must be fulfilled:

1. Starlings must be able to pick up the disease, either externally (e.g. on the feathers or feet) or internally (e.g. via the alimentary tract).
2. TGE virus must be able to survive in or on the starlings during the time they take to travel between pig farms.
3. Starlings must be able to pass on the virus to recipient pigs in a dose sufficient to be infective.
4. Starlings must visit more than one pig farm.

Gough and Beyer (1980), working in Iowa (USA), isolated TGE visus on the external surface of 4% of starlings at farms with and without TGE, and from the alimentary tract of 17% of birds. Pilchard (1965) had shown earlier that starlings excreted the virus in their faeces up to 32 hours after experimental infection, suggesting that viral replication was occurring in this species. The fact that starlings can be infected implies that there will be a longer period over which they are a threat to pigs than if they carried the TGE virus only mechanically. Gough and Beyer (1980) found that starlings visited 2.1 pig farms per day and that, under artificial conditions, an infected starling could pass on sufficient TGE virus to a pig to constitute an infectious dose. They concluded that starlings do have the potential to serve as vectors of TGE.

Although the studies in Iowa clearly implicate starlings in the spread of TGE, it is possible that the above conditions are not fulfilled elsewhere. For example, in Hampshire, England, Feare (1980) found that few starlings fed more than 400 m from a calf-rearing unit where they were originally caught and tagged. That study suggested that starlings ranged over such short distances that they were unlikely to be vectors of diseases of farm livestock. However, the pattern of movement of starlings may show regional variations within England so our first aim was to establish the extent of starling movements in a TGE infected area in East Anglia.

TGE virus is relatively fragile, being readily inactivated by heat, light and disinfectants (Bohl, 1981). Several studies have shown that pigs are more susceptible to infection during colder weather (Shimizu et al., 1978; Shimizu and Shimizu, 1979) when it has been observed that starlings are more prevalent on pig farms, particularly when snow covers the ground (Ferris, 1973; Gough and Beyer, 1980). Winter is also the season when a large immigrant population visits Britain (Feare, 1984). Hence contact between birds and pigs is likely to increase at a time when TGE virus is more able to survive and the pigs are most susceptible. However, the cause and effect relationship between these factors is not known. Our second aim was to describe the pattern of attendance of starlings at a pig farm in relation to weather.

STUDY AREA AND METHODS

The study was carried out from November 1982 to March 1983 around the town of Swaffham, West Norfolk, England (52° 39 N, 0° 42 E). Starlings were marked at an extensive pig farm in Shingham (Figure 1) where TGE had been intermittently active for the two years prior to the commencement of these observations. Birds were caught in Chardonneret traps (Spencer, 1972) set on tracks between the paddocks of the farm, and baited with pig food. Most captured birds (344) were aged and sexed (Kessel, 1951), and fitted with single-coloured "Saflag" wing-tags (Feare, 1978). A small number of birds (31) were fitted with radio transmitters (Biotrack SRI) clamped to the base of the four central tail feathers. An AVM LA 12 receiver and a three element hand-held Yagi antenna were used to locate radio-tagged birds.

Local pig farmers were personally informed of the study and asked to report sightings. A radio and television broadcast and an article in a pig-farming journal were also used to publicize the study. Marked birds were also searched for along a selected road transect within 10 km of Shingham. This was carried out around mid-day at regular intervals throughout the study period. It was possible that a major change in the

dispersion and searches were therefore also made for night-time roosts, and their locations noted.

Air temperature records were obtained from RAF Marham, 7 km from Shingham.

RESULTS

The percentages of adult males, adult females, first-year males and first-year females in the trapped samples were 19.2, 16.8, 40.8 and 23.2 respectively, showing a bias towards first-year males. It was not known if this age and sex distribution was representative of the whole population present at the farm. The proportions of each age/sex class captured during November and December were not significantly different from those for January and February ($\chi^2_3 = 4.82$, $p > 0.10$) suggesting a relatively stable population structure throughout the winter.

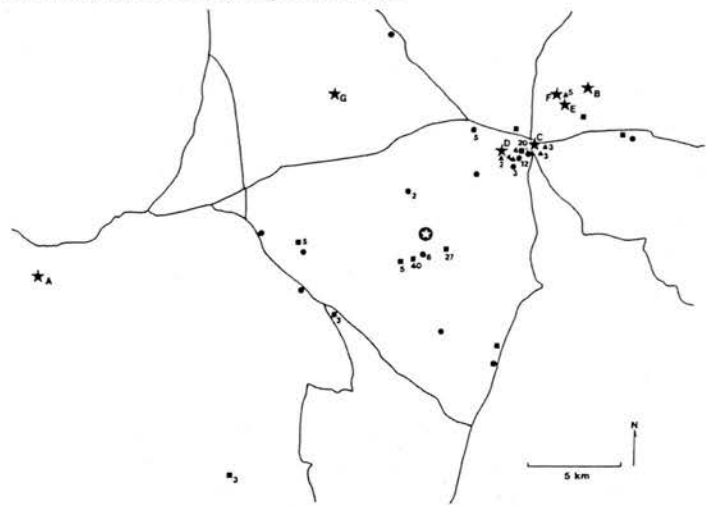

FIGURE 1. The study site in west Norfolk showing the pig farm at Shingham (white star), roost sites (black stars) and localities where birds marked at Shingham were subsequently seen · other pig farms (squares), at pre-roost gatherings (triangles) and other locations (circles). Numbers indicate the number of sightings at a given locality. Lines show main roads.

The starlings fed in a variety of situations in and around creep-feeders and open troughs. They also foraged on patches of grass in the pig paddocks and along the tracks between the paddocks. There were marked diurnal and seasonal variations in the populations of starlings on this farm. The largest numbers were present shortly after daylight and numbers decreased, on average, by 71% (SD ± 23%, n = 26) by late morning. The peak in numbers coincided with the feeding of the pigs in the open troughs. The seasonal pattern of numbers can be seen in Figure 2. Generally there were about a thousand birds present throughout the winter with a period in mid-December when between 3,000 and 5,000 were present soon after daybreak.

We found no relationship between the numbers of birds and minimum air temperatures ($r^2 = 0.02$). Furthermore, we found no correlation between the changes in numbers from one day to the next and changes in the minimum or mean air temperatures ($r^2 = 0.10$ and 0.08 respectively). However, the winter of 1982-83 was relatively mild, especially during January (Figure 2) and snow was scarce. There was a

when paddock fences were taken down and re-erected. This may have affected the number of birds at the farm. However, paddocks were only shifted to a neighbouring field and there was no change in the distance the birds had to fly from the roost to reach the farm in the morning.

Each day starlings left the farm at dusk to roost in a wood. When the study commenced two roosts were known, one in a mixed plantation (Figure 1A) and the other in hawthorn scrub (Figure 1B). Two birds which were radio-tagged at Shingham were relocated at roost A (a distance of 21 km) and one was again located at Shingham. It was not known if birds from Shingham were also using roost B at this time. Roost A was disturbed by farmers on 8 November and abandoned by 10 November 1982 and roost B was also abandoned by 12 November 1982.

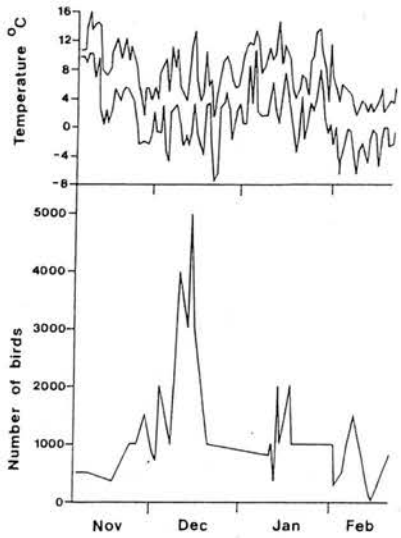

FIGURE 2. Seasonal changes in the number of starlings present at Shingham pig farm within an hour after sunrise (lower graph), and minimum and maximum air temperatures (upper graphs).

The next major roost to be used (C) was in conifers in a large garden at Swaffham. Approximately 100,000 birds used this wood for 14 weeks until it too was disturbed by farmers on 14 February 1983. All 22 birds which were marked with radio-tags at Shingham during this period used this roost. Thus we concluded that for much of the study period the population which fed at Shingham roosted at or near Swaffham, 8 km away. The birds then roosted at localities D, E, F and G over the next few weeks, each shift in location being due to disturbance by farmers. Seventeen tagged birds were relocated in the pre-roost gatherings within 2 km of these roosts (Figure 1). These gatherings were located on grass, stubble and ploughed fields, at a pig farm, a cattle farm and in trees.

During the day, 143 records of tagged starlings were made at 25 localities away from Shingham (Figure 1). These included 12 other pig farms (extensive and intensive), two duck farms, cattle farms, surburban gardens and grass and ploughed fields. The 12 pig farms included seven which had reported TGE outbreaks during the previous two years. Most of the records were made within 10 km of Shingham and the longest recorded movements were 16 km to the south-west and 32 km to the east

DISCUSSION

Gough and Beyer's (1980) study in the USA showed that starlings have the potential to serve as vectors of TGE. In west Norfolk we showed that starlings do move between pig farms during the winter period and if we accept that the other conditions, mentioned in the Introduction, are unlikely to vary between countries, then it would indicate that starlings have the potential to act as vectors of TGE in Britain. The only weakness in the argument is that we do not know, as yet, the rate at which starlings visit other pig farms, and how this is related to the survival times of TGE virus in and on starlings. This will be the basis of further research.

The observed difference between our results and those of Feare (1980) may have been due to different patterns of food availability at the two farms or to differences in age/sex distribution of the captured birds in the two studies. At Shingham, the pig food was super-abundant in troughs for only a short period in the morning, though continually present in the creep-feeders where it was less accessible to the starlings. At the calf unit in Hampshire, studied by Feare, food was continually abundant and he found that 67-77% (in different winters) of his trapped sample were males and most of these (76%) were adults. In our study 60% were males but only 32% of these were adults. Adult birds, which predominated in the Hampshire study, tend to show greater fidelity to particular localities than do young birds (Baker, 1978).

Although disease transmission is most likely to occur directly from one farm to another, there is also the possibility that it might be routed via the roost. The communal roosting behaviour of starlings involves the congregation of birds which have fed over an area of an average 13 km radius (Wynne-Edwards, 1929). At dusk, the huge flocks combine at localities around the roost in pre-roost gatherings in order to feed or rest prior to entering the roost. Thus, both at these pre-roost gatherings and in the roost itself birds may be able to pass disease between themselves. Should a pre-roost gathering occur at a pig farm, as was the case in this study, then this farm would be highly susceptible to receiving infection through contaminated birds from other farms.

SUMMARY

The pattern of attendance and dispersal of starlings was studied in west Norfolk in the winter of 1982-83 at an extensive pig farm where transmissible gastro-enteritis (TGE) had been a recurrent problem. Starling numbers were largest in the early morning, coinciding with the times the sows were being fed in open troughs. No relationship was found between starling numbers and air temperatures, possibly because of the relatively mild winter. A total of 375 starlings (36% adult, 60% male) were tagged and 143 re-sightings were made at 25 localities including 12 other pig farms (seven of which had had TGE in the past two years). The findings from this study supported the contention that starlings are potential vectors of TGE in Britain.

ACKNOWLEDGEMENTS

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Transmissible gastroenteritis in endemically infected breeding herds of pigs in East Anglia, 1981-85

G. C. Pritchard

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Endemic infection was a common sequel to primary outbreaks of transmissible gastroenteritis in large breeding herds of pigs in East Anglia. The main clinical features of the disease were diarrhoea affecting sucking piglets aged about six days or more, diarrhoea among recently weaned pigs and brief episodes of overt clinical recrudescence in part of the herd. Post mortem and bacteriological findings were often more suggestive of colibacillosis than transmissible gastroenteritis. In some herds, endemic infection remained clinically mild or inapparent for long periods. Evidence of endemic transmissible gastroenteritis infection was found in 43 (50·6 per cent) of 85 herds of pigs studied prospectively between 1981 and 1983. There was a significant correlation with herd size; the disease recurred during the 12 months after primary outbreaks in 36 (65·5 per cent) of 55 herds with over 100 sows compared with seven (23·3 per cent) of 30 herds with less than 100 sows ($P<0·001$). In the larger herds it occurred more commonly where finishers were kept ($P<0·05$). Sow morbidity and management factors during the primary outbreak had no statistically significant effect on the incidence of recrudescence. Epidemiological aspects of the findings are discussed with emphasis on the difficulties associated with the diagnosis and control of endemic transmissible gastroenteritis infection.

THE clinical features of classical acute outbreaks of transmissible gastroenteritis in fully susceptible breeding herds of pigs are well recognised by veterinarians and producers alike. However, the milder clinical picture seen in partially immune herds is less readily identifiable. This syndrome has been described as 'endemic' transmissible gastroenteritis (Bohl and others 1972, Morin 1976, Bohl 1981, Hogg 1982). Endemic transmissible gastroenteritis is a significant problem in the USA (Haelterman 1981) and in parts of Europe but has received little attention in Britain. Pritchard (1982b) and Pritchard and Cartwright (1982) reported a high incidence of recrudescence and of persistent infection in large herds in East Anglia and Pritchard (1983) commented that endemic transmissible gastroenteritis was a common sequel to primary outbreaks in large breeding herds.

This paper presents the main clinical, diagnostic and epidemiological features of the disease in Norfolk and Suffolk (East Anglia) after the major series of primary outbreaks in 1980-82 (Wood and others 1981, Pritchard 1981, 1982a, 1982b) and discusses the implications of the findings. Further details of these and related studies have been described by Pritchard (1984).

Materials and methods

Information concerning outbreaks of transmissible gastroenteritis in East Anglia during the five years between December 1980 and December 1985 was obtained from reports received at the Norwich Veterinary Investigation Centre through the voluntary notification and early warning system (State Veterinary Service 1972) and from submissions for routine laboratory diagnosis and field visits.

Terminology

In this paper the terms 'primary outbreak' and 'epidemic outbreak' are used synonymously to describe classical acute transmissible gastroenteritis. 'Endemic transmissible gastroenteritis' is used to describe the persistence of the disease in partially immune herds. 'Recrudescence' is defined as the overt clinical recurrence of the disease at least six weeks after the clinical signs associated with the primary outbreak have been judged to have ceased, and its occurrence is taken to indicate that the herd has become endemically infected with the disease (Pritchard 1983). Recrudescence is not used to describe outbreaks resulting from the reintroduction of infection from outside sources.

Prospective investigations

Although the voluntary notification and early warning system provides a reliable indication of the incidence of epidemic outbreaks of transmissible gastroenteritis, it fails to indicate the prevalence of endemic disease. Prospective studies were therefore undertaken on 85 breeding units in East Anglia which experienced primary outbreaks of transmissible gastroenteritis between December 1980 and October 1982 to investigate any subsequent development of endemic infection.

The farms were visited during the primary outbreaks and details of the outbreaks were recorded. Subsequently regular telephone contact was maintained for at least 12 months with the herd managers or their veterinary surgeons, details of recrudescence and other features of endemic infection were recorded and the farms were revisited as appropriate. Suitable specimens (piglets, sera, faeces) were collected for laboratory examination where possible.

Using the information collected at the time of the primary outbreak, the effect of various factors on the recrudescence of the disease was examined. These factors included the herd size (number of sows), herd type (breeding or breeding and finishing), sow morbidity (less than or more than 60 per cent seen scouring) and management procedures (early weaning, isolation) adopted during the primary outbreak (Pritchard 1982b). When appropriate the results were analysed by standard statistical methods.

Further herd studies

Between March 1981 and May 1983, the clinical and epidemiological features of transmissible gastroenteritis in 13 endemically infected breeding herds were examined in greater depth, mainly by the serological monitoring of individual sows or gilts and of sequential batches of finished pigs at slaughter. Limited investigations elsewhere in East Anglia continued until December 1985.

Laboratory examinations

Laboratory confirmation of the disease was based mainly on

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TABLE 1: Effect of herd size on the incidence of recrudescence of transmissible gastroenteritis

Herd size (number of sows)	Number of herds	Number of herds reporting recrudescence (%)
0–50	7	1 (14·3)
51–100	23	6 (26·1)
101–200	27	16 (59·3)
201–300	12	7 (58·3)
301–500	12	10 (83·3)
>500	4	3 (75·0)

TABLE 2: Effect of herd type on the incidence of recrudescence of transmissible gastroenteritis

Herd size (number of sows)	Incidence of recrudescence: Breeding and finishing	Incidence of recrudescence: Breeding only
0–100	5/20	2/10
>100	21/26*	15/29
All herds (%)	26/46(56·5)	17/39(43·6)

*P<0·05

TABLE 3: Effects of early weaning, isolation and sow morbidity during the primary outbreak of transmissible gastroenteritis on the incidence of recrudescence

Herd size (number of sows)	Incidence of recrudescence: Early weaning* Yes	Early weaning* No	Isolation† Yes	Isolation† No	Sow morbidity‡ <60%	Sow morbidity‡ >60%
0–100	0/2	7/28	0/2	7/28	5/23	2/7
>100	11/17	25/38	7/12	29/43	16/22	20.33
All herds (%)	11/19 (57·9)	32/66 (48·5)	7/14 (50·0)	36.71 (50·7)	21.45 (46·7)	22/40 (55·0)

* From about six days of age
† Isolation of imminently farrowing sows and their litters
‡ Proportion of sows seen scouring

the fluorescent antibody test (Lucas and Napthine 1971) carried out on portions of snap frozen jejunum and ileum taken from affected piglets submitted live to the veterinary investigation centre for post mortem examination. In addition the diagnosis was sometimes confirmed by the serological examination of blood samples by the serum neutralisation test (Cartwright 1966) using microtitre techniques; the detection of positive titres (≥1/2) in home-bred pigs aged over four months provided retrospective evidence of endemic transmissible gastroenteritis in the herd (Bohl 1981) since, by this age, maternally derived antibodies are absent (Derbyshire and others 1969).

In addition to virological examinations, bacteriological culture of small intestinal contents onto sheep's blood agar (Oxoid CM271) and MacConkey agar No 2 (Oxoid CM7) was routinely carried out for differential diagnosis; the isolates were identified by standard bacteriological methods (Ministry of Agriculture, Fisheries and Food 1978).

Results

Epidemiological findings

Evidence of endemic infection was found in 43 (50·6 per cent) of the 85 herds studied prospectively; there was a significant correlation between herd size and the incidence of recrudescence (Table 1). Seven (23·3 per cent) of the 30 herds containing 100 sows or less experienced recrudescence compared with 36 (65·5 per cent) of the 55 larger herds studied (P<0·001). Endemic infection of varying duration appeared to be an almost inevitable sequel to primary outbreaks in herds of more than 300 sows. The presence of finishing pigs had little effect in herds of 100 sows or less, but it resulted in a significantly greater incidence of recrudescence in larger herds (P<0·05) (Table 2). Statistical analysis failed to reveal any significant relationship between the incidence of recrudescence and either low morbidity among sows, the isolation of susceptible pigs or early weaning during the primary outbreak (Table 3).

Clinical findings

For much of the time the endemic disease was mild or inapparent and was difficult to diagnose clinically. However, diarrhoea commencing about a week after weaning and persisting for several weeks within the affected batches of weaners was a common finding. In other herds a mild scour in sucking piglets from about six days old was the predominant feature. Some herds experienced problems with diarrhoea both before and after weaning. The faeces of affected piglets varied in colour and consistency from clear and watery to white and creamy or grey and pasty. Although very few piglets died, concurrent infection with enteropathogenic strains of *Escherichia coli*, rotavirus or salmonella (Sojka and others 1982) sometimes led to unthriftiness and mortality occasionally reached 20 per cent. Adverse husbandry factors and extensive outdoor systems of management increased the mortality.

Adult pigs – particularly purchased gilts – sometimes scoured briefly and seroconverted to transmissible gastroenteritis within a few weeks of joining endemically infected herds. More commonly, however, they failed to show any clinical signs despite efforts to expose them to the virus by 'feed-back' (Pritchard 1982b, 1983). Such gilts often remained seronegative for several months although they usually seroconverted after they had farrowed or the herd had experienced overt recrudescence.

Recrudescence

Periods of recrudescence represented discrete episodes during which mild or inapparent disease gave way to overt disease in part of the herd. They were often precipitated by the simultaneous farrowing of several susceptible gilts in the same house. Endemically infected herds almost invariably experienced some degree of recrudescence within about nine months of the end of the primary outbreak, and most did so by four months. Some herds experienced only a single recrudescence, others had several, usually at intervals of three or four months.

Only one endemically infected herd failed to exhibit recrudescence during the period studied. This herd had a persistent low grade scour among piglets in the farrowing house. Endemic infection was diagnosed by demonstrating rising serum neutralisation titres to transmissible gastroenteritis among the sows and seroconversion among bought-in replacement gilts. In two other herds the recurrence of clinical signs more than a year after the initial outbreak was attributed to the reintroduction of infection rather than to its persistence within the herd.

Recrudescence was always of much shorter duration than the primary outbreak and usually lasted only six to 10 days. It normally became apparent as a sudden resurgence of severe diarrhoea among sucking piglets. Usually only a few litters were affected, frequently those of gilts, and the outbreak was often confined to one or two farrowing houses. The affected piglets were usually aged six days or more at the onset of clinical signs and as a result the mortality was always lower than during the primary outbreak. However, the mortality in the few litters that became affected younger was as high as that seen in typical epidemic outbreaks. The litters of older sows, including those whose piglets had developed the disease during the primary outbreak, were usually unaffected unless they were housed close to litters that were scouring profusely. Adult pigs were generally unaffected during periods of re-

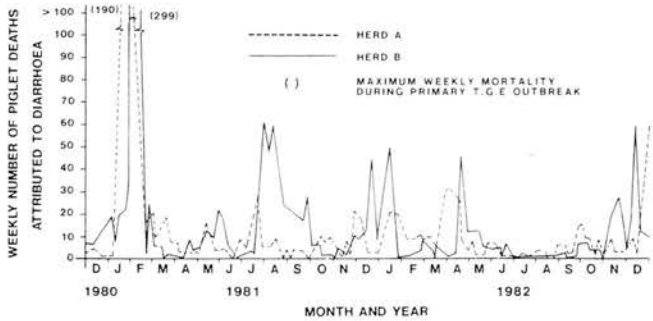

FIG 1: Numbers of piglet deaths per week due to diarrhoea in two endemically infected breeding herds in Norfolk (from Pritchard 1984)

crudescence but those in the same farrowing house as scouring piglets occasionally showed anorexia and transient looseness of the faeces.

The mortality among piglets during a typical recrudescence was rarely more than about 10 per cent of that during the initial outbreak, but the total mortality from several recrudescences often represented a significant financial loss to the producer.

Fig 1 shows the typical pattern of piglet mortality attributed to diarrhoea in two endemically infected herds of 500 sows in Norfolk during the two years after primary outbreaks; the peaks represent overt recrudescence. Transmissible gastroenteritis was reconfirmed by the fluorescent antibody test in herd A in July 1981 and March 1982 and in herd B in July 1981. Serological monitoring of finished pigs at slaughter from both herds confirmed that the virus was continuously present until sampling ceased in December 1982.

Laboratory findings

Gross pathology.— The majority of baby piglets affected by classical outbreaks of transmissible gastroenteritis, seen by the author during the past few years, have shown fairly characteristic post mortem features of thin-walled translucent intestines – associated with severe villous atrophy – distended with watery, greenish-yellow flocculent intestinal contents, stomachs full of firm clotted milk and prominent urate crystals in the kidneys. However, these features were seen in only about 25 per cent of the piglets from endemically infected herds which were normally submitted for examination during periods of recrudescence. Instead, the pathological findings were very variable and frequently resembled those of enteric colibacillosis. The small intestines were often congested with creamy contents, extensive villous atrophy was often not macroscopically evident and urate crystals were rarely seen in the kidneys.

Bacteriology.— Results from the routine bacteriological culture of the small intestinal contents of 240 piglets affected with transmissible gastroenteritis from 78 farms between December 1980 and December 1982 were analysed during these studies. Significant growths of beta-haemolytic or typable enteropathogenic strains of *E coli* were isolated from 22 (32·8 per cent) of 67 piglets from recrudescences compared with only 10 (5·8 per cent) of 173 piglets from primary outbreaks ($P<0·001$). These results suggested that enteric colibacillosis was a common complicating factor in endemic transmissible gastroenteritis.

Fluorescent microscopy.— The fluorescent antibody test was the method of choice for the laboratory confirmation of the disease in unweaned piglets. However, it often proved technically more difficult to perform and interpret when used on piglets from endemically infected herds than when used on piglets from primary outbreaks; the affected piglets were older and their intestines were larger in diameter. Fewer villous enterocytes appeared to be infected with virus and it was often necessary to examine several levels of small intestine.

Serology.— When endemic transmissible gastroenteritis manifested itself essentially as a post-weaning diarrhoea, the preferred method of laboratory confirmation was the serological examination of paired blood samples usually collected at weaning and four weeks later, to detect rising serum neutralisation titres. On breeding units which finished pigs to slaughter weight, retrospective confirmation of endemic disease was conveniently established by the detection of positive serum neutralisation titres in blood samples collected at the abattoir.

Discussion

The observed clinical features of endemic transmissible gastroenteritis were similar to the findings of previous authors. The main clinical signs have often been unrecognised by veterinary practitioners and even a severe recrudescence has been misdiagnosed because the clinical features differ from those of a primary outbreak. Hogg (1982) made similar observations as did Bohl (1981) who commented that the endemic disease could resemble colibacillosis or rotaviral diarrhoea. In the investigation of persistent scour problems on pig units, the possibility of endemic transmissible gastroenteritis should always be considered, particularly in large herds with a history of a primary outbreak in the previous two or three years. However, the laboratory diagnosis of endemic transmissible gastroenteritis can be difficult and the findings may instead seem to support a primary diagnosis of enteric colibacillosis.

Classical transmissible gastroenteritis is unlikely to be confused clinically with porcine epidemic diarrhoea, the other similar coronavirus disease of pigs (M. Pensaert, unpublished data) because the latter is usually much less severe. However, although Wood (1977) suggested that piglet mortality in outbreaks of epidemic diarrhoea affecting unweaned piglets ('epidemic diarrhoea type II') did not normally exceed 30 per cent, Pritchard and Marriott (unpublished data) encountered two confirmed outbreaks in Norfolk in 1982 to 1983 which resulted in a clinical picture and piglet mortality indistinguishable from epidemic transmissible gastroenteritis. Nevertheless, there is more likely to be clinical confusion between epidemic diarrhoea and endemic transmissible gastroenteritis. Fortunately, there are specific diagnostic tests for epidemic diarrhoea based on immunofluorescence and an enzyme-linked immunosorbent assay (ELISA) technique (Callebaut and others 1982) has now been developed for use in Britain (Cartwright and Brown, unpublished data).

The severity of clinical signs among sucking piglets in endemically infected herds appears to reflect their level of exposure to the virus relative to their degree of passive protection from colostrum and milk (Bohl and others 1972). Bohl and Saif (1981) suggested that a mild or subclinical infection in unweaned piglets receiving partial lactogenic immunity might allow active immunity to develop with minimal side effects. The present observations support this hypothesis.

Herd size was the most important factor in the development of the endemic disease and Pritchard (1982b) also showed that primary outbreaks persisted longer in larger herds. Management procedures adopted during the primary outbreaks appeared to have little bearing on the outcome. However, there was a slightly higher incidence of recrudescence in herds where sow morbidity was low. Efforts to isolate imminently farrowing sows and unaffected litters during primary outbreaks failed to lead to a significant increase in endemic disease – possibly because such efforts were usually unsuccessful. It would still be advisable to expose isolated dams and their piglets to infection when the piglets are about two weeks old.

In a large herd it is more likely that a significant number of susceptible pigs will escape infection during the primary outbreak and be available to maintain the virus in the herd. In

East Anglia the large herds almost always used continuous farrowing programmes rather than farrowing in batches, thus ensuring that potentially susceptible piglets – such as those sucking seronegative dams or partially immune dams with a poor milk supply – were always available for infection. Furthermore, a reservoir of transmissible gastroenteritis virus is also maintained among the weaner population (Pensaert 1971, Haelterman 1981) because piglets lose the protection of lactogenic immunity when removed from an immune dam. This hypothesis was supported by the fact that post weaning diarrhoea was common among endemically infected herds.

Another factor which favours the perpetuation of the disease in large herds is the fact the virus can spread slowly through a susceptible population (Maes and Haelterman 1979). In the present studies replacement gilts entering endemically infected herds frequently took several months to seroconvert. Such observations may explain the higher rate of recrudescence seen in large herds where finishing pigs were also kept, for these would contribute to the reservoir of susceptible pigs on the unit.

Large herds with a rapid turnover of breeding stock and using continuous farrowing and early weaning (the current trends in the pig industry) are likely to perpetuate endemic transmissible gastroenteritis whereas small herds, particularly those with less than 50 sows, are likely to inhibit the development of the endemic disease because of the long breaks between farrowings.

The nursing and supportive therapy advised for primary outbreaks (Pritchard 1982b) proved equally applicable to recrudescence; cross suckling and litter switching were particularly successful. Some farmers tried to control the effects of the endemic disease by feeding diarrhoeic faeces from weaned and unweaned piglets to sows and gilts in late pregnancy in an attempt to boost lactogenic immunity by stimulating the gut-mammary gland link (Bourne and Newby 1981). This approach often seemed successful in the short term, particularly during periods of severe recrudescence, but it probably perpetuated the endemic condition and did not control post-weaning scour. Occasionally it increased the levels of virus being introduced into the farrowing house and precipitated a further recrudescence of disease.

Endemic transmissible gastroenteritis sometimes disappeared for no apparent reason although it more often persisted, particularly in very large herds. Haelterman (1981) suggested that it could be eliminated by deliberately infecting all the pigs in a herd simultaneously in order to leave the virus without any susceptible hosts to perpetuate the infection. However, the clinical observations and serological findings in East Anglia indicate that it is not normally possible to achieve 100 per cent infection, especially in large herds.

A strategy that has proved successful (T.J.L. Alexander, personal communication) is to remove all the pigs from the affected breeding unit at weaning for finishing on a separate farm so that the virus maintained in the weaner population cannot return to the farrowing house. However, since the virus could be maintained among unweaned piglets that are receiving insufficient antibodies, it may also be necessary to provide a break in the farrowing cycle. The vacated pig buildings should also be thoroughly disinfected because the virus might survive in the environment if conditions were favourable (Ferris 1973). Vermin and birds should be controlled because mice (Pritchard 1982b) and starlings (Gough and Beyer 1980) can act as short term reservoir hosts. On the few units with less than 100 sows where the endemic disease persisted the standards of hygiene and pest control were poor.

Despite all these recommendations, endemic transmissible gastroenteritis might still maintain itself by the slow and incomplete spread of the virus among adult pigs, particularly herd replacements. There is also the possibility of long-term carriers (Underdahl and others 1974, 1975, Morin and others 1978) although there is some doubt whether such pigs shed infective virus (Haelterman 1981). Very few pig producers were prepared to make serious efforts to eliminate the endemic disease because of the cost and the disruption to management and because of the loss of herd immunity which would be important in the areas of East Anglia with dense populations of pigs, where the risk of reinfection would be high. An effective vaccine for the active immunisation of sucking piglets seems to be the only practical solution to the problem of endemic transmissible gastroenteritis. Although vaccines are used in other parts of the world none are currently licensed for use in Britain.

It is generally accepted (Bohl 1981, Haelterman 1981) that the virus can be excreted in the faeces for up to three weeks after infection; as a result recently recovered weaners could carry infection on to finishing units. In seroepidemiological studies over a 12 month period in 1981 to 1982, Pritchard and Cartwright (unpublished data) showed that outbreaks of transmissible gastroenteritis were much more likely to occur on larger finishing units, particularly those housing over 1000 pigs, than on smaller units (Pritchard and Cartwright 1982). The large units received weaners from many different sources, mainly within East Anglia, and in consequence a significant proportion came from endemically infected herds. Endemically infected breeding herds are therefore likely to provide important reservoirs for the maintenance of the virus and to transfer infection regularly to finishing units, thereby helping to maintain the disease.

In spite of the five to seven yearly cycle of epidemics in Britain, the records indicate that transmissible gastroenteritis has been diagnosed in East Anglia virtually every year since 1956. The region therefore helps to maintain an endemic focus of infection for Britain as a whole. It will probably continue to do so even though one effect of the recent Aujeszky's Disease Eradication Scheme was to eliminate many of the large herds which were endemically infected with the transmissible gastroenteritis.

Addendum

During 1986, serconversion to transmissible gastroenteritis virus was detected in Britain in a large number of pig herds with no history of the disease (Brown and Cartwright 1986). Similar findings were also reported from elsewhere in Europe including Denmark (Danish Veterinary Service 1984) and Belgium (Pensaert and others 1986). An apparently new respiratory non-enteric coronavirus related to transmissible gastroenteritis has now been isolated (Pensaert and others 1986, Brown and Cartwright 1986).

While not detracting from the findings described in this present paper, these recent developments mean that a differential serological test is now needed to distinguish between seroconversion to transmissible gastroenteritis virus and the new porcine coronavirus.

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Transmissible gastroenteritis of pigs

SIR, – Transmissible gastroenteritis (TGE) largely disappeared from Britain during the mid to late 1980s, coinciding with the emergence of infection with a now common and closely related respiratory variant – porcine respiratory coronavirus (PRCV). Classical TGE unexpectedly reappeared in a large herd in Lincolnshire in January 1996 (Jones and Paton 1996), and this letter provides an update on developments since then.

The Lincolnshire outbreak was typical of epidemic cases seen in naive herds affected with acute TGE in the early 1980s (Pritchard 1982). Losses continued for six weeks: 750 piglets and weaners and five sows died. The origin of the infection remains obscure. All pig movements on to the farm for several months were from a single breeding herd with no evidence of TGE. Starlings have been implicated in spreading the disease in the past and were frequent visitors to the farm at the time of the outbreak. Although they had no obvious direct contact with the pigs, they could have contaminated the bakery waste used as feed.

Preliminary characterisation of the virus at the Central Veterinary Laboratory showed it to be fully virulent for neonatal pigs and detectable by a reverse transcriptase-polymerase chain reaction specific for enteric TGE virus (TGEV). From a partial genetic comparison it appeared more distantly related to reference strains of TGEV than other isolates, and most closely related to a British isolate from 1983. However, the number of recent isolates of TGEV available for study are too few to pinpoint its origin with certainty (Paton and Lowings 1997).

The unit in Lincolnshire was seropositive for PRCV before the outbreak and blood samples taken from the piglets in which TGEV infection was first detected were seropositive for PRCV at the time of investigation. Therefore, the widely held view that PRCV infection protects against TGE may not always be correct. This is in accordance with some laboratory studies that have failed to demonstrate cross-protection in experimentally challenged piglets (Van Nieuwstadt and others 1989, Paton and Brown 1990). Unfortunately, antigenic typing and cross-neutralisation tests with PRCV and the Lincolnshire TGEV isolate have been hampered by the poor growth of the new isolate in cell cultures.

Despite intense publicity, particularly locally, there were no further reports of similar outbreaks. Stringent efforts were made to contain the outbreak, including removing all dead pigs to a knackery for incineration. Following confirmation of the diagnosis, efforts were also made to delay slurry spreading for as long as possible. Limited follow-up serology and close monitoring of the clinical picture after the outbreak have surprisingly shown little or no evidence to indicate that infection has persisted. This was unexpected because Pritchard (1987) had previously noted that TGEV usually continued to circulate for some time after cessation of overt disease in large herds.

Surveillance activities were subsequently increased in East Anglia – the traditional heartland of TGE – including revisiting a large outdoor herd which had experienced infection, both epidemic and endemic, throughout most of the period from 1970 to 1987. In this herd, apart from a few weaners which were exhibiting a mild diarrhoea of an indeterminate cause, there was no recent history suggestive of TGE. However, faeces collected from the weaners were positive for TGE with an antigen ELISA and TGEV was subsequently isolated in cell culture. Further studies in this herd, including serology using a TGEV-specific differential ELISA (Ingenasa) confirmed that the virus is circulating in the absence of overt clinical disease and without any detectable effect on productivity. More recently, another outdoor herd in East Anglia experienced a fairly mild scour in adult pigs with 80 to 90 per cent morbidity, which then spread to weaners; a small proportion of the sucking piglets also scoured and a few died. Although virus isolation and antigen detection tests were undertaken, TGEV was not identified in the affected pigs. However, all of 13 sows blood sampled were seropositive for TGEV with the differential ELISA and there was also evidence of recent seroconversion from paired samples.

Serology for PRCV and TGEV was carried out at the Central Veterinary Laboratory as part of a package of tests for the investigation of porcine respiratory disease. Over a 17-month period from January 1996 to June 1997 a total of 1140 sera were examined from 112 herds. Forty-six samples (4 per cent) derived from 27 (24 per cent) herds were specifically antibody positive for TGEV. In the absence of overt enteric disease it is uncertain whether this represents subclinical infection or non-specific cross reaction.

The absence of reports of clinical TGE during the past decade is generally attributed to several factors (Pritchard 1987), including mild winters, elimination of endemically infected herds as a result of the Aujeszky's disease eradication scheme and, particularly, the emergence, in 1986, of PRCV. It is too early to say whether these new incidents of TGEV infection are merely sporadic events likely to be restricted by widespread immunity to PRCV or if they represent the start of a new epidemiological situation. More detailed characterisation of the new TGEV isolates is required and a fuller account of our findings will be submitted for publication in due course.

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11:40 A **Recent experience in the control of *Salmonella sp.* in broiler chicken production.**

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The UK has one of the most sophisticated regimes in the world for the testing and reporting of *Salmonella* infections in food producing animals and their environment. In addition to official tests required by legislation a large number of tests are carried out in accordance with agreements between producers and retailers and under voluntary monitoring exercises. The pattern of occurrence of different serotypes of *Salmonella* in chickens is actually quite different from that which is reported to occur in the human population. As control programmes have been implemented in chickens, the number of isolates from parent stock and their environment has reduced steadily. This progress is particularly notable in the case of *S. enteritidis*. Progress has also been made in reducing the level of contamination of feeds. As this has happened, it has made it possible for us to discern a pattern of occurrence of differing serotypes on specific broiler farms. This allows the prioritising of decontamination activities in accordance with the specific farm history. Once farms are effectively decontaminated, and appropriate barrier systems installed, indoor rearing provides real benefits in that the stock may be protected from environmental sources of infection.

11:40 B **Recent developments with transmissible gastroenteritis in Britain.**

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Classical transmissible gastroenteritis (TGE) returned in January 1996 as a single outbreak in Lincolnshire having apparently been absent from Britain since the mid 1980s. Recent studies in East Anglia indicate that TGEV infection is still present in the pig population but the pathogenicity is extremely low or negligible. In a serological survey of 635 slaughterhouse sera from 64 finishing units, 14.2% were seropositive for TGEV by differential ELISA. Seropositive sows were subsequently detected in three breeding herds supplying weaners to the seropositive finishing units. Seroconversion to TGEV was confirmed amongst purchased gilts within four months in one of the infected herds. TGEV was detected by antigen ELISA on slightly diarrhoeic weaner faeces in all three herds and isolated in tissue culture from one. Seroconversion to TGEV was seen elsewhere in East Anglia amongst sows exhibiting high morbidity diarrhoea. About 4% of samples submitted to the Central Veterinary Laboratory for respiratory virus serology between January 1996 and June 1997 was seropositive for TGEV; routine checks in several high health herds have also identified TGEV seropositive pigs in the absence of overt scour. The emergence of a novel virus, closely related to TGEV or porcine respiratory coronavirus (PRCV) cannot be excluded.

11:40 C **The Computer as a Research Tool.**

Martinelli, M.J.[1], Hoggar, S.G., [2] and Carragher, B.[3] (introduced by Professor S. Love)**
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The computer has become a very important part of daily life for many individuals and continues to provide a valuable service for both academic clinicians and researchers. The purpose of this paper is to demonstrate our use of the computer as a research tool. The system consisted of a Macintosh™ 6200 connected to a flatbed scanner for image acquisition, Macintosh 7500 and 8500 for image processing, and external SCSI devices and a server for data storage. The more sophisticated image manipulations were carried out on a Unix machine accessed remotely via public domain software (Telnet, Fetch). Processing software included a commercial graphic artist's package (Adobe PhotoShop™), a public domain image processing package (NIH-Image) and spreadsheet programs. Colour images were acquired in a fixed raster format at 800 ppi for computerised histomorphometry. Bone identification and quantification were carried out by converting to bitmap format so as to exclude all extraneous information within the image via mathematically based pixel manipulation. Measurements were carried out with custom algorithms and automatically placed into spreadsheet programs for analysis. The computer is most appropriately used for rote calculations and repeatability of measurements. Whenever possible, these should be standardised through the application of algorithms, scripts and macros.
We would like to acknowledge funding from The Home of Rest For Horses.

I.13

TRANSMISSIBLE GASTROENTERITIS IN BRITAIN: A CHANGING SITUATION?

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Introduction

Transmissible gastroenteritis (TGE) is an important pig disease in many parts of the world. It is characterised by watery diarrhoea, vomiting and inappetence amongst pigs of all ages and a high mortality in baby piglets. It presented a serious and recurring threat to the pig industries of many European countries until the mid 1980's when its virtual disappearance appeared to coincide with the emergence, in 1986 (1) of the closely related porcine respiratory coronavirus (PRCV). In Britain, major TGE epidemics occurred every five to seven years between 1956 and 1983. Sporadic outbreaks were confirmed up to the late 1980's and less severe endemic infection lingered in East Anglia where clinical recrudescence, mainly in weaners, was last confirmed in 1989. There were no further convincing reports in Britain until classical TGE unexpectedly returned in January 1996 as a single isolated outbreak in a large herd in south Lincolnshire (2). Following this outbreak, surveillance activities were increased in East Anglia - a pig dense area traditionally associated with TGE (3). The preliminary results of epidemiological and virological investigations (4) are described here.

Materials and Methods

(a) A large outdoor breeding herd (A) in which TGE had been frequently diagnosed since the 1970s and where it had last been confirmed in 1989, was re-visited for investigations in February 1996. Four (19%) of 21 faeces collected from weaned pigs aged 3 - 8 weeks were positive for TGEV by antigen ELISA (5) and 14 (70%) of 20 blood samples collected from cull sows were seropositive for TGEV by differential ELISA (Ingenasa). There was no recent clinical evidence of TGE in this herd although mild non-specific looseness, with low morbidity occurred intermittently amongst weaners. A longitudinal study of nine newly purchased three-month-old gilts commenced 3/96 to follow the TGEV seroconversion pattern in herd A.

(b) A serological survey of blood samples (10 per herd) collected at slaughter from 64 finishing units belonging to a large finishing scheme in East Anglia was carried out between 5/96 and 1/97. The weaners supplying the finishing units came from 18 breeding herds including herd A.

(c) Breeding herds supplying weaners to farms with TGEV-seropositive finishers identified from (b) were visited to collect blood and faeces samples and obtain clinical history. Similar field investigations were also undertaken in another outdoor breeding herd where a suspected viral scour was reported by a local veterinary practitioner.

(d) Differential ELISA serology and faecal antigen ELISA results from samples submitted to the Central Veterinary Laboratory (CVL) for disease diagnosis from Veterinary Investigation Centres (VICs) in England and Wales in 1996 and 1997 were collated. Additional virological investigations, including virus isolation and fluorescent microscopy were undertaken from selected herds.

Results

(a) The nine gilts in the cohort study were all seronegative for TGEV when sampled within an hour of arrival but three were seropositive for PRCV. They remained on the same paddock, which was 200 metres from outdoor weaner kennels where TGEV antigen had been identified in faeces, and were re-sampled 7/96. Two were seropositive for TGEV and one was inconclusive; they had all seroconverted to PRCV. By 1/97, after farrowing and moving to another paddock, a further sow had seroconverted to TGEV and two others, previously seronegative, were inconclusive. When the seronegative/ inconclusive sows were finally re-sampled 3/97 they had all either seroconverted or were inconclusive.

(b) From a total of 635 sera, 45 (7.1%) were seropositive and 54 (8.5%) inconclusive. Viral diarrhoea had not been observed on any of the 18 (28%) positive finishing units and it was suspected that seroconversion to TGEV had probably occurred in the herds of origin.

(c) The seropositive finishing units received weaners from three breeding herds, including farm (A). The two other herds were large units also with weaners in outdoor kennels. Neither had any significant history suggestive of TGE although, as with herd A, there was some mild diarrhoea amongst recently weaned pigs but this had not been identified as a significant problem. In one herd three (8.3%) of 36 weaner faeces were positive for TGEV and in the other 11 (50%) of 22 faeces samples were positive. Seropositive sows were also detected in both herds. Investigations 5/97 in another outdoor herd in East Anglia which experienced a high morbidity, although clinically mild, diarrhoea amongst sows, showed serological evidence of TGEV in all 13 convalescent sows sampled with recent seroconversion demonstrated in two animals. Concurrent infection with *Salmonella typhimurium* was also detected. TGEV antigen was not detected in faeces samples from this herd. Outside East Anglia, TGEV infection was confirmed by antigen ELISA and differential serology in 2-3 week old piglets with enteritis.

(d) Overall, including positive samples from East Anglia, TGEV antigen was detected in 32 (6.1%) of 521 faeces tested at CVL 1996/97 (9 herds). To date, all attempts to isolate TGEV in tissue culture have failed, but samples from two herds were positive by nested RT-PCR (6). Surveillance in 1996/ 7 revealed 111 TGEV positive sera from 45 herds without a history of diarrhoea. Follow-up stratified testing of one high health status breeding unit indicated 5.4% TGEV seropositive animals (77% were seropositive for PRCV).

Discussion and Conclusions

These recent studies indicate that TGEV (or a closely related coronavirus apparently distinct from PRCV) is present in the pig population in Britain. Pathogenicity is very low or negligible in most cases. Although not confirmed, circumstantial evidence suggests an enteric rather than respiratory tropism for the virus. Low level seroconversions in the absence of enteric disease may reflect poor specificity of the TGEV tests. The ability of coronaviruses to mutate is well recognised. Although studies to date have failed to isolate and characterise the infectious agent, the possible emergence of a novel coronavirus closely related to TGEV or possibly PRCV, cannot be excluded.

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1.14

ATTEMPTED ISOLATION AND CHARACTERISATION OF RECENT BRITISH ISOLATES OF TRANSMISSIBLE GASTROENTERITIS

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Introduction

Since the late 1980's, there has been little evidence of transmissible gastroenteritis virus (TGEV) in pigs in Britain. As in other parts of Europe, this change has been ascribed to the emergence of the respiratory variant - porcine respiratory coronavirus (PRCV). In 1996, a single but severe outbreak of TGEV was diagnosed in Lincolnshire (1). Subsequently, TGEV surveillance activity was increased, and several herds were identified in which there was evidence of an enteric TGEV-like agent, albeit of low pathogenicity (2).

This report describes attempts to confirm TGEV diagnoses based on immunoassays, and to characterise the Lincolnshire TGEV isolate.

Materials and Methods

Primary TGE diagnoses of field cases were based on detection of virus in faeces by antigen capture ELISA (3) and/or serology. Antibodies specific to TGEV (and not PRCV) were detected by a commercial ELISA (Ingenasa). Evidence of TGEV was found in several herds as described elsewhere (1, 2, 4).

Attempts were made to confirm these immunological diagnoses by virus isolation and RT-PCR. In most cases, only faeces samples were available. For virus isolation, clarified homogenates were inoculated onto primary pig kidney cell cultures and onto a pig kidney cell line (LLCPK1). Cultures were examined during three blind passages, and any showing cytopathic effect were immunostained using TGEV-specific antibodies. RT-PCR was carried out as described (5) or using the same extraction procedure, followed by a one tube nested RT-PCR. For the latter a new set of primers were used in the first round PCR, followed by nested PCR using the previously described primers as an internal pair (5). The one tube reaction was achieved by sealing the internal PCR primers and reagents within a trehalose plug attached to the lid of the PCR tube (6). After completion of the RT-PCR, the nested PCR was initiated by re-mixing of the tube contents.

Characterisation of the Lincolnshire TGEV isolate involved (a) a pathogenicity study in neonatal and weaned pigs, and (b) genetic characterisation by RT-PCR amplification and sequencing of the 3' end of the genome, encoding the structural proteins.

Results

For the severe Lincolnshire case, the presence of the TGE virus was confirmed by antigen detection and RT-PCR in gut and faeces, and by the development of TGEV-specific antibodies in recovered pigs. The virus grew very poorly in cell cultures. Faecal and intestinal material from the affected herd was fed to four neonatal piglets resulting in acute signs of TGE, and a high level of virus excretion. Four seven-week old pigs were also infected with intestinal homogenate collected from the experimentally challenged neonatal animals. They developed only transient and mild diarrhoea, shed low levels of virus, and seroconverted weakly.

The 3' third of the virus genome was amplified in five segments, each of approximately 1800 nucleotides. Sequencing results indicate that all of the genes are TGEV-like. The spike gene (ORF 2, 4350 nucleotides) contains no insertions or deletions compared to that of the 1970 British TGEV isolate FS772/70 (7), the genes of the two viruses showing a nucleotide similarity of 95.7%. Just downstream of the spike gene is a 9 nucleotide deletion, identical to that found in the 1986 British PRCV isolates 86/137004 and 86/135308 (8). A series of larger deletions obliterate the transcription signal and start codon of ORF 3a as well as most of ORF 3a itself. The remaining ORFs from 3b onwards appear to be unaffected by deletions or insertions, other than a 3 nucleotide insertion in the sM gene (ORF 4).

Apart from the Lincolnshire case, all attempts to isolate TGEV in cell culture or to amplify viral RNA by a single RT-PCR were unsuccessful. Using the nested RT-PCR, an amplicon of the expected size for TGEV was amplified from faeces collected from three herds with immunological evidence of low-grade enteric TGEV. Unexpectedly, smaller amplicons of the size expected for PRCV were obtained from some other faeces samples from these farms, as well as from nasal swabs.

Discussion

The virulence of the Lincolnshire TGEV isolate was demonstrated by the severity of the outbreak from which it originated and the experimentally induced illness in neonatal pigs. Genetic characterisation confirmed that there was no deletion in the spike protein, consistent with the serological findings from the farm. The size of the deletions affecting ORF 3a is greater than has been reported in other TGEVs or even PRCVs, demonstrating that this ORF is not essential for enteric virulence. The phylogenetic significance of the 9 nucleotide deletion found between ORF 2 and 3a in both the Lincolnshire isolate and two PRCV isolates will have to be addressed by examining this region in other field isolates.

It proved very difficult to confirm TGEV detection made by antigen capture ELISA, in cases of low-grade enteritis affecting weaned pigs. Neither virus isolation nor single RT-PCR gave positive results. A nested PCR was therefore developed using a one-tube method to minimise false positive results due to cross-contamination. Using this method, a number of PCR products were amplified, with sizes suggestive of either TGEV or PRCV. Work is underway to characterise these amplicons by sequencing, in order to confirm their uniqueness, and to compare them to other TGEV isolates including that obtained from the Lincolnshire case.

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1.15

SHORT COMMUNICATIONS

Transmissible gastroenteritis and porcine epidemic diarrhoea in Britain

G. C. PRITCHARD, D. J. PATON, G. WIBBERLEY, G. IBATA

TRANSMISSIBLE gastroenteritis (TGE) and porcine epidemic diarrhoea (PED) are infectious diseases of pigs caused by antigenically distinct coronaviruses. They cause anorexia, diarrhoea and vomiting in all ages of pigs, with high mortality in baby piglets. Together with rotavirus infection, they constitute the three main viral causes of diarrhoea in domestic pig herds.

Since it was first described in the USA in the 1940s, TGE has been seen in most countries where intensive pig production is practised. Until the early 1980s, most published accounts of disease outbreaks were from western Europe, Britain and the USA, whereas more recent reports have come from the Asian countries of Taiwan and Korea and from eastern Europe. The decline of TGE during the past 10 to 15 years in Britain and elsewhere has been largely attributed to the emergence of porcine respiratory coronavirus (PRCV) in the mid-1980s (Brown and Cartwright 1986). This virus, which is a TGE virus mutant with a partially deleted receptor binding protein and a tropism for the respiratory tract, has spread rapidly through the pig population, apparently resulting in cross-protection against TGE virus (Pensaert and Van Reeth 1998). Possible additional factors contributing to the disappearance of TGE in Britain include the elimination of endemically infected herds as part of the eradication programme for Aujeszky's disease during the 1980s and a succession of milder winters (Jones and others 1997). PRCV remains widespread, as determined by serological evidence, but there is a lack of information on the tissue tropism and genetic characteristics of currently circulating strains.

TGE epidemics occurred in Britain (mainly in the densely pig populated areas of East Anglia and Humberside) for two to three consecutive winters every five to seven years between 1956 and 1980. After the 1980-82 TGE epidemic (Pritchard 1982) sporadic outbreaks of declining frequency and clinically milder endemic disease in large herds continued until the late 1980s (Pritchard 1987). There were no more convincing reports of TGE in Britain until its unexpected return in 1996 as a single isolated outbreak of classical disease in a large breeding herd in south Lincolnshire (Jones and Paton 1996, McGoldrick and others 1999). A further outbreak was subsequently diagnosed in east Yorkshire in February 1999 (Anon 1999) and at the time of writing (March 1999) there was no evidence of spread to neighbouring farms. There was no obvious explanation for these isolated sudden outbreaks. A similar unexplained outbreak occurred in Eire in 1984 (Gunn 1996).

In Britain and elsewhere in Europe in the early 1970s, a disease now termed PED was initially defined clinically as being similar to, although less severe, than TGE. Wood (1977) subsequently differentiated 'epidemic diarrhoea type I' (diarrhoea and anorexia in adult pigs) from 'epidemic diarrhoea

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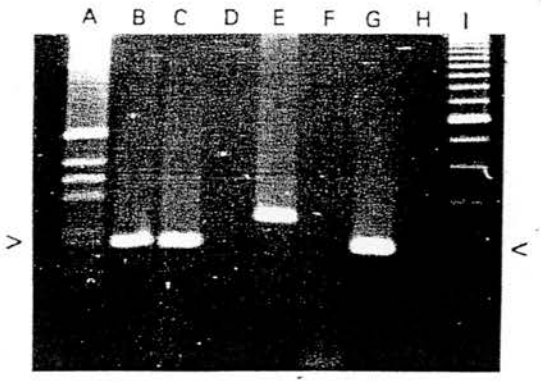

FIG 1: Demonstration of RT-PCR products separated by electrophoresis in a 1 per cent agarose gel, and visualised by ethidium bromide staining and ultraviolet light transillumination. Lane A, 1 kilobase pair DNA ladder; B, C, D, Faeces from field case (two out of three positive); E, TGE virus positive control (strain Purdue); F, H, Negative controls; G, PED virus positive control (strain CV777); I, 100 base pair DNA ladder. Arrows indicate 200 base pair DNA markers

type II' which also affected unweaned piglets. However, following identification of a coronavirus 'CV777' in Belgium (Pensaert and DeBouck 1978) it was realised that both clinical manifestations were caused by the same virus, which was in turn antigenically distinct from TGE virus. Outbreaks of PED, clinically virtually indistinguishable from those caused by TGE, continued in Britain, primarily in finishing units, into the early 1980s but PED among unweaned piglets was relatively uncommon. Nevertheless, one large breeding herd in East Anglia known to the authors experienced a confirmed outbreak of PED affecting unweaned piglets several months after a confirmed outbreak of TGE had occurred on the same unit (G. C. Pritchard, unpublished data).

Like TGE, PED subsequently virtually disappeared from Britain apart from a few unsubstantiated clinical reports. However, an outbreak of PED was diagnosed in February 1998 in a large finishing herd in East Anglia and presented as high morbidity diarrhoea and vomiting in three consecutive batches of incoming eight- to 15-week-old weaners over a two-month period. Laboratory diagnosis was based on the identification of PED viral antigens in the faeces of affected pigs and of PED-specific antibodies in convalescent sera. Both tests used were enzyme linked immunosorbent assays (ELISA) similar to those previously described by Callebaut and others (1982). Confirmation was obtained by RT-PCR amplification from faeces of a short stretch of the PED virus spike protein gene based on the sequence of Duarte and Laude (1994): forward primer, TTTATTCTGTCACGCCATGT; reverse primer, CCAGATTTACAAACACCTATGTTA; annealing temperature 60°C. This RT-PCR test detects both PED and TGE viruses, resulting in different sized products of 199 and 247 nucleotides, respectively. Two out of three faeces samples from the above herd gave a clear product of the size expected for PED virus (Fig 1). PED virus-seropositive sows were subsequently identified in both breeding herds supplying the weaners although neither had a history of suspicious clinical disease (J. D. Wilkinson, personal communication).

Following the TGE outbreak in 1996, surveillance for both TGE and PED was increased, particularly in East Anglia where a serological survey of blood samples (10 per unit) collected at slaughter from 64 different finishing units belonging to a large finishing scheme was undertaken between May 1996 and January 1997. Weaners supplying the finishing units came from 18 local breeding herds. The results (Table 1) showed the anticipated very low seroprevalence (1·9 per cent) for PED. However, the prevalence of TGE virus infection (7·1 per cent) using a competitive differential ELISA (Ingenasa) was higher than expected (particularly when inconclusive positives were also included) in view of the paucity of clinical reports. The test used is designed to be specific for TGE virus and not to detect antibodies to PRCV. A serological survey of sow sera in Britain in 1990 showed a seroprevalence of only 0·6 per cent using a similar competitive ELISA, based on the same monoclonal antibody (Brown and Paton 1991). Further investigations in 1997 indicated that the TGE virus seropositive weaners, none of which had a history of diarrhoea while in the finishing units, were derived from three unrelated breeding herds, including one large outdoor herd (A) where clinically mild endemic TGE had last been confirmed in 1989. None of these herds had a recent history suggestive of TGE but TGE virus antigen was nevertheless detected by ELISA (Paton 1996) in faeces samples collected within a month after weaning on all three units. Although TGE virus was not isolated in tissue culture, samples from one herd were positive by nested RT-PCR (Paton and others 1998). The weaners did not show any significant diarrhoea but a non-specific looseness, not unlike that seen in very mild endemic TGE (Pritchard 1987) was evident in some of the groups sampled. Seropositive sows were present on all three breeding units, including 14 of 20 sampled in herd A.

TABLE 1: Prevalence of TGE virus and PED virus antibodies in 64 finishing units surveyed in East Anglia 5/96 to 1/97

Virus	Sera tested	No of herds	No (%) positive sera	No (%) seropositive herds
TGE	635	64	45 (7·1) *99 (15·6)	14 (21·9)
PED	634	64	12 (1·9)	9 (14·1)

*Including inconclusive positives

A prospective serological study of nine newly purchased three-month-old gilts commenced in March 1996 on farm A (Pritchard and others 1998). They were all seronegative for TGE virus when blood sampled on arrival but three were seropositive for PRCV. They were kept on a paddock which was about 200 metres from outdoor weaner kennels where TGE virus antigen had been identified in weaner faeces. When tested again in July 1996, two were seropositive for TGE virus and one was inconclusive; they had all become PRCV seropositive. By January 1997, after farrowing and moving to another paddock, a further sow had seroconverted to TGE virus and two others, previously seronegative, were inconclusive. When the seronegative and inconclusive sows were finally re-tested in March 1997 they had all either seroconverted or were inconclusive. These findings confirmed that active, albeit subclinical infection with a virus serologically indistinguishable from TGE virus, was present in the herd. Seroconversion to TGE virus was also demonstrated in 1997 in outdoor adult pigs elsewhere in East Anglia following an outbreak of clinically mild high morbidity diarrhoea (Jones and others 1997).

To put the above findings into context, overall, including positive samples from East Anglia, TGE virus antigen was detected in about 6 per cent of more than 500 diagnostic faeces samples tested by the Veterinary Laboratories Agency (VLA) between 1996 and 1997 (nine herds). Serological evidence of TGE was found in 45 herds without any history of diarrhoea and follow-up stratified testing of one high health status breeding unit indicated 5·4 per cent TGE virus seropositive animals (Pritchard and others 1998). Only about 10 herds from which sera were submitted to the VLA during the same period have yielded PED seropositive animals. PED antigen has been detected in only the one herd referred to earlier, since 1995.

In conclusion, although PED remains prevalent in Asia, it appears to be very uncommon in Britain and outbreaks are rarely seen elsewhere in Europe (Pensaert and Van Reeth 1998). The situation with TGE is somewhat different, since there is serological evidence of more widespread infection, as well as sporadic cases of clinical disease. How these findings relate to PRCV infection is an important, but unanswered question. A TGE virus-like virus was detected in pig intestines from the two severe outbreaks of TGE in 1996

and 1999. Whether a similar virus is involved in less marked or subclinical infections remains to be established. Alternatively, could some of the apparently clinically silent seroconversions to TGE virus actually be caused by infection with variant forms of PRCV? Given the known propensity for coronaviruses to mutate by deletion and recombination of their RNA genomes, the possibility of new variant viruses having emerged needs to be evaluated. Further characterisation of circulating TGE virus and PRCV is therefore necessary. There is also a need for improved understanding of serological test results, particularly with respect to interpretation of inconclusive TGE virus seroreactors. Otherwise, there are likely to be potential problems with differential diagnosis and international trade.

Veterinary surgeons in general practice should continue to suspect TGE or PED if suspicious clinical signs, particularly high morbidity diarrhoea affecting all ages of pigs, are seen. In mild cases, where there is a wide range of possible differential diagnoses, serology is a useful and inexpensive starting point. Direct demonstration of the virus is justified where clinical signs are more pronounced, or to confirm serological findings. For TGE, fluorescent antibody tests are available on 'snap frozen' lengths of small intestine taken from freshly killed affected baby piglets; for PED, faecal antigen ELISA is used. RT-PCR tests can provide a final confirmation since virus isolation is difficult. Attempts to isolate and characterise the 1999 Yorkshire isolate are proceeding together with other related studies.

ADDENDUM

During April 1999 two more outbreaks of PED were confirmed by antigen ELISA and PCR in finishing units. Characterisation of the TGE virus isolated from the 1999 east Yorkshire outbreak showed it was similar, but not identical to that from Lincolnshire in 1996. No further clinical outbreaks of TGE were reported despite the close proximity of other pig units in the area. However, retrospective serological evidence of TGE virus infection was confirmed in a nearby breeding herd which had experienced an upsurge in diarrhoea in pregnant sows about two months earlier but without the high piglet mortality associated with classical disease (M. White, personal communication).

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Porcine influenza outbreak in East Anglia due to influenza A virus (H3N2)

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CLASSICAL swine influenza (H1N1) was responsible or partly responsible for a widespread respiratory condition among pigs in England during 1986 (Tofts 1986, Roberts and others 1987). Antibodies to H3N2 influenza A virus strains have been found in pigs in Great Britain since 1968 (Roberts and others 1987), but until now no evidence of clinical disease has ever been reported.

The clinical severity of the influenza outbreaks diagnosed during 1986 varied considerably and appeared to depend largely on intercurrent disease factors and secondary bacterial infection. In many of the outbreaks there was also serological evidence of infection with porcine coronavirus (Brown and Cartwright 1986). The coronavirus is suspected of causing mild respiratory illness in pigs but it is not known whether it contributed significantly to the severity of the influenza outbreaks.

In February 1987, a particularly severe outbreak of respiratory disease occurred on a large (1600 pigs) finishing unit in Suffolk. A pen of 22 pigs aged about 12 to 13 weeks purchased from a local market eight days previously was the first to show signs of illness. Infection spread rapidly through the unit and within three weeks at least 60 per cent of pigs had been clinically affected. Initially, the pigs were seen lying about, failing to rise when roused. They were very dull, lethargic and pyrexic, with rectal temperatures of up to 109°F (42·8°C). Anorexia was marked. Pronounced redness of the skin was often present, particularly around the perineal area. Sneezing was not a major feature but many affected pigs had a barking cough. Recovery occurred over a period of about a week as rectal temperatures returned to normal and appetites improved.

Seven pigs died during the outbreak. These were all in the first pen to be affected and there was a possibility that the losses may, in part, have been associated with water deprivation. The condition is usually referred to as water deprivation/salt poisoning because of the inaccessible position of the drinkers.

Post mortem examinations carried out on four pigs submitted to Norwich Veterinary Investigation Centre showed extensive erythema of the skin. The lungs, trachea and nasal turbinates were extremely congested but pulmonary consolidation was absent. The upper respiratory tracts contained mucopurulent and fibrinous exudate. No significant growths of pathogenic bacteria were isolated from the lungs but an influenza A virus designated A/Swine/Weybridge/163266/87 (H3N2) was isolated from lung tissue taken from one pig that had been submitted live to the laboratory.

Convalescent blood samples were collected at slaughter from 20 pigs approximately two months after recovery. Seventeen (85 per cent) of these were seropositive for A/Swine/Weybridge/163266/87 (H3N2) virus but they were all seronegative for the A/Swine/Belgium/1/79 (H1N1) and A/Swine/Weybridge/117316/86 (H1N1) strains of virus with the haemagglutination inhibition test (Roberts and others 1987). The samples were all seropositive for transmissible gastroenteritis/porcine coronavirus with the serum neutralisation test.

It was of particular concern to the farmer that nine (10 per cent) of the first 90 recovered pigs to go for slaughter after the outbreak were condemned by the meat inspector as a result of 'septic pleurisy'. This was in spite of water medication with therapeutic levels of chlortetracycline and the inclusion of therapeutic levels of potentiated sulphonamide in the feed during the main period of the outbreak. In view of the condemnations, a further seven days' in-feed medication with potentiated sulphonamide was provided to all the remaining pigs on the unit and no further rejections occurred. However, at least 30 per cent of carcases required pleural stripping at the abattoir. It was not clear whether the bacterial infection occurred during, or as a consequence of the outbreak or whether it was merely incidental. There had been no evidence of secondary bacterial infection in the pigs necropsied in the laboratory at the beginning of the outbreak, although extensive pneumonia and pleurisy was present in one pig subsequently necropsied on the farm.

The direct cost of the outbreak to the farmer was in excess of £3000. This comprised an increased medication cost of £1800 and a further loss of £1200 from deaths and condemnations. In addition, there was the unquantifiable cost of an extra two weeks to slaughter due to loss of growth during the period of inappetence and fever.

There has long been an apparent association between influenza A virus infection in man and infection in pigs. H1N1 and H3N2 strains circulating in the human population are thought to provide a source of subsequent infection for pigs (Schild 1984). There was some public health concern with this present outbreak because both the owner and the pigman's son experienced a severe influenza like illness at about the same time that the outbreak started in the pigs. However, convalescent sera examined at the Public Health Laboratory, Colindale, failed to indicate exposure to the porcine H3N2 isolate, but they did show high antibody titres to the currently circulating human influenza virus (A/Taiwan/1/86 H1N1). During the past year, 30 serum samples from humans in close contact with outbreaks of swine influenza in Britain have been tested and found negative for porcine strains of influenza A virus (H1N1 and H3N2) (P. Chakraverty, personal communication).

There were several other unconfirmed clinical reports of apparently similar outbreaks on nearby pig units in Suffolk at about the same time as the outbreak described above. A clinically mild outbreak in May 1987 on a fattening unit about a mile away, was subsequently confirmed by demonstrating rising antibody titres to H3N2 virus. The H3N2 swine influenza A virus is known to have caused recent problems elsewhere in Europe (Haesbrouck and Pensaert 1986).

The clinical severity of swine influenza (H1N1 or H3N2) in Britain appears to be somewhat variable. In outbreaks of the severity described above, the possibility of swine fever needs to be considered as part of the differential diagnosis. However, this can normally be eliminated at post mortem by the absence of petechial and ecchymotic haemorrhages. At the other extreme, it seems likely that many of the milder outbreaks of swine influenza are passing unrecognised by producers and veterinarians or are perhaps being attributed to enzootic pneumonia or other respiratory infections.

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Papers and Articles

Zinc toxicity, copper deficiency and anaemia in swill-fed pigs

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Zinc toxicity, copper deficiency and an associated anaemia among weaned swill-fed pigs is described. The source of zinc was flaking galvanising from the inside of bins used to store swill before processing. The disease condition readily responded to supplementation of the diet with copper sulphate. The interactions between dietary zinc, copper and calcium are discussed with reference to the swill-feeding industry. It is concluded that supplementary copper should be routinely included in the ration of swill-fed pigs and that the use of galvanised bins for storing swill should be avoided.

ZINC toxicity in farm livestock is a rare occurrence. In pigs, zinc deficiency is well documented but there appears to be no account of a field case of toxicity.

Experimentally induced zinc toxicity in pigs has been described by Sampson and others (1942) who confirmed earlier observations (Grimmett and others 1937) that feeding young weanling pigs 0·1 per cent of zinc (as zinc lactate) in largely milk diets for an extended period produces poor appetite, unthriftiness, lameness and 'arthritis'. However, Sampson and others (1942) observed no ill effects in older pigs (35 to 50 kg) fed the same level of zinc in a diet of ground meal and skim milk. Brink and others (1959) found that 0·1 per cent zinc (as zinc carbonate) was the maximum level tolerated in the diet of weanling pigs (from 16 to 18 kg bodyweight initially) fed on meal for up to 42 days but that higher levels (0·2 to 0·8 per cent) produced clinical signs including depressed appetite, weight loss, haemorrhages, an arthritic condition and death.

This paper describes zinc toxicity, copper deficiency and an associated anaemia in swill-fed pigs in a herd in Norfolk. The source of zinc was flaking galvanising from the inside of bins used to collect and store swill before processing. The findings are discussed with particular reference to their significance to the swill-feeding industry.

Case history

System of husbandry and feeding

A swill plant (designated A) was licensed under the Diseases of Animals (Waste Food) Order 1973. The swill was fed to a herd of about 50 Hampshire cross large white sows and 550 finishing pigs, all kept on the same holding. During pregnancy the sows were managed outside in paddocks and fed entirely on swill. They were brought inside to farrow and received only proprietary meal while in the farrowing house.

All piglets were routinely injected with a commercially produced iron dextran solution at three days of age. They were weaned at eight weeks on to a proprietary concentrate containing 175 mg/kg added copper. At 10 to 12 weeks, the growing pigs were given a diet consisting entirely of swill, with no mineral supplement. However, a group of about 50 pigs managed by the owner's brother, were also fed a daily 'handful' of proprietary meal containing supplementary copper.

Clinical features

Clinical signs were first seen in May 1982 and were restricted to finishing pigs aged about three to four months that had been fed only on swill for at least two weeks. Most pigs were affected but the 50 pigs managed by the owner's brother and receiving supplementary copper were unaffected.

The pigs were pale, lethargic, unthrifty and anorectic. About a third of them had conspicuous subcutaneous haematomas, particularly over the limb joints and involving the aural pinnae. In addition, about 10 per cent of pigs showed evidence of lameness and several were recumbent. Mortality was about 4 per cent.

In view of the apparent anaemia, some of the affected pigs were injected with iron dextran solution by the owner but no response was seen.

Necropsy

Four pigs, weighing between 17 and 30 kg and aged between 10 and 14 weeks, were examined post mortem at the veterinary investigation centre, Norwich in June 1982. One had a large haematoma over the right hock joint while another had a large aural haematoma.

The skeletal musculature and viscera of all four pigs were very pale; the blood was watery in consistency and failed to clot readily. The spleens were enlarged and the lymph nodes congested or haemorrhagic. Sanguinous fluid was present in most joints but articular surfaces and synovia appeared normal. There was excessive pericardial fluid and epicardial petechiation, particularly adjacent to the coronary groove. Haemorrhagic foci, about 2 to 3 mm diameter, were distributed throughout the lungs. In addition, the lungs of two of the four pigs had typical gross lesions of enzootic pneumonia.

Histopathology

Histopathology of liver, kidney and bone marrow from two clinically affected pigs was carried out. Kupffer cells throughout the livers were laden with haemosiderin. In the kidney of one pig haematopoietic cell foci were sparsely distributed at the corticomedullary junction and the bone marrow was cellular with evidence of granulocytic maturation; normoblasts and megakaryocytes were also present.

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TABLE 1: Haematological findings and blood copper levels

Pig identity and clinical status	RBC ($\times 10^{12}$/litre)	Hb (g/dl)	PVC (litres/litre)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Nucleated red cell (%)	WBC ($\times 10^{9}$/litre)	Corrected WBC	Copper supplement status	Blood copper level (mg/100 ml)
Two of four severely affected pigs submitted for necropsy	1·26	2·5	0·053	–	–	–	–	–		Nil	
	1·31	2·5	0.077	–	–	–	–	–		Nil	
Affected											
Pig 1	2·72	5·7	0·200	73·52	20·95	28·50	12	20·40	17·95	Nil	0·01
Pig 2	2·44	4·7	0·095	38·93	19·26	49·47	2	6·50	6·37	Nil	0·01
Recovering											
Pig 3	2·32	5·0	0·225	96·98	21·55	22·22	15	11·60	9·86	Treated with copper after signs of anaemia developed (0·5g $CuSO_4$/pig/day in swill)	0·02
Pig 4	8·32	10·8	0·370	44·47	12·98	29·18	1	15·30	15·14		0·08
Normal											
Pig 5	5·97	12·1	0·390	65·32	20·26	31·02	5	16·10	15·29	Supplemented with copper daily in diet as standard practice	0·08
Normal haematological values* (age matched) in 3½ to 4-month old Duroc-Jersey pigs	6·4–8·0	11·5–12·0	0·380–0·440	53–61	–	28·0–31·0	–	18·9–33·8			Normal blood copper level† 0·12–0·18

*Schalm and others (1975)
†Central Veterinary Laboratory, Weybridge data
RBC Red blood cell count
Hb Haemoglobin
PCV Packed cell volume
MCV Mean corpuscular volume
MCH Mean corpuscular haemoglobin
MCHC Mean corpuscular haemoglobin concentration
WBC White blood cell count

Haematology

Table 1 presents haematological findings from two of the pigs submitted for necropsy; two clinically affected pigs from which supplementary copper was withheld (pigs 1 and 2); two pigs that were recovering following copper supplementation (3 and 4) and one unaffected pig (5) belonging to the owner's brother. The latter pig had received each day a small quantity of proprietary pellets containing copper to supplement the swill. Five of the six affected pigs showed clear evidence of anaemia. Only pig 2, in which leucopenia was evident, had a neutropenia.

Biochemical analysis of tissues

The blood copper concentrations in pigs 1 to 5 were all lower than normal values; those in pigs 1, 2 and 3 were particularly low (Table 1).

The levels of iron, copper and zinc in the livers of three of the four affected pigs necropsied and the kidney of one of these three pigs are given in Table 2. Compared with normal, iron and copper levels were somewhat depressed but zinc levels were considerably elevated.

Sources of swill and its biochemical analysis

The swill used on plant A was collected from Air Force bases and from hotels and restaurants. A few weeks before the onset of the disease problem, about 30 extra galvanised bins had been purchased – from a different supplier to that used previously – for the transportation and storage of swill from an additional Air Force base. The owner kept these new bins in use even though the galvanising was flaking off into the swill.

A composite sample of swill taken from the processing tank, and separate composite samples taken from the old bins and from the new bins (with the flaking galvanising), were analysed for copper, zinc and calcium (Table 3). Although the swill stored in the old bins also contained a high level of zinc this was only about half of that in the new bins. Calcium and copper levels were similar in all three samples.

Response to copper supplementation

Once copper deficiency and anaemia had been confirmed, the swill was supplemented at the rate of 0·5 g of copper sulphate ($CuSO_4.5H_2O$), ie, approximately 100 mg copper per pig per day. The clinical response was rapid and dramatic; appetite returned, the pigs regained their colour and the incidence of haematomas, lameness and recumbency fell within a few days.

TABLE 2: Concentrations of zinc, iron and copper (mg/kg dry matter) in the tissues of affected pigs examined post mortem at the veterinary investigation centre

Pig identity	Liver Zinc	Liver Iron	Liver Copper	Kidney Zinc	Kidney Iron	Kidney Copper
Three of four severely affected pigs submitted for necropsy	2100	170	4·9	770	38	18
	2400	130	3·8	–	–	–
	1400	–	10·0	–	–	–
Common adequate levels (Central Veterinary Laboratory, Weybridge data)	100–300	>300	>20	50–150	150–350	20–30

TABLE 3: Levels of copper, zinc and calcium in the swill used on plant A during the disease outbreak

Swill sample	Copper (mg/kg dry matter)	Zinc (mg/kg dry matter)	Calcium (g/100 g dry matter)
Processing tank*	4·2	4300	0·46
Old bins	3·2	2580	0·37
New bins†	3·7	5100	0·37

* Containing a mixture of swill from both old and new bins
† Those with flaking galvanising

TABLE 4: Levels of copper, zinc and calcium in the swill used on plant A in December 1982, six months after resolution of the disease problem

Swill sample	Copper (mg/kg dry matter)	Zinc (mg/kg dry matter)	Calcium (g/100 g dry matter)
Processing tank*	7·1	130	0·5
Old bins	1·1	38	0·3
New bins†	1·8	170	0·2

* Containing a mixture of swill from both old and new bins. Copper sulphate supplement added
† Those from which the galvanising had flaked off

TABLE 5: Levels of copper, zinc and calcium in the swill used on four other licensed premises

Plant sampled*	Copper (mg/kg dry matter)	Zinc (mg/kg dry matter)	Calcium (g/100 g dry matter)
B	5·0	130	1·83
C	9·0	90	0·67
D	3·5	98	0·24
E	4·7	540	0·60

* Composite sample of swill taken from the processing tank

Copper supplementation of the swill was maintained after clinical signs were no longer present. The owner continued using the new bins, which, by this time, had lost most of their galvanising.

In December 1982, the blood copper levels of five finishing pigs aged four to seven months were checked and found to be satisfactory (0·08 to 0·20 mg/100 ml). The swill was also re-analysed at this time and the zinc content was found to have fallen very markedly (Table 4).

There was no further evidence of either zinc toxicity, copper deficiency or anaemia on this unit. In fact, the owner frequently commented on how well the pigs were growing since the introduction of supplementary copper to the ration.

Analysis of swill used on other plants

Swill samples collected from four other licensed swill plants (designated B, C, D and E) in Norfolk were analysed for copper, zinc and calcium to compare levels with those in the swill from plant A. Swill on these four plants were derived from hospitals, hotels, restaurants, holiday camps and Air Force bases. In each case it was collected and stored in galvanised bins.

The biochemical findings are given in Table 5.

Discussion

Liver zinc concentrations in pigs are commonly of the order 100 to 300 mg/kg dry matter (Central Veterinary Laboratory, Weybridge data) but reflect dietary intake; Sampson and others (1942) found similar levels in their control pigs. Kidney levels remain more constant (50 to 150 mg/kg dry matter) unless dietary intake is very high. In the zinc toxicity trials of Sampson and others (1942) where 0·1 per cent zinc was included in the diet of weanling pigs, liver zinc concentrations of 3500 to 4320 mg/kg dry matter were found after about nine months on experiment. Brink and others (1959) found liver concentrations ranging from 1600 mg/kg wet weight (approximately 4800 mg/kg dry matter) for a pig fed 0·2 per cent zinc and 3100 mg/kg wet weight (approximately 9300 mg/kg dry matter) in a pig fed 0·4 per cent zinc over a period of about 40 days. The values found in the present case (Table 2) might therefore be expected in pigs receiving 0·25 to 0·5 per cent zinc over a period of two to three weeks.

The main clinical findings in experimental chronic zinc toxicity were described by Sampson and others (1942) and Brink and others (1959) as depression of growth and appetite. According to Brink and others (1959) the pathology featured subcutaneous axillary haematomas, ulcerative gastritis, haemorrhagic enteritis and haemorrhages into the mesentery, cerebral ventricles, lymph nodes and spleen. Lesions of the major limb joints were reported by both groups of authors but lameness was evident only in the affected pigs studied by Sampson and others (1942). In these the synovia were distended and the joints contained sanguinous synovial fluid. Neither Sampson and others (1942) or Brink and others (1959) demonstrated reduced haemoglobin values but the latter reported prolonged clotting time associated with reduced prothrombin time in some animals fed 0·8 per cent zinc. Both the clinical features and the pathology in the present case are similar to those referred to above with the additional feature of anaemia in this field outbreak.

Zinc interacts competitively for absorption with several elements, notably iron, copper and calcium, and the relationship and antagonisms between dietary copper, iron, zinc and calcium in the pig have been described by several authors including Ritchie and others (1963). There seems little doubt that these interactions played a significant part in this field case and complicated the findings beyond those of simple zinc toxicity.

Zinc induced copper deficiency anaemia is recorded in rats (Grant-Frost and Underwood 1958). Rats fed 0·4 per cent zinc developed a hypochromic, microcytic anaemia which was overcome by supplements of both copper and iron (Underwood 1979). Hill and others (1983) produced copper deficiency, with depressed tissue stores of copper and reduced haemoglobin and haematocrit, in piglets born to dams fed a diet containing 5000 ppm zinc. In the present incident, the two pigs sampled before necropsy and pigs 1, 2 and 3 had haematological evidence of severe anaemia (Table 1). Red cell morphology examined in the latter pigs suggested a chronic anaemia. In pigs 1 and 3 this was moderately macrocytic in type whereas in pig 2 it was moderately microcytic. The hepatic haemosiderosis and renal haematopoietic activity seen histologically are consistent with internal haemorrhage and chronic anaemia, respectively. Unfortunately insufficient material was examined to fully characterise the haematological changes in relation to the complexities of the dietary abnormalities. It was therefore not possible to evaluate the relative roles of zinc toxicity and copper deficiency in the resulting anaemia. Both acute and chronic blood loss anaemia might be anticipated to develop in pigs receiving high levels of zinc and it is also likely that copper deficiency would modify the haematological findings.

Iron deficiency is well recognised as a cause of anaemia in sucking piglets kept under intensive systems of husbandry (Seamer 1955). In contrast, field occurrence of anaemia in the pig associated with copper deficiency is poorly documented although Brooksbank (1954) reported a hypochromic anaemia with neutropenia in piglets which had received parenteral iron therapy and which responded to oral copper sulphate administration. A similar incident was described by Smith and Deans (1969). Liver iron concentrations in two pigs in the present studies (Table 2) were lower than normal. There is no information to indicate whether this was a result of a low dietary intake or a consequence of reduced availability in the presence of high levels of zinc as shown by Cox and Hale (1962).

Lahey and others (1952) showed that pigs experimentally rendered deficient in copper or iron developed a microcytic, hypochromic anaemia but only the copper deficient pigs showed a neutropenia. Pigs deficient in both copper and iron showed all the features of copper deficient pigs. In copper deficient pigs, despite the morphological similarity to that of iron deficiency, the anaemia is not corrected by administration of iron, but responds rapidly to copper supplementation (Lahey and others 1952, Cartwright and others 1956) as was clearly demonstrated in this present case.

The minimum requirement for copper in the pig is low. The

Agricultural Research Council (1981) give an estimate of 4 mg copper/kg dietary dry matter as the requirement for growing pigs up to 90 kg liveweight and Hill and others (1983) confirmed that the dietary copper requirement of the baby pig is about 5 mg/kg diet. Despite its low minimum requirement, copper is normally added to commercially produced pig meal at rates of about 125 to 180 mg/kg (145 to 210 mg/kg dietary dry matter) because of its low cost growth promoting effect (Braude 1975). Hence, in pigs fed a conventional proprietary ration, copper deficiency is extremely unlikely to occur. However, despite a low requirement, swill fed pigs are potentially at risk from copper deficiency. This was demonstrated by the marginal levels detected in the swill used on plants A, B, D and E (Tables 3, 4 and 5). Moreover, a survey in 1983 involving 14 licensed swill plants in Norfolk (E. J. McDonald, personal communication) indicated that the swill was routinely supplemented with copper on only three premises (21·4 per cent) although proprietary meal containing added copper was occasionally fed for short periods when swill was in limited supply or when weaners were first introduced into the finishing herd.

Despite the marginal level of copper in the swill used on plant A, there were no relevant clinical signs until high levels of zinc were inadvertently introduced into the ration. Ritchie and others (1963) showed that the addition of just 100 mg/kg of zinc to the diet of pigs dramatically reduces liver copper levels. In this present incident much higher levels of zinc intake were imposed on low copper diet and the liver copper concentrations in the pigs appeared to reflect this interaction. The liver copper levels detected (10 mg/kg dry matter or less) (Table 2) can be considered as deficient and are similar to those reported by Lahey and others (1952) in their experimental pigs showing copper deficiency anaemia. Furthermore the clinically affected pigs, 1, 2 and 3, (Table 1) were very hypocupraemic.

Calcium is usually added to commercially produced pig meal at the rate of 0·8 to 1·2 g/100 g. The Agricultural Research Council (1981) recommended a level of 0·8 g/100 g dry matter for pigs weighing 20 to 55 kg. The concentration of calcium in the swill used on plant A (Table 3) was well below these levels as it was also in the swill used on the other plants with the exception of plant B. High dietary calcium reduces zinc availability (Underwood 1979) and can therefore alleviate the toxic effects of high dietary zinc (Hsu and others 1975). Conversely the potential toxic effect of high dietary zinc will be enhanced by a low level of calcium in swill.

Undoubtedly the source of zinc in the swill was the galvanising. The usual zinc content of human food – from which swill is derived – rarely exceeds 50 mg/kg (Ministry of Agriculture, Fisheries and Food 1981). The Agricultural Research Council (1981) give the dietary requirement of zinc in the growing pig as 50 mg/kg. However, most commercially prepared pig food contains 100 to 150 mg/kg of added zinc because the high levels of added calcium and copper reduce the availability of zinc. The Agricultural Research Council (1981) give the toxic level of zinc in pig feed as 2 g/kg dry matter. Hence, the levels present in the swill fed on plant A (Table 3) were well into the toxic range. The levels of zinc present in the swill analysed from plants B, C and D (Table 5) were at an acceptable level, as was the level in the swill from plant A when sampled in December 1982 (Table 4). However, it was interesting to note that the zinc content of the swill from plant E (Table 5) was well above the recommended Agricultural Research Council requirement although it was not toxic.

The authors conclude that the combination of factors present on plant A which led to zinc toxicity, copper deficiency and anaemia could readily occur on other swill plants. Galvanised bins are in common use for collecting and storage of swill before processing and galvanising is frequently seen to have worn away from the inner surfaces of the bins (E. J. MacDonald, personal communication). Hence, levels of zinc in swill may frequently be high, while levels of both copper and calcium may be low or marginal. Therefore, it is suggested that the swill producer should routinely add a proprietary copper supplement to the swill. Not only may this improve growth rate (Braude 1975), it should also protect against copper deficiency and zinc toxicity. Furthermore, galvanised bins should be avoided for the storage of swill and plastic or polythene containers should be used instead where possible.

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Zero disease risk on farms is unachievable and no biosecurity system is perfect. However, while breakdowns will inevitably occur, particularly in pig-dense areas, adherence to sound basic biosecurity principles will reduce risks to a minimum

Biosecurity: reducing disease risks to pig breeding herds

GEOFF PRITCHARD, IAN DENNIS AND JAKE WADDILOVE

DISEASE imposes considerable constraints on the productivity and profitability of the livestock industry. Pig producers have probably suffered more than other sectors from the devastating effects of a succession of infectious disease outbreaks over the past 30 years. Many of these have been highly contagious viral diseases, including transmissible gastroenteritis (TGE), swine influenza (SI), Aujeszky's disease (AD), and porcine respiratory and reproductive syndrome (PRRS). Postweaning multisystemic wasting syndrome (PMWS), which is linked to porcine circovirus (PCV-2) infection, has challenged the very survival of the pig industry in Britain and elsewhere. Even long established endemic diseases, such as enzootic pneumonia (EP) and swine dysentery (SD), still cause significant losses if introduced into naive herds. The recent reappearances of classical swine fever (CSF) and foot-and-mouth disease (FMD) in Britain were timely reminders that there is no place for complacency in disease prevention programmes at both national and herd level. The concept of biosecurity, which gained prominence in Britain during the FMD epidemic, encompasses the full range of measures aimed at disease prevention. In fact, it is one of the cornerstones of the Government's Animal Health and Welfare Strategy for Great Britain, which aims to promote biosecurity to a livestock industry that, in the past, has not always given it high priority. This article reviews the most important sources of disease on pig units – and, thus, the key areas for consideration in terms of farm biosecurity.

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DISEASE RISKS AND SOURCES OF INFECTION

Diseases exhibit a broad spectrum of infectivity and farms vary widely in their ability to institute preventive measures. Hence, each unit needs to be evaluated individually with particular reference to herd location, preventable risks and financial constraints. Procedures need to be

Principal considerations for farm biosecurity

- Live pigs
- Contacts
- New stock (including semen and embryos)
- Local spread
- Herd location
- Vehicles (especially if transporting pigs or other livestock), equipment and consumables
- Humans (eg, visitors and farm staff)
- Feed, water and bedding
- Wildlife and vermin

Fomites

Infection present in faeces (the main source), saliva, nasal secretions, blood, milk or semen may be mechanically transmitted between animals on a variety of different inanimate objects – or fomites. These include contaminated clothing, boots, vehicles, equipment, bedding and feed.

The period of time that fomites remain infectious depends on the nature of the agent and environmental factors such as temperature, exposure to ultraviolet light and the efficacy of disinfection procedures (see box on page 233). Porcine parvovirus (PPV) and PCV-2, for example, are very resistant and survive well for several months under common UK environmental conditions. *Brachyspira hyodysenteriae* – the cause of swine dysentery – is reported to survive in moist faeces for up to 40 days. PRRS virus has been isolated from trailers which have been cleaned and disinfected and rested overnight in cold conditions without drying; there are other recorded examples of PRRS virus surviving in the environment for several weeks in buildings.

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Transmissible gastroenteritis is a highly contagious disease of pigs that was prevalent in the 1970s and 1980s, but is now apparently absent from Britain

Postweaning multisystemic wasting syndrome was first confirmed in Britain in 1999 and remains a very serious disease problem for the pig industry

heightened during notifiable disease alerts. The Hazard Analysis Critical Control Point (HACCP) system, introduced originally for the food industry, offers some useful principles that can be adapted for use on farm to deal with disease risks. This identifies hazards, establishes critical limits and introduces monitoring and recording systems to deal with the risks. The principal considerations with respect to biosecurity on pig farms are listed in the left-hand box on page 230, and are discussed, in turn, below.

LIVE PIGS

Direct contact with other live pigs (including feral wild boars) presents the main specific risk for acquiring new infections. Pigs may be clinically affected, apparently normal but incubating disease, convalescent carriers or long-term excreters of pathogens. The likelihood of disease transmission is exacerbated by stress due to loading, mixing and transportation. Semen and embryos also present a risk in relation to viral diseases such as PRRS and CSF (as illustrated by recent experiences in the Netherlands).

Guidelines for obtaining breeding replacements

- Know the disease status of both the recipient and source herds
- Consider the location of both herds and endemic disease risks
- Acknowledge the limitations of recipient herd biosecurity
- Select an appropriate single source for replacements
- Seek both genetic improvement and sound disease control
- Assess the biosecurity status of the breeding pyramid
- Consult with the breeding company's veterinary surgeon
- Check company claims, monitoring records and back-up service
- Confirm that there has been no recent disease outbreak on the farm of origin
- Check what diseases have not been tested for
- Ensure a continuous supply of pigs of appropriate status

Most herds buy in breeding replacements for genetic improvement. It is important that these animals are sourced appropriately and health-matched with the recipient herd, and undergo a period of isolation (see box below). Health status is essentially an assessment of herd immunity and current disease activity. Ideally, incoming replacements should be of similar or higher health status than the recipient herd to prevent them from introducing infections not already present in the recipient herd. Conversely, if they are inappropriately 'disease free' and

Isolation of incoming animals

Irrespective of how reliable the health status of the herd of origin is, it is important that incoming gilts or boars undergo a period of isolation (or quarantine) for at least three to four weeks. The duration will depend on the particular diseases of concern (eg, eight weeks for EP). The feasibility of adopting a worthwhile isolation period is improved by reducing the number of intakes and bringing gilts in at a younger age (eg, 30 to 100 kg in one batch). Isolation allows pigs to recover from the stress of a journey and adapt to a new environment, and any incubating infections to become evident in a contained environment. This should be followed by gradual acclimatisation and challenge with resident pathogens, ultimately leading to full integration into the herd, ideally combined with the use of sentinel pigs. In the USA, it is commonplace to follow 60 to 90 days of isolation with a 30-day 'cooling-off' period. At present, in Britain, the majority of herds – to their cost – do not routinely isolate replacements.

Isolation provides an opportunity for clinical inspection, laboratory testing and vaccination, if appropriate. For example, examinations for sarcoptic mange, tests for *Salmonella* infection or serology for diseases such as PRRS could be undertaken. Laboratory techniques are constantly being upgraded, particularly with the rapid advances in biotechnology and the introduction of new PCR tests and ELISAs. Veterinary surgeons should check with the laboratory to which samples are to be sent to establish which tests best meet current requirements.

Guidelines for isolation units

- Ensure that biosecurity of an isolation unit is at least as good as that of the main herd
- Locate isolation units at least 50 m from the main herd, preferably on another site
- Consider the risks from effluent and drainage outputs
- Ensure that buildings are easy to clean and disinfect
- Provide clean dry bedding and readily available water
- Ensure good lighting and accessibility of animals for visual inspection
- Use dedicated boots and clothing (and, ideally, personnel)
- Isolate animals for at least three to four weeks
- Make regular clinical inspections and observe animals closely
- Operate isolation premises on an all-in, all-out basis

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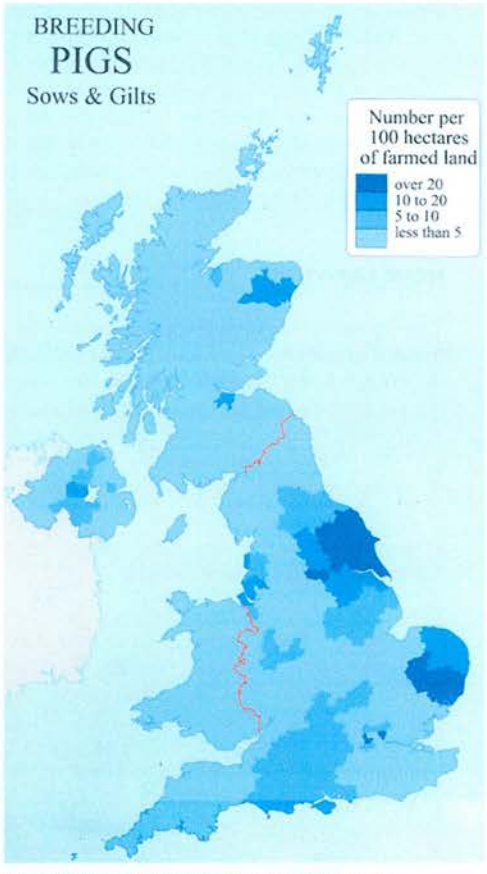
immunologically naive to common infections, they may need protecting by vaccination or acclimatisation before entering the new herd. This also applies if a new herd is likely to suffer a disease breakdown because of location (see later).

Replacement profiling requires knowledge of the disease status of the herd of origin and the recipient herd. Disease status information can be obtained from various sources, including records of clinical disease history, performance records, serological monitoring, necropsy findings, lung/snout scores and other abattoir reports. An enhanced abattoir monitoring programme for the pig industry in Britain (British Pig Health Scheme) has been developed by the British Pig Executive (BPEX) and is due to be launched in July 2005. Its recently introduced Zoonoses Action Plan (ZAP), based on meat juice ELISA testing at the abattoir, can indicate exposure to *Salmonella* species and gives a potentially useful guide to status although it mainly measures zoonotic risk rather than clinical disease. Most reputable breeding companies have in-house health-recording programmes and the company veterinary surgeon will be responsible for liaising with the customer's veterinary surgeon.

Pig units should be sited away from potential sources of infection such as landfill sites, abattoirs and other pig herds



Demographics of the UK pig population showing concentration in areas such as East Anglia and Humberside. Picture, British Pig Executive

LOCAL SPREAD

The generic term 'local spread' is often used in areas of high local livestock density where it is impossible to determine precisely how an infectious agent enters a herd. Unexplained disease transmission over short distances is often attributed to aerosol infection, but it is very difficult to exclude the possibility of local spread by other routes, particularly wildlife. Aerosol spread appears to occur to a variable extent with a number of diseases including EP, PRRS and PMWS; long distance airborne spread in viral plumes is well recognised with FMD and AD. Aerosol and airborne spread depend on

Checklist of physical biosecurity measures

- Remote location and single approach road (no public rights of way)
- No nearby livestock farms, abattoirs, landfill sites, sewage works, and so on
- Clear farm identification
- Farm office or reception area off-site
- A bell or phone to attract attention
- 'Keep out' warning signs
- Controlled access with single, well located farm entrance
- Strategically placed, well maintained disinfectant footbaths
- Off-site car park, with easily cleaned and disinfected hard standing
- Well defined farm boundary and wildlife-proof perimeter fence
- Changing/showering facilities and separation of clean/dirty areas
- Good hand washing facilities for visitors and workers
- Dedicated farm equipment, protective clothing, boots and vehicles
- Loading/unloading bay for live pigs away from the main herd
- Completely separate bay for carcase removal or on-farm incineration facilities
- Good separate isolation facilities for added stock
- Feed bins sited on farm perimeter, with farm-dedicated own blower pipes
- Bird- and vermin-proof buildings, as far as practicable
- Good facilities for slurry or manure collection and disposal
- Off-site lorry wash facilities with trained operatives
- 'Don't feed the pigs' sign for outdoor herds

Good, controlled access to a pig unit showing the farm office and security gates

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numerous factors such as the type of pathogen, number and density of animals excreting and susceptible to infection, housing, droplet size, humidity, ambient temperature, ventilation fans, wind strength and direction, sunlight and topography. In France, the danger of aerosol spread when spraying slurry onto agricultural land is well recognised and, hence, slurry injection is recommended instead of surface spreading.

HERD LOCATION

Geographical location, particularly proximity to other live pigs, is probably the overriding factor which dictates the risk of a herd acquiring new disease. The type, number and density of pig units in a 2 km radius are crucial. The position of major roads, prevailing wind direction, drainage, vegetation and biosecurity measures adopted on nearby premises are all significant. Ideally, new pig units should be sited in areas of low pig density away from obvious risk factors such as other pig herds, slaughterhouses, slurry lagoons, refuse tips and roads used by pig transporters. However, for commercial reasons, pig production in Britain and elsewhere is concentrated in a few regions where endemic diseases are inevitably prevalent.

A minimum distance of at least 500 m between pig farms may reduce the risk of acquiring common infections but, in many commercial herds in high pig density areas, it is very difficult or impractical to maintain disease freedom from common endemic diseases such as PRRS, EP, SI and, particularly, PMWS. This is no excuse for poor biosecurity, but an appreciation of what is realistically achievable is essential. For some diseases, the use of vaccination (if available) may be more appropriate and cost effective than relying solely on other biosecurity measures which are impractical due to factors beyond the owner's control.

Disinfection procedures

Disinfection is a targeted procedure aimed at reducing disease transmission via contaminated protective clothing (particularly boots), vehicles or equipment. It is an important line of defence in good biosecurity practice, but should be considered as an adjunct measure rather than a stand-alone procedure. Disinfectants require the effective removal of organic matter to be fully efficacious. It is essential to use the correct concentration (a minimum of DEFRA General Orders rates). Adequate prior cleaning of buildings and equipment should be carried out. These should be allowed to dry after washing and disinfectant should be applied to saturation point. Thorough application, including the undersurfaces of equipment, should be ensured. Care should be taken not to recontaminate recently disinfected surfaces.

Too much reliance is often placed on disinfectant footbaths, particularly in farm situations with basically poor hygiene and scrapethrough slurry handling systems, which facilitate the spread of pathogens. Accumulating faecal matter, dilution by rainwater, incorrect concentration and infrequent replenishment may render footbaths more a source of contamination than a means of preventing mechanical transmission of infectious agents. Merely standing in or walking through a disinfectant footbath with minimal contact time does little more than lull staff into a false sense of security.

However, if instituted properly at strategic locations on the farm, footbathing is a very useful adjunct to other measures and also heightens awareness of the importance of biosecurity in a tangible way that most people understand. Soiled boots must be cleaned thoroughly by scrubbing, perhaps using a dilute detergent solution and all gross faecal matter should be removed before immersing them in the disinfectant. Combined boot brushes/washers are useful, but can become heavily contaminated and require regular cleaning. Disinfectant mats are somewhat cosmetic unless the solution is regularly replenished and any build up of faecal contamination is removed. However, they also increase awareness and are worth considering outside relatively clean areas such as the entrance to off-site farm offices or changing rooms.

The ideal disinfectant

- Proven broad-spectrum activity and efficacy under farm conditions
- Fast acting to rapidly kill highly infectious agents
- Temperature stable and active at extremes of heat or cold
- Stable after dilution, especially for footbaths
- Suitable for porous and non-impervious surfaces
- Active in the presence of organic matter
- Versatile for use in footbaths, vehicles and surfaces
- Safe for environment, animals and operative staff
- Government approved for notifiable diseases
- Easy to store
- Cost effective

Combined boot brushes and footbaths can become heavily contaminated

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Thorough cleaning of vehicles, particularly wheel arches, played a crucial role in limiting the spread of infection during the 2001 FMD epidemic in Britain

A farm-dedicated pig transporter is ideal, but it should be cleaned and disinfected if it is used off the farm or between units

Vehicles presenting a biosecurity risk

- Vehicles delivering and collecting live pigs
- Vehicles delivering feed
- Employee vehicles
- Engineers' and maintenance services' vehicles
- Vehicles delivering bedding
- Waste removal vehicles
- Vehicles collecting dead pigs
- Vehicles delivering equipment, pharmaceutical products and semen

In Britain, the Pigs (Records, Identification and Movement) Order 2003 (PRIMO) and The Disease Control Order 2003 require a 20-day standstill period when pigs move to another herd. However, nucleus, multiplier, grow-out and weaner-breeder herds can apply for exemption from the 20-day rule if they restrict their sources of breeding replacements to approved herds higher up the pyramid. They must also demonstrate high standards of biosecurity

VEHICLES, EQUIPMENT AND CONSUMABLES

Contaminated vehicles (particularly those used to transport livestock) and their drivers represent an important means of disease transmission, as was evident during the recent outbreaks of FMD in Britain and CSF in Holland.

Vehicles, trailers and other equipment should be farm-dedicated, if possible. The degree of risk depends on how recently the vehicle has been used on other pig or livestock farms or for conveying pigs from market to the slaughterhouse. The increasing trend towards two- and three-site production systems has led to more transportation of live pigs. Transporting finishers to slaughter in the morning and weaners in the afternoon leaves insufficient time between journeys for satisfactory cleaning and disinfection. Adopting a three-week batch weaning system reduces the need to move pigs as frequently and facilitates the all-in, all-out approach, which promotes good hygienic practice.

External vehicles should not be permitted onto a farm unless essential (eg, for urgent repairs). These vehicles must be visibly clean, washed and disinfected under supervision on hard standing areas off-site. The effectiveness of this approach was demonstrated during the recent FMD epidemic in Britain. Particular attention should be paid to wheel arches and the underside of the vehicle. It is also important to ensure that personnel do not become contaminated during washing – disposable plastic boots should be worn, if practicable. Vehicle footwells or floors inside the lorry cab are difficult to clean and disinfect adequately, although the provision of rubber mats and disinfectant sprays may help. Fixed wheel washes are helpful if they are of good design, but disinfectant mats for vehicles are of limited value and soon become heavily contaminated.

Feed lorries must be visibly clean and make deliveries from outside the perimeter fence or at the periphery of the farm using farm-dedicated blower pipes. High-health status and nucleus units should try to arrange feed deliveries for the first drop of the day at the beginning of the week. Contractors' boxes and equipment that might have been used on other livestock premises should be disinfected.

The anteroom or office delivery point area close to the main entrance to a farm is a potential focus for contamination by pathogens on cardboard containers used for items such as pharmaceutical products or semen coolers. Surface disinfection and hygienic disposal of packaging should therefore be instituted, as appropriate.

Livestock loading and unloading

Loading and unloading of live pigs are procedures which offer opportunities for new infections to be introduced

A well designed and easily cleaned loading bay with good drainage facilities

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An appropriate and effective loading bay on an outdoor unit

by infected stock or fomites (contaminated vehicle, equipment or clothing). Vehicles delivering pigs should be thoroughly cleaned and dried before transporting pigs onto a unit. Pig transporters should not be allowed onto the main farm premises, if possible – a purpose-built loading bay with good washing, disinfecting and drainage facilities, located as far away as practicable from pig accommodation, is ideal. Outgoing pigs should be moved into the loading bay, with farm staff observing strict clean and dirty area protocols to avoid contact with the collection vehicle. The loading bay and surrounding area should be kept clean and disinfected.

HUMANS

The potential for the transmission of infectious aerosols by humans appears to have been greatly exaggerated. A policy of two or three pig-free days or 'downtime' for visitors and veterinary surgeons is widely imposed, particularly on high-health status and nucleus breeding units. This concept has its origins in work undertaken in the 1970s on the persistence and transfer of FMD virus from the mouth and nose of humans 28 hours (but not 48 hours) after exposure. However, contrary to common perceptions, recent scientific evidence – particularly from the USA – and field observations from experienced research workers suggest that the likelihood of people actually transmitting pathogens from their nose, mouth or pharynx is minimal. Imposing a blanket downtime requirement causes great inconvenience and is expensive to maintain.

Nucleus herds and others of high-health status will understandably wish to maintain 48 to 72 hours downtime as an insurance policy and a deterrent for unwanted visitors. Where possible, it is prudent for veterinary surgeons and other essential farm visitors to attend herds higher up a breeding pyramid at the beginning of the week. However, it is important that such measures are kept in perspective and do not assume more importance than they merit. Provided a visit to a pig herd is followed by a complete change of clothing, showering, hand (particularly fingernails) and hair washing, and nose blowing, a single overnight pig-free period of at least 12 hours, as now suggested by several authorities, should be more than adequate for most situations assuming other precautions are adopted. Notifiable disease outbreaks are an exception and are covered by national rules. Additional showering-in on arrival may be unnecessary, but it increases awareness and presents a useful physical barrier in that street clothes are replaced by dedicated clothing. In selected circumstances, if there are major concerns, the additional use of a disposable paper dusk mask that adheres to British standard EN149 with the highest (P3) dust protection factor may be worth considering.

Formulating basic biosecurity rules for visitors (including vehicle drivers) and farm employees costs little and can greatly reduce the risks of introducing infection by fomites, including dirty boots, contaminated clothing or personal equipment. Protocols will inevitably vary with the type of unit and circumstances, being higher and more onerous on high-health status nucleus herds at the top of the breeding pyramid and during notifiable disease outbreaks.

FEED, WATER AND BEDDING

Salmonella species may potentially be introduced by contaminated feed (in addition to the risk of feed delivery vehicles acting as fomites). Feed should be obtained from mills operating in accordance with relevant DEFRA and Agricultural Industries Confederation codes of practice and using ingredients obtained from sources with a consistently satisfactory bacteriological record. There is a small risk of acquiring infection (mainly *Salmonella* species) from contaminated water. Ideally,

Biosecurity precautions for farm workers and visitors

- Provide staff training programmes on biosecurity and hygiene
- Institute standard operating procedures and check compliance regularly (using HACCPs)
- Apply reasonable, but strict, protocols after contact with other pigs
- Ensure good personal cleanliness (hands, fingernails, hair)
- Provide farm-dedicated protective clothing and boots or insist on spotless and disinfected boots/clean washable protective clothing
- Consider using hats and disposable plastic gloves
- Do not allow visitors (excluding veterinary surgeons and similar) to approach or handle stock
- Use farm-dedicated equipment, notebooks (washable), mobile phones, and so on, or adopt a disinfection protocol (laptops, etc) between visits
- Keep office and staff accommodation clean and uncluttered
- Be aware of potential disease risks linked to international travel
- Do not allow any pig products or other unauthorised material onto the farm or into the canteen
- Ensure that human food is eaten only in the staff canteen or dedicated area (also see above)
- Make sure visitors/employees sign a book (see below) to confirm that precautions have been undertaken
- Adopt strict protocols for loading/unloading pigs, carcase disposal, and so on

Information to be recorded in a visitors' book

- Date
- Name of visitor
- Company name/address
- Purpose of visit
- Date of last contact with pigs
- Time of arrival
- Time of departure
- Signature to confirm that biosecurity precautions have been observed

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Carcase disposal

Disposal of carcases has always been a potential problem, particularly on units experiencing high mortality during severe outbreaks of disease such as TGE or PMWS. Difficulties have increased following the imposition of The Animal By-Products Regulations 2003, which prohibit on-farm burial and pig disposal pits. A new National Fallen Stock Scheme with a chargeable farm collection service started operating at the end of 2004. This has major biosecurity implications and, ideally, requires a separate isolated pre-collection carcase storage facility on a concreted area, which can be easily disinfected, located at the periphery of the farm. Instead, many farmers may prefer on-farm incineration. Low capacity incineration units (for burning less than 50 kg of animal by-products an hour), inspected and approved by the State Veterinary Service, may be suitable. For further information on carcase disposal, see the DEFRA website.

Disposal of dead pigs is an important issue with significant biosecurity implications

mains water should be used; water from boreholes and wells should be tested regularly for bacteriological quality.

The risks of straw, shavings and other bedding substrates acting as fomites should be reviewed. These materials should not come from sources which might have been exposed to livestock or excrement, and should be stored under cover and protected from contamination by birds or vermin.

WILDLIFE AND VERMIN

The risk of rodents, feral cats, birds, insects and other animals acting as vectors of disease is much more difficult to control than disease spread by humans, equipment and vehicles. TGE virus was notorious in Britain for its transmission by starlings and gulls, especially on outdoor units and in uncovered feeding areas. Complete bird proofing was attempted with partial success on some pig farms during the TGE epidemic of 1980 to 1983 in Britain. Other infections spread by birds include salmonellosis and avian tuberculosis (via infected peat used as bedding). Even on outdoor units, practical measures such as installing rubber flaps on feeders reduce disease risks and also allow considerable savings in feed costs. Mechanical transmission by insects, particularly flies, has been implicated in the spread of several diseases including TGE, *Streptococcus suis* infection and PRRS, but control can be problematic.

The increasing number of feral wild boar in some parts of Britain, and the potential for their expansion into new areas, pose very specific disease threats to outdoor herds – as recently illustrated by the CSF outbreaks in several European countries, such as Luxembourg. Commercial wild boar production units must be licensed under the Dangerous Wild Animals Act 1976 and secure escape-proof perimeter fencing is essential. Conventional pig units in the vicinity need to adopt heightened precautions.

Slurry disposal and waste management

If a slurry pit is to be used, it should be large enough to hold four to six months' of waste to reduce the need for frequent emptying. Ideally, the pit should be positioned outside the unit with channels running from different buildings. Storing slurry for as long as possible (at least four weeks, preferably several months) will kill most *Salmonella* species and other organisms. Farm-dedicated vehicles should be used for slurry removal. Solid manure should be stacked and composted in fenced pads before spreading it onto arable land. If slurry is to be spread onto pasture, pigs should be kept off the land for at least three weeks.

Wild boar farms require very secure perimeter fencing

Essentials of vermin control

- Adopt a regular, effective pest control programme (using a contractor or in-house)
- Ensure frequent and correct baiting and inspection
- Adopt intensive baiting when pens are empty
- Review house design, farm layout and general hygiene procedures
- Reduce favourable habitats for wildlife (weed overgrowth, rubbish and debris)
- Avoid feed spillage – if spillages do occur, ensure that they are cleared up promptly; cover feed barrows
- Repair buildings to prevent access and nesting, and remove refuge sites

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Close contact between pigs and birds on outdoor units inevitably presents increased disease risks

Rubber flaps on outdoor feeders help to discourage wild birds

Feral wild boar pose a significant risk to outdoor pig units

Perimeter fencing should be secure to prevent the escape and entry of feral wild boar or other pigs

Outdoor pigs are inevitably exposed to *Leptospira* serovars, particularly *Leptospira bratislava*, from various wildlife species including hedgehogs, foxes and rats. Rodents can introduce disease such as *Salmonella* infection (which is also spread by feral cats), swine dysentery and *Lawsonia intracellularis* infection. As with birds, a large rodent population represents a significant amount of food wastage, which is an added incentive for control, even on outdoor units. Independent consultancy advice on pest control is available from organisations such as ADAS. It is advisable not to allow domesticated pets onto the farm premises, but if guard dogs are used they should not be fed marrow bones. The veterinary surgeon should also remain alert to potential disease transmission risks from domestic cats.

VALUABLE INITIATIVES

The pig industry has been very proactive in promoting the benefits of biosecurity and implementation of its Strategy for British Pig Health and Welfare, launched in December 2003. As part of this initiative, the National Pig Association (NPA) and BPEX have developed (funded by DEFRA) a personalised self-assessment audit to help producers improve biosecurity on their own farms. This should become available on the NPA website during 2005. Such an approach offers an ideal starting point for veterinary surgeons and producers seeking to identify cost-effective measures appropriate to particular farm circumstances.

Acknowledgements
The authors would like to thank veterinary and DEFRA colleagues, and members of the pig industry for their valuable comments and for the use of the illustrations appearing in this article.

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- British Pig Association: www.britishpigs.org.uk
- National Pig Association: www.npa-uk.net
- British Pig Executive: www.bpex.org
- ADAS: www.adas.co.uk

CORRECTION

Heartworm and lungworm in dogs and cats in the UK (*In Practice*, March 2005, pp 147-153)

On page 147, under the section on *Angiostrongylus vasorum*, the first sentence should have read '*Angiostrongylus vasorum* is a metastrongyloid nematode which inhabits the right ventricle and pulmonary arteries of its definitive host, the dog and related Canidae.', and not as stated. The author regrets the error.

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Papers and Articles

Maedi-visna virus infection in commercial flocks of indigenous sheep in Britain

G. C. Pritchard, J. B. Spence, M. J. Arthur, M. Dawson

Veterinary Record (1984) **115**, 427-429

The recent identification of maedi-visna virus infection in three commercial flocks of indigenous sheep in Britain is described. In one flock the evidence suggested that overt clinical disease was present. The implications of the findings are discussed.

PREVIOUS reports of maedi-visna in Britain have involved imported exotic sheep and their progeny or indigenous breeds that had had contact with exotic sheep. Most of the evidence to date has been serological although Grimshaw and others (1981) and Markson and others (1983) found gross and histological lesions. In addition, Jones and others (1982) reported a case of clinically patent maedi in a Texel ewe.

This paper describes the suspected occurrence of clinical maedi in a commercial flock of indigenous sheep in East Anglia (flock 1) and serological evidence of the disease in two other commercial indigenous flocks, one in East Anglia (flock 2) and one in the south of England (flock 3).

Case histories

Flock 1

Flock 1 is an established flock of about 800 mules (blue faced Leicester cross Scottish blackface) tupped by Suffolk rams for finished lamb production. Replacement mule ewe lambs are purchased annually from the Scottish border counties through a dealer and no progeny are retained. The sheep are kept outdoors throughout the year and are not housed for lambing. For several years there has been a high culling rate among the ewes because of emaciation and chronic respiratory disease.

An aged cull ewe was submitted alive to the Norwich veterinary investigation centre in June 1983. It was afebrile, weak, emaciated and severely dyspnoeic, particularly when made to walk. At necropsy, the lungs were heavy, uncollapsed and adherent to the thoracic wall. The ventral borders of the diaphragmatic lobes and virtually the entire apical and cardiac lobes were grey, firm and consolidated. The bronchial and mediastinal lymph nodes appeared oedematous and were grossly enlarged. On section, the cut surface of the lungs showed distinct grey cellular areas interspersed with apparently normal aerated tissue. These gross lesions were suggestive of maedi (Jensen and Swift 1982).

Histopathological examinations showed widespread lymphoreticular hyperplasia and smooth muscle hypertrophy. The alveolar walls were infiltrated with lymphocytes and histiocytes. Although these lesions were not inconsistent with maedi infection (Markson and others 1983) histological interpretation was hampered by concurrent chronic suppurative bronchopneumonia and infestation with lungworm (probably *Muellerius capillarius*). Markson and others (1983) also found evidence of mixed pulmonary disease in sheep infected with maedi-visna virus. No significant growths of bacteria were obtained following cultural examination of the pneumonic lesions. A blood sample collected at euthanasia gave a positive result to the agar gel immunodiffusion test for maedi-visna (Dawson and others 1979).

Another two emaciated aged cull ewes were examined at the VI centre in July 1983. A blood sample taken from one that was live but moribund on arrival was seropositive for maedi-visna. At post mortem examination, its lungs had a patchy greyish appearance; areas of consolidation were confined to the ventral parts of the apical lobes. The bronchial and mediastinal lymph nodes were enlarged, pale and oedematous.

The other ewe was received dead and was not examined serologically. Necropsy showed gross pulmonary lesions suggestive of pasteurellosis. There was extensive purple coloured consolidation of most of the apical and cardiac lobes and of the dependent parts of the diaphragmatic lobes. Histological examinations of the lungs of both ewes showed some features consistent with maedi, including lymphoreticular hyperplasia. The main lesions, however, were those of suppurative pneumonia. Bacteriological cultures yielded *Pasteurella haemolytica* (T strain) from both sets of lungs.

In view of the recent report by Houwers and Terpstra (1984), it is noteworthy that there was no gross or histological evidence of pulmonary adenomatosis in the three ewes examined.

The farm was visited in October 1983 and blood samples taken randomly from 12 of the poorer ewes of various ages. All were serologically positive for maedi-visna. Subsequently 450 animals, representing over half the flock, were blood sampled to obtain a more precise estimate of the prevalence of infection. The sheep sampled included all 200 of the recently purchased ewe lamb replacements. These were found to be serologically negative but of the other 250 samples, 177 (70·8 per cent) were seropositive. The sheep were aged by examining their incisor teeth: the proportion of positives in each age group is shown in Table 1. Most animals appeared to seroconvert between the 'two tooth' and the 'four tooth' stage.

The fact that the ewe lamb replacements were serologically negative did not preclude the possibility that these purchases may have been the source of infection in the past, since their farms of origin varied from year to year. The high proportion of serologically positive animals in the flock suggested that there may have been a number of clinical cases. In view of this it was significant that the sheep were unhoused. They did, however, have close contact when folded intensively with a diet of arable byproducts in the autumn and winter.

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TABLE 1: Number of animals seropositive for maedi-visna virus in each age group in flock 1

Sheep	Number sampled	Number positive (%)
Ram lambs	6	0
Ewe lambs*	200	0
2-tooth rams	3	2(66·7)
2-tooth ewes	57	15(26·3)
4-tooth rams	2	1(50·0)
4-tooth ewes	45	40(88·9)
6-tooth rams	1	0
6-tooth ewes	24	23(95·8)
Full mouth rams	5	3(60·0)
Full mouth ewes	81	71(87·7)
Broken mouth ewes	26	22(84·6)
Total	450	177(39·3)

*These were recently purchased and had not been mixed with the main flock

TABLE 2: Number of animals seropositive for maedi-visna virus in each age group in flock 3

Age (years) and sex of sheep	Number sampled	Number positive (%)
Rams (1)	2	0
Ewes (2)	189	23(12·2)
Rams (3)	1	0
Rams (4)	3	1(33·3)
Ewes (4)	32	10(31·3)
Rams (5)	2	0
Ewes (5)	114	47(41·2)
Rams (6–7)	1	0
Ewes (6–7)	244	110(45·1)
Total	588	191(32·5)

There were no three-year-old ewes in the flock. No ewes under two years of age were blood sampled

Flock 2

Flock 2 is a long established commercial flock of about 600 ewes of mixed indigenous breeds but predominantly Scottish halfbred (Border Leicester cross Cheviot). These are tupped by Suffolk rams for finished lamb production; Hampshire rams are used on those Suffolk cross ewe lambs which are retained as flock replacements. Further replacement Scottish halfbred gimmers are usually purchased every second year at a sheep fair in the Midlands.

As with flock 1, the sheep were unhoused throughout the year and had close contact only when folded on a diet of stubble turnips or sugar beet tops.

For many years there has been a history of poorness and emaciation in older ewes but the flock had been somewhat indifferently managed in the past making it difficult to assess the significance of this feature. Chronic respiratory disease has not been a particular problem.

Maedi-visna virus infection was suspected following routine histological examinations of lung lesions from two ewes that had shown chronic abscesses and pleurisy when submitted to the Norwich VI centre in August, 1983. *P haemolytica* (A strain) was isolated from one and *Corynebacterium pyogenes* from the other. A total of 16 blood samples was taken randomly from the flock in October 1983: nine of 10 ewes and four of six rams sampled were serologically positive. The sheep tested were of mixed ages and included 'two tooth' animals.

Flock 3

Flock 3, comprising 588 adult sheep, was formed in 1978 from mule (blue faced Leicester cross Swaledale) shearlings. Subsequently, more mules and a number of Suffolk cross ewes were brought in to expand the flock. Rams are mainly Suffolks but also include Cambridge, Dorset Down and British milk sheep. Unlike flocks 1 and 2, this flock is routinely housed for several weeks each year over the lambing period.

Although maedi infection was not suspected in this flock, three serological positives were detected among 10 ewes sampled at the owner's request in January 1983. Subsequently, a flock test in May 1983 revealed 191 (32·5 per cent) serologically positive animals. Since the sheep had been ear tagged they could be accurately aged: the detailed serological results are shown in Table 2. As with flock 1, significantly fewer young ewes had seroconverted than older ewes.

The seropositive animals were removed from the flock and 389 seronegative animals were resampled in November 1983. Of these, a further 67 (17·2 per cent) were found to have seroconverted, suggesting that active infection was present in the flock.

Discussion

It seemed inevitable that clinical maedi would eventually be diagnosed in indigenous flocks (Markson and others 1983). From 1978, when the presence of maedi-visna virus infection was first detected in Britain (Dawson and others 1979) until 1983, all 170 flocks shown to be infected in this country had connections, direct or indirect, with imported breeds. The evidence has always supported the view that maedi was introduced into Britain with imported exotic breeds of sheep during the 1970s.

The breeding replacements for flocks 1 and 2 were obtained through dealers. It was not possible to trace the farms of origin and because of this it could not be determined whether the replacements had been in contact with imported sheep, the progeny of imported sheep or others which had been in contact with imported sheep or their progeny. However, there is evidence that flock 3 may have been in contact with sheep of the Texel breed.

Although lateral transmission of maedi-visna virus occurs more readily when sheep are kept intensively, its presence in flocks 1 and 2 shows that infection can also become well established among traditionally managed unhoused sheep.

The pathological features described for the cases in flocks 1 and 2 emphasise the difficulty in interpreting the lesions produced when lungs are subjected to an insult of multiple aetiology. The contribution of maedi-visna virus to the clinical signs recognised in flocks 1 and 2 is not clear but the high prevalence of infection in these flocks suggests that it was probably a significant pathogen. Experience suggests that practitioners and diagnosticians should be aware of the insidious nature of the disease, the likelihood of it being masked by other pulmonary diseases such as pasteurellosis and pulmonary adenomatosis, and the need to include maedi in the differential diagnosis of debilitating and wasting disease.

The present findings suggest that on occasion flocks may experience rapid spread of maedi-visna infection and illustrate the difficulties in collecting adequate epidemiological information to determine the origin.

The three flocks referred to in this paper were not identified as a result of testing carried out under the maedi-visna Accredited Flocks Scheme (Anon 1982). However, to put the findings into perspective, it should be noted that only one reactor – a crossbred ewe in a flock in East Anglia – has been identified to date among indigenous scheme flocks. Currently (April 1984) there are approximately 940 indigenous flocks, representing about 89,000 breeding sheep, accepted into membership of the scheme. Of these, 105 (13,000 sheep) are accredited and approximately 520 (48,000 sheep) have undergone clear qualifying testing. The membership of the scheme comprises mainly pedigree breeding flocks: the prevalence of maedi infection in commercial 'halfbred-type' flocks is not known.

Acknowledgements.— We acknowledge the cooperation of the farmers and veterinary surgeons involved. The assistance of Dr I. F. Keymer and of the pathology department at the Central Veterinary Laboratory, Weybridge, for histological examinations of the lungs is also gratefully acknowledged. Information concerning the maedi-visna Accredited Flocks Scheme was obtained from the Animal Health Division, Ministry of Agriculture, Tolworth, Surrey.

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Addendum

One hundred and ninety four of the 200 ewe lambs in flock 1 that had been seronegative in October 1983 were resampled in June 1984 at the age of about 15 months. Two (1·0 per cent) were found to have developed positive titres for maedi-visna virus. The lambs had been managed separately from the rest of the flock from their arrival in September 1983 and there had been no direct contact between the two groups of sheep at any time. However, there had been opportunity for indirect contact via the pasture, communal farm tracks and sheep collection points.

Although the possibility that the lambs had acquired infection from the older ewes cannot be completely excluded, it seems much more probable that they were infected before arrival in East Anglia although at the age of seven months they had not seroconverted. These latest serological findings therefore appear to indicate an origin for the maedi infection in this flock.

1.20

Maedi-visna in indigenous sheep

From Mr G. C. Pritchard and Mr J. B. Spence

SIR,— With reference to our recent paper (*VR*, October 27, p427) we feel that, while in no way detracting from the intent, it is desirable to correct some of the statements made in your Editorial (*VR*, October 27, p421).

While our report concerns maedi-visna in indigenous sheep, we could not say with certainty that there had been no connection with the imported sheep. As stated in the paper the replacements for flocks 1 and 2 were obtained through dealers and the farms of origin could not be traced. In flock 3 there may have been contact with Texels.

It is regrettable that Oldenburg and Texel were the only breeds cited. In a previous paper (Markson and others 1983) it was suggested that other foreign breeds may have been implicated in the introduction of maedi to Britain; indeed, although Oldenburg crosses were reactors there was clear serological evidence that the original imports of purebred stock were not infected.

Regarding the reference to pulmonary adenomatosis, which was a complicating factor in the previously cited paper, in the limited examinations carried out in our recent study of maedi-visna in the three indigenous flocks pulmonary adenomatosis was not diagnosed.

Incidentally, this investigation serves to highlight the vital surveillance role played by veterinary investigation centres, throughout the United Kingdom and is highly pertinent to your Editorial (*VR*, November 3, p449).

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Maedi-visna virus infection in commercial flocks of sheep in East Anglia

G. C. Pritchard, M. Dawson

Veterinary Record (1987) **120**, 208-209

IN a previous paper, Pritchard and others (1984) reported the presence of maedi-visna virus infection in three commercial flocks of indigenous sheep in Britain, two of the flocks being in East Anglia. This present paper reports the findings of a follow-up serological survey to determine the prevalence of maedi-visna virus infection in commercial flocks in East Anglia.

Between May and September 1985, blood samples were collected from 23 commercial breeding flocks in East Anglia (Norfolk and Suffolk). The farms sampled were randomly selected from those suggested by veterinary practitioners as being likely to cooperate in a survey. None of the flocks had any history of maedi-visna infection.

At the time of sampling, information was sought regarding flock replacement policy, contact with imported stock or derived progency during the past five years and history of wasting disease or chronic pneumonia in old ewes. Details of housing and husbandry were also recorded.

The number of animals sampled in each flock ranged from 12 to 60 (average 52) and was based on the requirements for

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TABLE 1: Summary of findings in the four seropositive flocks

Flock (number of ewes)	Main breeds	Number of blood samples taken	Number of seropositive ewes (%)	Comments
A (800)	Scottish halfbred, Suffolk cross	56	19 (34)	Replacement ewes lambs from Scotland; rams from maedi-visna accredited flocks*; not housed
B (520)	Scottish and north country mules	56	3 (5)	Replacement shearlings and ewe lambs from local sheep sales and sheep fair in the Midlands; rams from maedi-visna accredited flocks*; housed for lambing and for two months in winter
C (500)	Scottish mules, Suffolk cross	54	2 (4)	Replacement shearlings from local market; not housed; status of rams not known
D (900)	Scottish mules	59	1 (2)	Replacement ewe lambs from Scottish Borders; not housed; rams from known infected flock

*Refers to ram purchases made since May 1982 when the maedi-visna accredited flock scheme commenced

detecting a minimum of 5 per cent positives with 95 per cent confidence (Cannon and Roe 1982). Where possible, cull or thin animals were sampled; in all cases the animals sampled were aged at least two years. Rams were not sampled. The blood samples were tested using the agar gel immunodiffusion test for maedi-visna (Dawson and others 1982).

Breeds represented were predominantly Scottish mules, Scottish halfbreds and Suffolk crosses. Other breeds and crosses sampled included north country mules, Lincoln longwools, Cluns, Lleyns, Swaledales and Mashams. Twelve (52 per cent) of the 23 flocks had current or past contact with imported stock or derived progency.

Of the 23 flocks sampled, four (17 per cent) contained one or more seropositive animals. Details of these are given in Table 1. Although the seroprevalence was low in three of these flocks, it was over 34 per cent in flock A. Despite the high proportion of seropositives in the latter flock there was no clinical history suggestive of maedi-visna apart from the few thin cull ewes that are a feature of most flocks.

There were no essential differences in housing, husbandry and flock replacement policy between the seropositive and seronegative flocks. In virtually all the flocks sampled, flock masters replaced 20 to 30 per cent of the flock annually with ewe lambs or shearlings – usually from the north of England or Scottish Border areas – via a dealer or markets. In most cases lambing was the only time that the sheep were housed although close contact often occurred when feeding on arable by-products.

The results of this survey support the findings of Pritchard and others (1984) and further demonstrate that maedi-visna virus infection is present in commercial flocks in East Anglia and that a few flocks are heavily infected. Ministry of Agriculture, Fisheries and Food census returns list some 653 breeding flocks in Norfolk and Suffolk. Assuming that about 15 per cent of these flocks breed their own replacements or produce rams, the remainder (555) are commercial flocks similar to those surveyed. From the results of the survey, the number of flocks in East Anglia with a 5 per cent prevalence of seropostitive sheep could therefore be between 30 and 205 (95 per cent confidence interval).

The low seroprevalence of maedi-visna in flocks B, C and D was presumably because of the purchase of a few seropositive animals. From the available evidence there was nothing to suggest that lateral transmission had occurred – even in flock D which contained rams from a known infected flock. However, the fact that at least three heavily infected but extensively managed commercial flocks have been detected in East Anglia – flock A of the current study, and flocks 1 and 2 described by Pritchard and others (1984) – raises important questions. Have these flocks been purchased from heavily infected sources or has lateral transmission occurred readily despite extensive management conditions? Concurrent infection with sheep pulmonary adenomatosis predisposes to increased maedi-visna virus replication and excretion (Dawson and others 1985), but sheep pulmonary adenomatosis has not been diagnosed in any of these present flocks in recent years.

Maedi-visna virus infection has probably become more widespread in commercial flocks in Britain than has previously been supposed. Perpetuation of the virus, often in the absence of overt clinical signs, is likely to occur in commercial breeding flocks such as those described in this paper. In these flocks a high culling rate is usually maintained and ewes may not be retained long enough for significant clinical disease to develop.

In a recent Dutch study (Van der Molen and others 1985), maedi-visna infection was associated with diffuse udder induration and reduced milk production. Attention was drawn to the problem by failure of unweaned lambs to thrive. Should such a problem emerge in commercial flocks in Britain, the economic significance could be considerable, especially in flocks where the majority of ewes are rearing twins.

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SOME FEATURES OF SHEEP HUSBANDRY AND DISEASE IN THE EASTERN REGION
2 : EAST ANGLIA

by

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Introduction

East Anglia - particularly Norfolk - has strong historical links with the sheep industry and much of the region's former wealth came from the wool trade. Nowadays, sheep production is relatively insignificant in this area dominated by arable farming, in which pigs are the main livestock species.

Flocks engaged in finished lamb production are generally small (Table 1) although there are a few large progressive farms. The main breeds are Scottish Mules and Suffolk crosses but most flocks contain sheep of various breed types.

Table 1 Breeding Ewes (including shearlings) in Norfolk and Suffolk

	Total Ewes	Total Flocks	Flocks >200 (%)	Flocks >700 (%)
Norfolk	55300	407	54 (13)	14 (3.5)
Suffolk	23000	322	34 (10.5)	4 (1.2)

An unknown but large number of store lambs come into the area during late summer and autumn for finishing on sugar beet tops, stubble turnips or fodder beet.

Disease Situation

Inevitably, submissions to V.I. Centres reflect the incidence of

those diseases and conditions which lend themselves to laboratory diagnoses. With this reservation, the main trends for sheep disease in East Anglia are listed in Table 2.

Table 2 Sheep diseases diagnosed at Norwich VIC (from Norfolk and E. Suffolk)

Diseases seen commonly/occasionally	Diseases seen rarely/never
Abortion (toxoplasma, EAE, campylobacter)	Border disease
Acidosis/beet top poisoning, etc.	Fascioliasis
CCN	Gid
Copper deficiency and copper poisoning	Salmonellosis (including Salmonella abortions
E. coli enteritis	Swayback
Keratoconjunctivitis	Tick-borne disease
Listeriosis	
Maedi	
Mastitis (particularly post-lambing)	
Metabolic disorders	
Pasteurellosis	
Periorbital dermatitis	
PGE/coccidiosis	
Pulmonary adenomatosis	
Watery mouth	
Scrapie	

Store Lambs

Diseases associated with intensive store lamb production on arable by-products feature fairly prominently but producers are generally aware of possible problems. Pasteurellosis in the autumn due to Pasteurella haemolytica biotype T can be serious. This is hardly surprising because of the stress of long-distance transportation - often from as far away as the Shetlands - and the dramatic changes in feeding and husbandry from extensive upland grazing to intensive fattening on beet tops on fields with little natural shelter.

Copper

Much of the pastures of East Anglia contain high sulphur concentrations. This, together with moderately high molybdenum, results in an induced copper-deficiency. A national project carried out by ADAS in 1985 on 18 known copper deficient farms, to assess the importance of copper in ruminant animal nutrition, concluded that there was little evidence of significant improvement in growth rate of lambs supplemented with one gram of copper oxide needles. However, in the particular trial carried out on the black fenlands of Norfolk, the copper supplemented lambs showed an increased live weight gain at slaughter of 1.52 +/- 0.62 kg compared with untreated controls ($P<0.05$). It does appear that in some parts of East Anglia induced copper deficiency can lead to reduced growth rate. Swayback is rarely seen.

Copper poisoning occurs occasionally due to grazing sheep on pasture spread with pig slurry containing high levels of copper. However, more cases are probably seen in small flocks which inadvertently feed cattle minerals or concentrate rations designed for species other than sheep.

Pulmonary adenomatosis

Whereas pulmonary adenomatosis is considered somewhat rare in the Bedford/Northampton area, cases are not uncommon in Norfolk and Suffolk and a few flocks are heavily infected. Recently, for example, six advanced clinical cases were confirmed in a group of 29 nine-month-old Cambridge cross lambs. It was thought that the introduction of a highly susceptible breed of sheep into an already endemically infected flock had led to clinical disease developing at an unusually young age.

Maedi

A recent somewhat disconcerting finding in East Anglia was the diagnosis of maedi in commercial flocks of indigenous breeds. In 1983, the disease was diagnosed at necropsy in an emaciated dyspnoeic aged mule ewe from a large flock in Norfolk. Subsequent serology revealed that over 80 per cent of older ewes in the flock were seropositive (Pritchard and others, 1984). In order to determine whether the disease was coming in with the flock replacements, that year's intake of 200 ewe lambs aged seven months, obtained through the regular dealer, were tested and found to be seronegative. The lambs were kept separate from the main flock and were re-tested at 15 months when two were found to have seroconverted. These were removed but when the remainder were re-tested at 24 months of age, 50 per cent were seropositive. Thereafter, all efforts at maintaining a maedi-free section to the flock were discontinued.

In 1985, a small survey was carried out in Norfolk and Suffolk to determine the prevalence of maedi-visna virus infection in commercial flocks (Pritchard and Dawson, 1987). Four of 23 flocks sampled (17 per cent) had one or more sero-positive animals including one with 34 per cent seropositives.

Although it is difficult to assess the economic significance of the disease in commercial flocks, the discovery of maedi in East Anglia inevitably raises concern about the situation elsewhere in Britain.

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Papers and Articles

Concurrent maedi-visna virus infection and pulmonary adenomatosis in a commercial breeding flock in East Anglia

G. C. Pritchard, S. H. Done

Veterinary Record (1990) **127**, 197-200

The seroprevalence of maedi-visna virus infection in thin potential cull ewes aged over two years in a flock in East Anglia increased from 3·7 per cent in August 1985 to 39·0 per cent in September 1987 and 93·3 per cent in May 1989. This increase coincided with the first appearance of sheep pulmonary adenomatosis in the flock. Four emaciated ewes which were dyspnoeic were necropsied between 1987 and 1989. Maedi and pulmonary adenomatosis were confirmed histologically in one of these ewes and pulmonary adenomatosis was confirmed in the other three.

THE presence of maedi-visna virus infection in commercial flocks of indigenous sheep in Britain has been described by Pritchard and others (1984) and Pritchard (1987). In a survey of commercial breeding flocks in East Anglia in 1985, further serological evidence of infection was demonstrated in four of 23 flocks which were statistically sampled (Pritchard and Dawson 1987). One of these seropositive flocks was the subject of further investigations between 1987 and 1989.

Materials and methods

The long established flock of Suffolk crosses (Suffolk × Scottish half-bred) and north country mules produced finished lambs intensively. The sheep were kept outside throughout the year, either at grass or on sugar beet tops or stubble turnips, and lambed in January. About 100 female shearlings were brought into the flock annually from markets in East Anglia and the midlands to replace the 70 to 90 ewes culled each year. Suffolk rams and teasers were obtained from sources of largely unknown disease status. The flock increased in size from about 500 ewes in 1985 to 735 ewes in 1989.

Disease history

Wasting disease with chronic pneumonia was not seen in the flock until December 1985. Between then and September 1989 about 15 ewes became markedly emaciated and dyspnoeic, usually with a profuse nasal discharge. Most clinical cases occurred during the autumn and winter. In 1988 and 1989 the number of ewes culled prematurely because of poor bodily condition increased by about 20 per year.

Serological findings in 1985

The flock was first blood sampled in August 1985, as part of a serological survey described by Pritchard and Dawson (1987). Two (3·7 per cent) of 54 thin potential cull ewes aged over two years were found to be seropositive for maedi-visna virus infection by the agar gel immunodiffusion test (Dawson and others 1982).

Further serological investigations

The flock was blood sampled in September 1987 and in May 1989 to examine the changes in the seroprevalence of maedi-visna virus infection, using the same test. The method of selecting ewes for sampling was the same as that used in 1985. The flock was put through a race and blood samples were collected from ewes aged two years or more which were judged to be in sufficiently poor bodily condition to merit culling. Additional blood samples were taken randomly from ewes in good bodily condition, from ewes purchased as shearlings the previous year and from rams and teasers. Their approximate ages were recorded by referring to coloured ear tags used by the shepherd to identify each year's intake of shearlings.

Post mortem examinations

The owner was requested to submit emaciated and dyspnoeic sheep to Norwich Veterinary Investigation Centre for laboratory examination. They were examined clinically and tested for maedi-visna antibodies with the agar gel immuno-diffusion test. Routine necropsies and bacteriological cultures were performed. Samples of lung, brain, spinal cord, and udder were taken for detailed histological examinations.

Results

Serological investigations

The serological findings from the thin potential cull ewes aged over two years are shown in Table 1; the 1985 results are

TABLE 1: Seroprevalence of maedi-visna virus infection in thin potential cull ewes, aged at least two years

Date	Number sampled	Number positive	(per cent)
August 1985	54	2	(3·7)
September 1987	41	16	(39·0)
May 1989	60	56	(93·3)

TABLE 2: Number of ewes seropositive for maedi-visna virus infection in each age group in May 1989

Age (years)	Number sampled	Number positive	(per cent)
2*	50	28	(56·0)
3	32	25	(78·1)
4	61	48	(78·7)
5	43	30	(69·8)
6	186	140	(75·3)
Total	372	271	(72·8)

*Purchased as shearlings in August 1988

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TABLE 3: Laboratory findings from ewes presented for post mortem examination

Date	Clinical history	'Wheelbarrow' test	Agar gel immunodiffusion test for maedi-visna	Significant bacteria isolated from lungs	Post mortem observations
September 1987	Thin three-year-old mule, 40·2°C. Hyperpnoeic. Severe cough with expulsion of fluid from nostrils	+	+	*Pasteurella haemolytica* biotype A	Severe pulmonary adenomatosis; suppurative lung lesions
September 1987	Emaciated, dyspnoeic, broken mouthed mule, 40·0°C. Watery fluid in mouth and nostrils	+/−	−	−	Fibrinopurulent pericarditis; multiple chronic lung abscesses. Small early lesion of pulmonary adenomatosis.
October 1987	Very emaciated six-year-old homebred Suffolk cross, 39·8°C. Severely dyspnoeic. Fluid exuding from nostrils	+	+	−	Severe pulmonary adenomatosis and maedi (see text)
September 1989	Very emaciated, five-year-old mule, 40·4°C Rapid breathing; collapsed with forced exercise	−	+	−	Very severe pulmonary adenomatosis involving virtually all lung tissue. Focal suppuration (as pasteurellosis) also present

'Wheelbarrow' test Hindlegs raised to check whether fluid poured from nostrils

included for comparison. In 1987 four of 12 ewes purchased as shearlings in 1986 were seropositive. Insufficient samples were taken from other age groups in 1987 to make valid comparisons with the 1989 data. The age-specific prevalences of maedi-visna in the ewes tested in 1989 are shown in Table 2.

There were three seropositive Suffolk rams among the 20 tested in 1987. One had been on loan from a local farmer for the previous three months, one was a ram lamb purchased locally a few months earlier and the other was a homebred teaser aged about 18 months. In 1989 seven of the 13 rams resident in the flock were seropositive; most of them were three to five years of age.

Post mortem examinations

Four ewes were examined. Three of them had lesions of pulmonary adenomatosis and the other had pulmonary adenomatosis and maedi. The detailed findings from the last case are described below.

Coexistent maedi and pulmonary adenomatosis.— The lungs were not collapsed and impressions of the ribs were evident. Numerous grey-brown consolidated foci were distributed throughout the pleural and cut surfaces, particularly in the lower borders of the lobes but also in dorsal areas (Fig 1). At least 50 per cent of the lung tissue was grossly affected. The bronchial and mediastinal lymph nodes were enlarged, grey and very oedematous. The total weight of the lungs and associated lymph nodes (excluding the trachea) was approximately 2 kg.

All the lobes of the lungs showed histological changes indicative of a mixed infection with pulmonary adenomatosis and maedi. Focal areas of pulmonary adenomatosis exhibited alveolar epithelialisation (Fig 2) progressing to bronchiolar-alveolar carcinoma with raised cuboidal and columnar epithelium. Many of the small tumours had coalesced to form confluent pan-lobular tumours. Epithelial hypertrophy and hyperplasia were present in bronchi and bronchioles.

Between the focal tumours were areas showing changes associated with maedi-visna virus infection. These comprised isolated lymphoid nodules (Fig 3) and widespread interstitial pneumonia (Fig 4) with moderate mononuclear cell infiltration; smooth muscle hypertrophy was present in a few areas. The tracheobronchial and mediastinal lymph nodes were congested with distended lymphatics, subcapsular sinus catarrh and reactive hyperplasia of lymphoid follicles. The udder showed mild lymphocytic infiltration. There were no significant lesions in the brain or spinal cord.

Discussion

When the flock was first blood tested in August 1985, it had no history of either pulmonary adenomatosis or maedi. Four

FIG 1: Gross appearance of lungs showing areas of consolidation. Rib impressions are just discernible

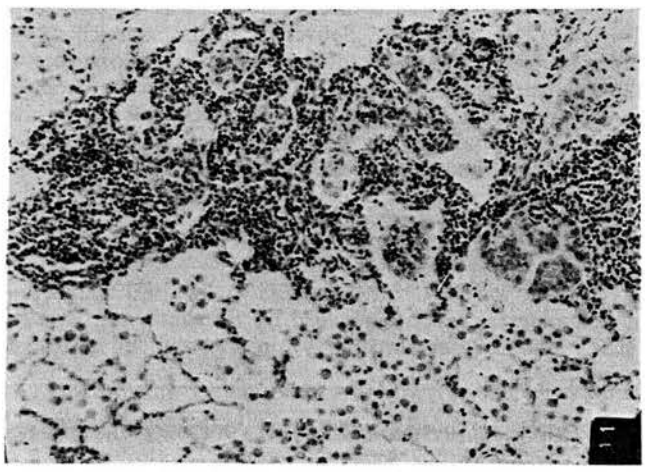

FIG 2: Focal adenomatosis with interstitial mononuclear cell infiltration and increase in alveolar macrophages. Haematoxylin and eosin × 90

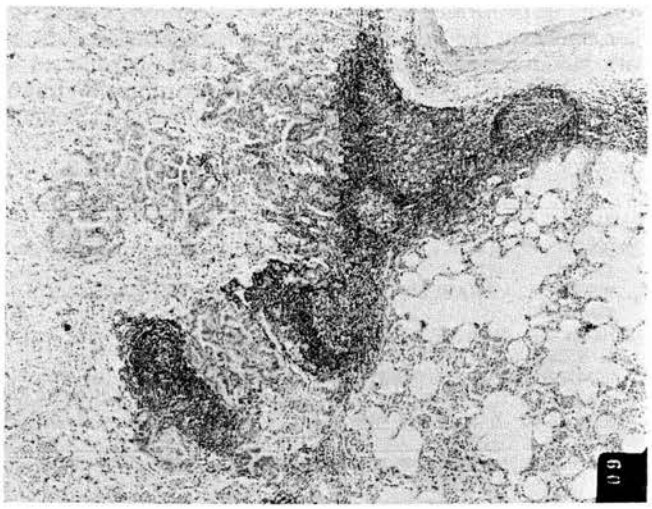

FIG 3: Adenomatous foci with multifocal isolated lymphoid nodules with germinal centre formation. Haematoxylin and eosin × 37

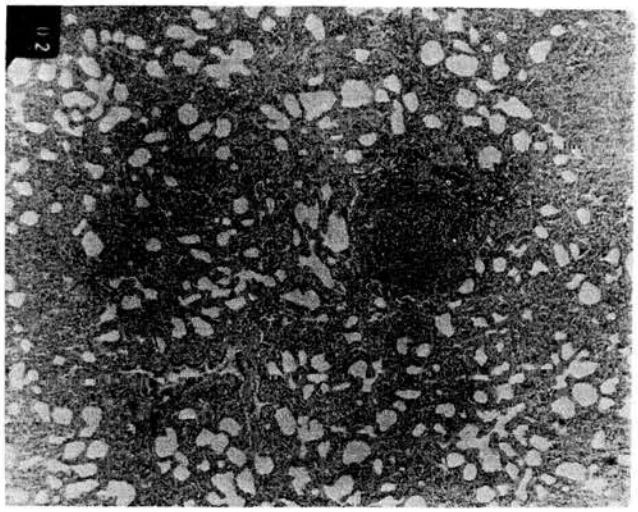

FIG 4: Interstitial pneumonia with lymphoid nodule. Haematoxylin and eosin × 37

months later pulmonary adenomatosis made its first clinical appearance, having probably been introduced with the annual intake of shearlings. During the next four years the seroprevalence of maedi-visna virus infection increased rapidly. These findings strongly support the observations of Houwers and Terpstra (1984) who reported 90 per cent seroconversion to maedi-visna infection within 18 months from lateral transmission within a flock heavily infected with pulmonary adenomatosis. Dawson and others (1985) have also commented on the synergism between infection with pulmonary adenomatosis and maedi-visna.

In 1989, 28 of 50 two-year-old ewes (56 per cent) that had been in the flock for less than a year, were seropositive for maedi-visna. Although some of these ewes could have been infected before they were brought into the flock it appeared more likely that they had become infected by lateral transmission after their arrival. Similar comments apply to the borrowed ram and the recently purchased ram lamb which were found to be seropositive in 1987.

There was less serological evidence of maedi-visna virus infection among the rams than among the ewes and no clinical cases were reported. Nevertheless, the reactor rate among the rams increased from three of 20 tested (15 per cent) in 1987 to seven of 13 tested (54 per cent) in 1989. The rams were kept separately in a small group throughout most of the year but it was possible that they had acquired infection from close contact with the ewes at tupping.

The pathognomonic histological lesions of sheep pulmonary adenomatosis include alveolar epithelialisation, tumour formation and a massive increase in macrophages with severe alveolar exudation. With maedi, there are no such pathognomonic lesions. Instead there are several non-specific changes that need to be considered together with the clinical history, gross post mortem findings and serology before making a presumptive diagnosis.

Histologically, maedi produces two major lung lesions. First, there is lymphoreticular hyperplasia unrelated to blood vessels, bronchi or bronchioles; the lymphocytic nodules do not necessarily have active germinal centres. Secondly, there is interstitial pneumonia comprising alveolar interstitial infiltration, smooth muscle hypertrophy, inflammatory cell infiltration and eventual fibrosis; alveolar exudation is absent or virtually absent.

In advanced mixed infections such as that described here, it can be difficult to distinguish between the lesions attributable to maedi and those due to pulmonary adenomatosis. Markson and others (1983) described mixed pulmonary disease (including pulmonary adenomatosis) in a maedi-infected flock that contained a high proportion of imported sheep. However, the difficulty in the attribution of specific histological lesions is reflected in the dearth of detailed descriptions of combined maedi and pulmonary adenomatosis in the literature.

In October 1989, the clinical and economic effects of maedi-visna virus infection in this flock were small and could not readily be separated from those due to pulmonary adenomatosis. About 15 clinical cases of maedi and, or, pulmonary adenomatosis were seen between 1985 and 1989. There was an increase of about 20 per year in the number of ewes culled prematurely for loss of condition in 1988 and 1989. The lambing percentage (lambs sold fat/ewes tupped) decreased from 175 per cent in 1985 to 150 per cent in 1989, but this decrease was probably due to an outbreak of enzootic abortion in 1988. The birth weights of the lambs were satisfactory and their subsequent growth rate on an intensive system, with supplementary feeding, was very good. Mastitis was not a significant problem, a particularly relevant observation in view of reports by Van der Molen and others (1985) and Van der Molen and Houwers (1987) of indurative lymphocytic mastitis in sheep after natural or experimental infection with maedi-visna virus.

In view of the lack of significant financial losses attributable to maedi-visna virus infection, the flock owner decided that it was not cost-effective to do more to control the disease than to cull the early clinical cases and most of the very old ewes, some of which were nine or 10 years old. There are no recognised sources of maedi-free crossbred female breeding replacements for commerical flocks in Britain, and the owner was therefore unwilling to attempt a costly eradication programme. The recently introduced category III membership of the National Sheep and Goat Health Scheme, operated by the State Veterinary Service, is an important step towards solving this problem.

This evidence from East Anglia suggests that maedi poses a greater potential problem for commercial flocks in Britain than has previously been supposed. Flocks with pulmonary adenomatosis and maedi are likely to present an increased risk of lateral transmission to other flocks. At present, there is no evidence of significant economic loss due to clinical or subclinical disease in British flocks. However, the reports of subclinical maedi-visna infection causing indurative mastitis (Houwers and others 1988) and other deleterious effects on productivity (Dohoo and others 1987) must be of concern to the sheep industry.

Postscript

During December 1989 and January 1990, three pregnant ewes developed nervous signs which were initially attributed to pregnancy toxaemia and hypocalcaemia. They failed to respond to any therapy and deteriorated over a period of five to six weeks despite remaining fairly bright and alert, and continuing to eat. The clinical signs were a fine facial tremor (particularly

of the ears) and hyperaesthesia, slowly progressing to incoordination, circling, hindlimb paralysis and terminal convulsions. The most severely affected animal, a three-year-old ewe that was seropositive for maedi-visna was submitted to the Veterinary Investigation Centre.

No significant gross lesions were detected post mortem. Histological examination of the brain revealed mild focal mononuclear infiltration of the meninges and choroid plexuses. The subependymal layers of the lateral ventricles showed extensive multifocal demyelination and there were also focal areas of lymphocytic perivascular cuffing in the white matter. By an oversight the lungs were discarded and not examined histologically because they appeared grossly normal.

Although the histological lesions in the brain were very mild, in contrast to those of experimental visna, when supported by the clinical picture and the flock history they strongly suggest that visna was also present in the flock.

In 1990 the lambing percentage declined further and there was also a sudden upsurge in post weaning mastitis cases. The entire flock has now been consigned for slaughter.

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Multiple cases of maedi and visna in a flock in East Anglia

SIR, – Cases of maedi and visna have recently been confirmed in a commercial flock of 1200 ewes (mainly Scottish and North Country mules) in East Anglia. There have been several previous reports – mainly from East Anglia – of maedi-visna (MV) virus infection in Britain. Maedi, usually complicated by concurrent disease, particularly pulmonary adenomatosis, has been confirmed or suspected in some of these including a recent report from Somerset (Gunning 1993). Visna, the neurological manifestation of MV virus infection, was not recorded in Britain until 1990 (Pritchard and Done 1990, Watt and others 1990).

The two initial cases of visna submitted to Bury St Edmunds Veterinary Investigation Centre in August 1995 were aged mule ewes in fairly poor condition and blind – one bilaterally and the other unilaterally. The latter was circling and had a marked head tilt. Other clinical signs of visna seen in this flock have included dazed aimless wandering, gait abnormalities (particularly dragging of the hindlimbs), ataxia and terminal recumbency. In addition, there have been typical cases of maedi with severe weight loss and dyspnoea. There appear to have been at least 30 cases of either maedi or visna during the past year.

The lungs of one of the initial cases necropsied were typical of uncomplicated maedi. They weighed 1·8 kg (three to four times normal) and were rubbery, uncollapsed and had a greyish-pink granular appearance. Unlike pasteurella pneumonia or consolidation associated with pulmonary adenomatosis, they floated in water. Histologically, the brains showed both demyelination with extensive white matter destruction particularly in the cerebellum. There was also intense perivascular cuffing, severe gliosis and focal leptomeningitis. In both animals extensive lymphoid aggregates were present in the udder, joint capsules and lungs which also exhibited widespread interstitial pneumonia and characteristic smooth muscle hyperplasia.

There do not appear to be any previous reports in the literature of blindness associated with visna but it is a significant feature here. Clinically, visna may be confused with conditions such as cerebral listeriosis, cerebrocortical necrosis and pregnancy toxaemia, all of which also occurred in this flock. Cases might also be confused with scrapie and if this cannot be discounted on clinical grounds the animal health office should be informed. Maedi should always be suspected in dyspnoeic emaciated older ewes but it needs to be differentiated from pulmonary adenomatosis.

The flock was blood sampled in 1985 as part of a serological survey to assess the extent of MV virus infection in East Anglia and 19 (34 per cent) of 56 ewes tested were seropositive although there was no history of clinical disease at that time (Pritchard and Dawson 1987). When a random selection of 42 ewes were sampled in August 1995, the seroprevalence had increased to 80 per cent. Investigations are continuing and we hope to publish further details later.

These findings must be of concern to the sheep industry of Great Britain. They emphasise the insidious nature of a condition that can remain inapparent in an infected flock for many years. In this instance overt disease emerged mainly in broken-mouthed ewes that were retained in the flock for longer than usual instead of being culled. Inevitably, diseased commercial flocks will maintain a reservoir of infection as successive intakes of ewe lambs and shearlings become infected. Owners who unknowingly purchase infected cull ewes for further breeding will introduce the infection into their flocks.

Based on a survey of almost 15,000 sera from 1500 non-health scheme flocks of sheep and goats selected randomly throughout Great Britain it was estimated that there were approximately 70,000 seropositive adult sheep in 1993 (Dawson 1994). Interestingly, there appeared to be a relative concentration of infection in East Anglia. The challenge to the industry and the profession must be to prevent the virus being spread between commercial as well as pedigree flocks by the trade in breeding replacements. All those who purchase breeding stock of whatever type need to be aware of the possibility of infection and its consequences.

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1.25

EXPERIENCES WITH MAEDI-VISNA IN EAST ANGLIA

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HISTORY OF MAEDI-VISNA IN BRITAIN

The first recognition of maedi-visna (MV) virus infection in Britain was in 1978. Reports during the ensuing five years all involved imported exotic sheep and their progeny or indigenous breeds that had contact with exotic sheep. By 1983, clinical and/or significant serological evidence of MV infection had emerged in three indigenous commercial flocks, two of which were in East Anglia. Since then, apart from two flocks in Scotland and one in Somerset (all identified between 1990 and 1993), reported clinical evidence of MV virus infection has been confined to East Anglia.

A serological survey in Norfolk and Suffolk in 1985 revealed infection in a further four out of 23 commercial flocks. One of these, which had only 3.7 per cent seropositive animals and no clinical history suggestive of maedi, subsequently developed clinical maedi and visna by 1989 and was depopulated. In this flock the introduction of pulmonary adenomatosis (SPA) appeared to have triggered a rapid increase in seroprevalence to over 70 per cent. Histological examinations carried out on clinical cases revealed predominantly mixed maedi/SPA lesions. Previous authors have commented on the synergism between SPA and maedi.

By 1995, another of the infected flocks from the 1985 survey that had not previously experienced any clinical disease, exhibited multiple cases of both maedi and visna. The seroprevalence in this flock increased from 34 per cent in 1985 to 80 per cent in 1995.

No further information is currently available from the two other infected flocks identified by the 1985 survey. However, a further heavily infected commercial flock was identified in 1994. This flock, which had a history of a high culling rate for poor condition, was tested because MV infection was detected in an associated pedigree flock. Of 20 commercial ewes sampled, 18 (90 per cent) were seropositive.

A serological survey by the Scottish Agricultural Colleges (SAC) in 1996/7, using 44,102 sera from randomly selected flocks throughout Britain (East Anglia appears to have been poorly represented), showed 0.195 per cent positives (1.4 per cent of flocks). This was very similar to the 0.39 per cent seroprevalence (1.6 per cent of flocks) in 14,675 sera in the previous survey in 1993.

Figure 1. Co-existent maedi and pulmonary adenomatosis. Rib impressions just evident. Numerous grey-brown consolidated foci, particularly in the lower borders of the lungs but also in dorsal areas. Total weight of lungs and associated lymph nodes, but excluding trachea, about 2 kg

MAIN CLINICAL FEATURES

The clinical features of maedi are predominantly progressive weight loss and dyspnoea in older ewes (mainly at least four years old) but cases have been seen in ewes as young as two and a half years. Despite this, in a heavily infected flock currently under investigation by the author, the number of ewes dying or culled specifically for maedi is estimated at only about one or two per cent of the flock, with most affected ewes being culled before clinical disease becomes markedly evident. In this flock visna cases have occurred much more sporadically. Clinical features of visna have included general incoordination, ataxia, circling, head-tilt, dragging of hind limbs, knuckling over of fore limbs, dazed confusion, and aimless wandering. Blindness has also been noted in several cases.

NECROPSY FINDINGS

At first glance, maedi-affected lungs can appear relatively normal, particularly when exhibiting non-specific generalised congestion with autolysis. Maedi can also be masked by secondary infections, such as pasteurellosis or lungworm infestation or obscured by SPA.

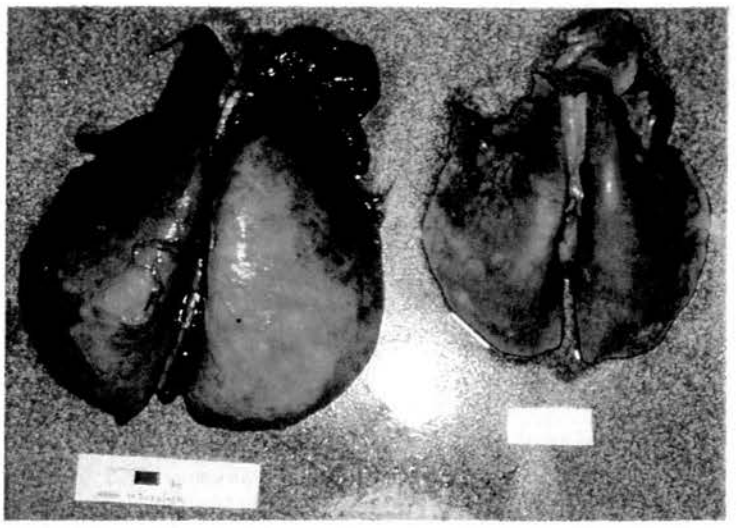

Figure 2. Autolysed, uncomplicated maedi lungs weighing 2.3 kg (left) with normal lungs (720g) for comparison

Uncomplicated maedi cases are likely to be relatively uncommon compared with mixed conditions.

The weight of the lungs provides a useful guide to the possible presence of maedi - in all confirmed cases examined by the author, the lungs (excluding heart and visible trachea) have weighed at least 1kg and are often at least 2kg or more in weight. This compares with 600-700 grams for normal ewe lungs. Furthermore, until maedi is fairly advanced, the affected lung tissue floats in water rather than sinking (unlike pasteurella pneumonia, for example). The lungs and associated lymph nodes are enlarged. The gross appearance of the lungs varies from uniformly red and congested, with a dense rubbery consistency, to a mottled pinkish red colour variegated with firm greyish granular tissue. Definitive confirmation requires histological examinations, preferably supported by serology. If maedi is present in a flock, at least 60 per cent of ewes are likely to be seropositive.

RECENT INVESTIGATIONS

In 1996, studies were carried out in a heavily infected flock in East Anglia to determine whether, as in the Netherlands (Houwers and others, 1988), there was a reduced milk yield and poor pre-weaning growth rate in lambs from affected ewes. The birth and weaning weights of twin lambs born to 56 seronegative ewes were compared with those from 94 seropositive ewes (the ewes were blood sampled at lambing).

Surprisingly, there were no statistically significant differences between the two groups, even though a number of cull ewes from this flock had shown significant histological evidence of indurative lymphocytic mastitis (I. B. Griffiths, personal communication). Lambs from seropositive ewes (which were mainly older) were actually slightly heavier (statistically not significant) than those from seronegative ewes (predominantly younger).

The possible explanation for these unexpected results may be that the lambs were kept intensively, and from birth were offered creep feed which could have compensated for any loss of milk yield by the ewes. Furthermore, the effect of older ewes usually having heavier lambs may have outweighed any effect of MV infection.

The findings from this study suggested that the main costs of maedi in an intensive lowland commercial flock were associated essentially with the cost of replacing ewes one or two years earlier through premature culling, the cost associated with lower lamb weights from younger ewes and perhaps some unquantifiable additional feed costs. The cost of culling ewes is strongly influenced by the cull ewe price and, as with caseous lymphadenitis for example, owners of infected sheep are under no obligation to dispose of them directly to slaughter.

CONTROL

Limited attempts by owners of two of the infected flocks in East Anglia to establish MV-free flocks from purchased replacements maintained close to, but apart from, infected flocks, have met with failure. This is probably because significant horizontal transmission undoubtedly occurs under intensive

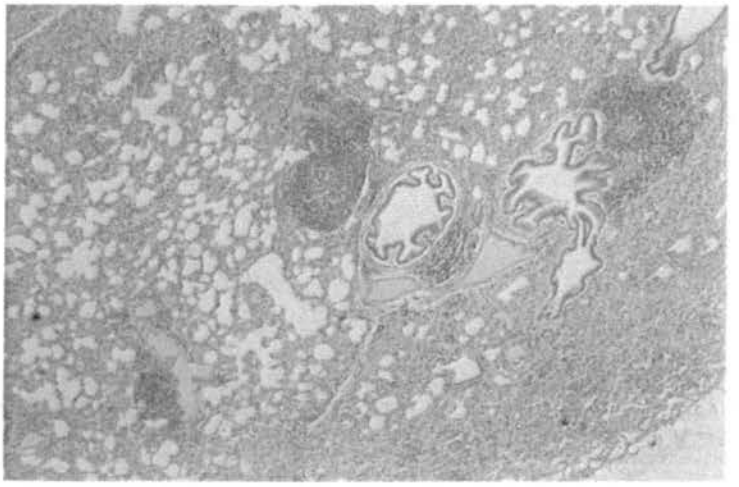

Figure 3. Low power slide showing lymphofollicular proliferation in perivascular and peribronchiolar sheaths. Note pulmonary vein with lymphofollicular proliferation

conditions. In view of the high proportion of infected ewes in these flocks, the only practical alternative to an early culling policy is to replace the entire flock. Such an expensive undertaking demands the assurance that any subsequent purchased breeding replacements are themselves uninfected. Hopefully, SAC's MV monitoring scheme will prove successful and provide a much-needed source of uninfected crossbred breeding replacements. The reservoir of the disease maintained within commercial flocks inevitably presents a significant risk to flock owners who unknowingly purchase infected cull ewes for further breeding, and to pedigree flocks run alongside commercial flocks.

CONCLUSION

Although MV virus infection is clearly widespread within the national flock, though hardly common, reports of clinical disease outside East Anglia remain elusive. One possible explanation is the lack of awareness, particularly amongst sheep farmers, combined with the fact that veterinary surgeons are only rarely called on to necropsy old ewes. It seems likely that the condition exists unrecognised in many other commercial flocks, with affected animals culled prematurely rather than submitted for veterinary investigation.

In conclusion, it seems inevitable that what is at present a problem for the sheep industry in East Anglia will be mirrored elsewhere unless concerted efforts are made to deal with the situation. Precisely why the infection is more common in East Anglia remains something of a mystery since the source of breeding replacements is similar to those used by flocks in many other parts of Britain. The possibility of a focus of MV infection in East Anglia pre-dating its apparent importation in the late 1970s cannot be completely excluded.

Figure 4. Typical 'congested' appearance seen with some cases of uncomplicated maedi which could easily be overlooked. Note rubbery texture; collapsed. Despite the weight (1.8 kg) the lungs float in water.

ACKNOWLEDGEMENTS

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1.26

Emergence of an apparently neurotropic maedi-visna virus infection in Britain

SIR, – Between September and December 2003, unusual flock presentations of maedi-visna (MV), presenting as neurological disease (visna) rather than respiratory disease (maedi), have been encountered in two flocks in Britain.

During the past 25 years since MV was first reported in Britain, clinical disease has been confirmed in less than 20 indigenous flocks, several of which have been in East Anglia (Pritchard and others 1995). The clinical expression has predominantly been maedi although coexistent visna was confirmed in two flocks (Watt and others 1990, Pritchard and others 1995).

Between September and November 2003, six of 200 ewes aged two to three years, in a commercial mule flock in the Midlands, developed neurological signs including ataxia, hindlimb paralysis and recumbency. Four ewes were examined postmortem and no significant gross lung pathology was observed. Visna was subsequently confirmed by histopathology, revealing characteristic lesions (multifocal markedly asymmetric severe non-suppurative encephalomyelitis accompanied by demyelination mainly involving the periventricular neuropil and white matter). Two of these ewes also had mild lymphofollicular pneumonia typical of low-grade maedi and two had minimal lymphocytic angiocentric pneumonia. Serological screening of 20 cull ewes (mainly in good body condition) revealed 13 to be seropositive by agar gel immunodiffusion testing (AGIDT). It is notable that respiratory disease was not reported to be a problem in the flock and culling was primarily for broken mouths or chronic mastitis.

Between November and December 2003, similar histological lesions were found in the brain of a three-year-old Suffolk cross ewe with a history of progressive head tilt, hyperaesthesia and ataxia leading to recumbency, and also in the brain and spinal cord of a further ewe presenting with ataxia and tremors. Approximately nine ewes had shown similar clinical signs, over a six-month period, in a large commercial flock in southern England. The first affected ewe was seropositive for MV by AGIDT, and subsequent serological screening of a further 25 animals, selected to assess exposure of different age cohorts, mostly between three and five years old, revealed 23 to be seropositive. There had previously been no clinical suspicion of MV in this flock.

The clinical presentation of MV virus infection primarily as a neurological disease marks a departure from previous experiences in Britain and suggests a more neurotropic virus strain may be involved, as was the situation in some flocks in Iceland during the last century (Palsson 1976). Maedi was also grossly evident at postmortem examination in these flocks despite the lack of overt respiratory disease.

MV virus infection is probably much more widespread in British sheep, particularly commercial flocks, than documented reports suggest. Most infection is subclinical and overt disease is not evident until the seroprevalence reaches about 60 per cent. Furthermore, under favourable lowland husbandry systems, as in these recent cases, MV can remain unrecognised for several years with no obvious effect on productivity. Such infected flocks present a major reservoir of infection of particular risk to pedigree flocks, with the associated financial consequences for sales of replacement breeding animals.

Concerns have been expressed that MV could become a serious issue for the health status of the national flock. Edwards (2002) commented on potential risks for dissemination following restocking after the 2001 foot-and-mouth disease epidemic and at least two other incidents investigated recently have supported this view (A. Otter, A. Holliman, personal communications). Furthermore, Synge and Baird (2002) highlighted an increased number of breakdowns seen in Scottish Agricultural College Sheep and Goat Health Scheme flocks attributed to contact with non-accredited stock.

Veterinary Laboratories Agency Regional Laboratories are keen to investigate flocks with suspected MV disease as part of the surveillance remit for emerging diseases.

The assistance and cooperation of the farmers and colleagues involved in these investigations are gratefully acknowledged.

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EMERGENCE OF APPARENTLY NEUROTROPIC MAEDI-VISNA VIRUS INFECTION IN BRITAIN

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INTRODUCTION

Between September and December 2003, unusual flock presentations of maedi-visna (MV), presenting as neurological disease (visna) rather than respiratory disease (maedi), were encountered in two flocks in Britain.

During the last 25 years since MV was first reported in Britain, clinical disease has been confirmed in less than 20 indigenous flocks, several of which have been in East Anglia (4). The clinical expression has predominantly been Maedi, although co-existent Visna was confirmed in two flocks (4, 6).

CASE PRESENTATION

Between September and November 2003, six of 200 ewes aged 2 to 3 years, in a commercial mule flock in the Midlands, developed neurological signs including ataxia, hind limb paralysis and recumbency. Four ewes were necropsied and no significant gross lung pathology was observed. Visna was subsequently confirmed by histopathology, revealing characteristic lesions (multifocal markedly asymmetric severe non-suppurative encephalomyelitis accompanied by demyelination mainly involving periventricular neuropil and white matter). Two of these ewes also had mild lymphofollicular pneumonia typical of low-grade maedi and two had minimal lymphocytic angiocentric pneumonia. Serological screening of 20 cull ewes (mainly in good bodily condition) revealed thirteen to be seropositive by agar gel immunodiffusion testing (AGIDT). It is notable that respiratory disease was not reported to be a problem in the flock and culling was primarily for dental or mammary problems.

Between November and December 2003, similar histological lesions were found in the brain of a 3 year old Suffolk-cross ewe with a history of progressive head tilt, hyperaesthesia and ataxia, leading to recumbency and also in the brain and spinal cord of a further ewe presented with ataxia and tremors. Nine ewes had shown similar clinical signs, over a six month period, in a commercial flock in southern England. The first affected ewe was seropositive for MV by AGIDT and subsequent serological screening of a further 25 animals, selected to assess exposure of different age cohorts being mostly between 3 and 5 years, revealed 23 to be seropositive. There had previously been no clinical suspicion of MV in this flock.

DISCUSSION

The clinical presentation of MV virus infection primarily as a neurological disease marks a departure from previous experiences in Britain and suggests a more neurotropic virus strain may be involved, as was the situation in some flocks in Iceland during the last century (2). Maedi was also grossly evident at necropsy in these Icelandic flocks despite the lack of overt respiratory disease.

Concerns were expressed that MV would become a serious issue for the health status of the national flock following restocking after the 2001 foot-and-mouth disease epidemic (1) and several incidents investigated recently have supported this view. Furthermore, Synge and Baird (5) highlighted increased numbers of breakdowns in the Scottish Agricultural College Sheep and Goat Health Scheme flocks attributed to contact with non-accredited stock (5).

MV virus infection is probably much more widespread in British sheep, particularly commercial flocks, than documented reports suggest. Most infection is subclinical and overt disease not evident until the seroprevalence reaches about 60%. Furthermore, under favourable lowland husbandry systems, as in these recent cases, MV can remain unrecognised for several years with no obvious effect on productivity. Such infected flocks present a major reservoir of infection of particular risk to pedigree flocks, with the associated financial consequences for sales of replacement breeding animals.

ACKNOWLEDGEMENTS

This abstract relates to work published in a letter to the Veterinary Record (3). The assistance and cooperation of the private veterinary practitioners and farmers involved in these investigations are gratefully acknowledged.

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28

Maedi-visna

G. C. Pritchard and M. Dawson

Maedi and visna are Icelandic words describing the clinical presentation of two apparently different syndromes that affected Icelandic sheep in the 1940s ands 1950s. Maedi, or 'laboured breathing', was a fatal, progressive pneumonia of mature sheep; visna, or 'wasting', was a meningoencephalitis, which caused progressive paralysis and death. The disease appeared in Iceland in the years following the importation of Karakul sheep from Germany in 1933. Its viral aetiology was established in the late 1950s, when it also became clear that maedi and visna were different syndromes caused by essentially the same virus. The disease was eventually eliminated from Iceland in 1965 following phased destocking of the sheep-rearing areas and repopulation with sheep from unaffected parts of the country. Iceland is the only country that has eradicated maedi-visna (MV) successfully.

MV-like diseases have been reported from many of the sheep-rearing countries of the world, but they have not been seen in Australia and New Zealand, although caprine arthritis encephalitis (CAE) virus infection occurs in both countries. In the USA, infection is endemic in some of the western range flocks, and the associated disease is termed ovine progressive pneumonia, or Montana sheep disease. In The Netherlands, the disease is known historically as *zwoergerziekte* and in France as *la bouhite*. Recent surveys in Spain indicated a high prevalence in some regions. In the UK, it is believed that infection was introduced in the early 1970s with the importation of breeding sheep from the continent.

CAUSE

MV virus is a non-oncogenic, exogenous, retrovirus. It belongs to the lentivirus sub-family of retroviruses and is related to human, simian, feline and bovine immunodeficiency viruses, equine infectious anaemia virus and CAE virus. The relationship between the latter and MV virus is close and cross-species transmission is possible, especially after feeding infected milk from one species to the other. MV and CAE viruses are commonly referred to as the small ruminant lentiviruses. Retroviruses are RNA viruses, which replicate via a DNA provirus that becomes integrated or closely linked to genomic DNA in the virus target cells. Antigenic variation in isolates of MV virus is well documented, and these changes in virus phenotype are mainly due to mutation in the gene encoding the surface glycoprotein.

CLINICAL SIGNS

The incubation period is protracted and clinical signs [1] develop insidiously, epitomizing slow virus infection. Disease is not evident until at least 2 years of age, or more usually, 4–5 years. Maedi is the most common clinical manifestation: affected animals remain bright and alert but exhibit gradual loss of condition; respiratory distress develops slowly and may initially only be evident on exertion. Pyrexia is not a feature unless secondary bacterial infection is present; auscultation of the thorax is generally unrewarding. Coughing and nasal discharge are occasional features; flared nostrils, neck extension and open-mouth breathing occur in more advanced cases.

Maedi is clinically less obvious in sheltered lowland flocks on a high plain of nutrition, where mildly affected sheep may pass unnoticed for several months and can survive for a year or more until culled or destroyed on humane grounds. In many flocks, the interval between introduction of infection and first

appearance of clinical disease can be as long as 10 years, by which time a high proportion of the flock is likely to be seropositive.

Although maedi is untreatable and ultimately fatal, mortality rates due to uncomplicated disease are generally very low – less than 2 per cent in a heavily infected flock studied in the East Anglian region of England. Deaths are commonly associated with secondary bacterial infection, particularly pasteurellosis, and there may also be an increased predisposition to other conditions, such as pregnancy toxaemia. Mortality rates can be higher under harsher climatic and environmental conditions, such as those encountered in an infected flock in the north-east of Scotland [1], where ewe mortality was 14 per cent, with associated losses in lambs due to poor viability and insufficient colostrum. However, the extent to which losses are due directly to MV virus infection or to secondary and opportunistic infections is not clear.

Visna, the nervous form of the disease, is also characterized by weight loss. It occurs much less commonly and is usually seen only sporadically in heavily infected flocks, often several years after the initial recognition of maedi. Both conditions may be present to varying degrees in the same animal or they can occur independently. Clinical signs of visna relate to lesions localized in either brain stem or spinal cord. Gait abnormalities of the hind limbs are the main initial feature, particularly unilateral dragging of the foot due to paresis of the affected limb, resulting in scraping of the hoof. Knuckling over and stumbling soon follow, leading to ataxia, general inco-ordination, collapse and death. Other features include circling, head tilt, fine tremor of the lips, dazed appearance, separation from the rest of the flock and aimless wandering. Visual impairment and blindness, features not recognized until recently, can also occur. Unlike maedi, visna cases progress relatively rapidly, resulting in death or euthanasia within a few weeks.

A slowly progressive, indurative mastitis [2] is a common feature in some heavily infected flocks. This can be detected only by careful palpation of the udder: there are no gross changes to the character of the milk. The main consequence seems to be delayed weight gains in unweaned lambs, particularly twins and triplets. Arthritis is a rare manifestation of MV virus infection. Carpal joints are mainly affected, and joint enlargement is not usually associated with acute lameness.

PATHOLOGY

Maedi is the manifestation of MV virus infection most likely to be suspected from gross pathology. The lungs have a voluminous, uncollapsed appearance (see Figure 28.1 in the colour plate section) and occasionally rib impressions are retained. The lungs and associated lymph nodes, which are also grossly enlarged (the caudal mediastinal may reach up to 10 times normal size), invariably weigh 1–2 kg, which is between two and four times the normal weight. In uncomplicated early cases, the lungs have a dense rubbery texture and are uniformly dull red, apart from a few pinkish-grey granular focal lesions, mainly evident on cut section. At this stage, the lungs remain aerated, float in water and superficially can resemble non-specific congestion. As the condition progresses, the lungs develop a more mottled appearance and become predominantly reddish-brown, variegated with firmer discreet, creamy-greyish granular spots up to 5 mm diameter, visible on the surface (see Figure 28.2 in the colour plate section) and on cut section. With increasing severity, these lesions, which are distributed throughout all lung lobes, extend to more confluent homogeneous consolidated grey areas. Frequently, secondary bacterial infection leads to broncho-pneumonia and the airways become obstructed with exudate.

In some affected flocks, uncomplicated maedi is the norm, whereas in other flocks, cases usually present as mixed infection with lungworm, pasteurellosis, purulent bronchopneumonia or, more notably [3], sheep pulmonary adenomatosis (SPA). In these cases, underlying maedi lesions can easily be masked and may not be detectable at routine necropsy unless suspected from the flock history.

Visna lesions are located in either spinal cord or brain and are not usually grossly apparent. When the udder is affected, the mammary glands are grossly small and firm to cut. Arthritis associated with MV virus infection produces gross thickening and sometimes a red/tan discoloration of the synovial membrane with joint enlargement, particularly of the carpus.

Histological lesions [4] essentially comprise immunologically mediated chronic active inflammatory changes, with lymphoid infiltration (Figure 28.3) and proliferation in one or more target organs including lung, central nervous system, mammary glands and joints. Main organ-specific changes consist of smooth

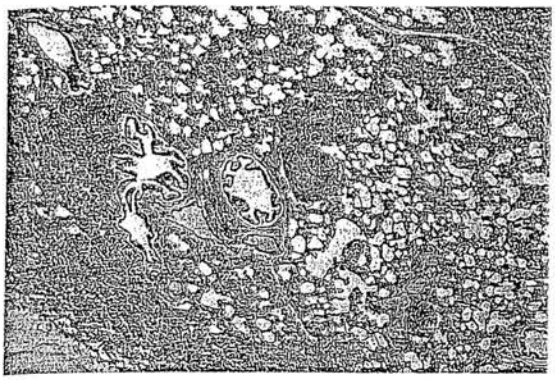

Figure 28.3: Histology of maedi lung, showing lymphoid infiltration in septa and lymphoid nodules.

muscle hyperplasia (lungs), demyelination (central nervous system), fibrosis (mammary gland) and proliferation of synovial membrane, and degenerative changes to the articular cartilage (joints). In advanced mixed pulmonary infections, particularly involving SPA, attribution of specific histological lesions can be problematical [3].

DIAGNOSIS

Most infected sheep within a flock show few overt signs of disease but remain clinically silent virus carriers. For control purposes, it is vital that such animals are identified as soon as possible after infection. Antibody detection tests are routinely used for diagnosis and, since virus infection persists, the presence of antibody signifies current infection. However, seroconversion can take several months. The agar gel immunodiffusion test has been the standard test for the last 10–15 years, but it is gradually being replaced by a range of ELISA methods, which employ purified virus, recombinant proteins or synthetic peptides as antigens. These offer the advantage of greater sensitivity and can therefore detect seroconversion earlier. Polymerase chain reaction (PCR) technology can be used for cases of special interest, such as when infection is suspected in a seronegative animal. PCR techniques permit the detection of virus-specific nucleic acid. Milk leucocytes or lung lavage cells can be useful substrates, but the virus copy number in peripheral blood leucocytes is usually too low to offer a reasonable prospect of a positive test.

MV virus infection should always be suspected in flocks with a history of progressive weight loss, emaciation and chronic pneumonia in older ewes, especially where breeding replacements are purchased from sources not monitored for MV virus infection. However, the condition can easily remain unrecognized in a flock for several years, particularly if mildly affected ewes are culled for other reasons before overt signs develop. Also, unless there is a serious flock problem, veterinary surgeons may not be consulted and, for economic reasons, necropsies are rarely undertaken routinely on older ewes. Clinically, maedi needs to be differentiated primarily from pulmonary adenomatosis, but atypical pneumonia, lungworm infection and chronic suppurative pneumonias also need to be considered.

Histological examinations are essential to confirm the diagnosis. However, it should be emphasized that there can be difficulties in reaching a definitive diagnosis in the presence of secondary infection [3]. Furthermore, the isolation of *Pasteurella* from the lungs, for example, does not exclude the possibility of underlying MV virus infection and associated pathology. Gross examination of the lungs can provide a useful guide, particularly checking whether the weight (excluding visible trachea) exceeds 1 kg. Although clearly non-specific, this approach offers a simple preliminary screening test, particularly for lungs not exhibiting obvious consolidation. If maedi is suspected in home-bred sheep, the overall within-flock seroprevalence is likely to exceed 60 per cent. Hence, blood sampling only a few aged poorer ewes should be sufficient to detect at least one seropositive animal.

Visna is unlikely to be the main initial presentation of MV virus infection in a flock with no previous history. Cases need to be differentiated from scrapie, space-occupying brain lesions and vestibular lesions. It may be necessary to differentiate visna from periparturient metabolic conditions, cerebrocortical necrosis and cerebral listeriosis, particularly if the flock has a previous history of these diseases. Definitive confirmation requires brain and spinal cord histopathological examinations, supported by serology.

EPIDEMIOLOGY AND TRANSMISSION

Lesions develop over a protracted period and sheep may be relatively old in relation to their productive

life span before overt signs appear. Following primary exposure, the virus establishes a persistent infection (by latent carriage in monocytes and macrophages) in peripheral blood, and infection extends to the lungs and other organs. Variations in immune response, genetic susceptibility and virus phenotype appear to dictate which target organ system is involved and the progress of lesion development. Despite mounting virus-specific humoral and cellular immune responses, infected sheep do not develop a protective immunity.

Intra-uterine and germ line transmissions are thought not to occur. The main route of transmission is from an infected ewe to its offspring via colostrum (including pooled colostrum banks) and milk. Horizontal transmission by respiratory secretions can occur between sheep of any age, particularly if intensively stocked or where concurrent SPA, which has a synergistic effect on transmission [3], is also present. Breed susceptibility and management practices also play significant roles. If naïve animals are introduced into a heavily infected flock, up to 50 per cent may seroconvert within 9 months. In the presence of SPA, seroconversion rates of 90 per cent within 18 months have been recorded: one such infected flock in East Anglia, England, for example, showed an increase in seroprevalence from 4 to 93 per cent within 4 years [3]. In all endemically infected flocks, there is an increase in seroprevalence with age, from about 25 per cent seropositive at 2 years to over 90 per cent at 5 years or more.

ECONOMIC ASPECTS

The economic effects of MV virus infection are poorly understood and difficult to quantify because of environmental and husbandry factors, genetic susceptibility, intercurrent disease, culling policy and the relative value of cull ewes compared with replacements. The potential cost is inevitably much greater in pedigree flocks selling breeding stock than in commercial flocks engaged in finished-lamb production. The effect on ewe mortality, lambing per cent, lamb birthweight, viability and weaning weight is very variable. Some studies have described a weight shortfall of up to 5 kg per lamb at weaning due to decreased milk production from indurative mastitis [2], but this has not been confirmed by other workers. The effect of age is a major confounding factor because older ewes generally have a higher lambing percentage and heavier lamb birthweights, regardless of MV status. Under extreme conditions, losses of 10–20 per cent of gross margin per ewe have been described in some affected flocks [1], whereas, under sheltered lowland conditions [3] the only economic effect of MV virus infection is the need to cull ewes a year earlier than usual.

PREVENTION AND CONTROL

For uninfected flocks, the first priority is to prevent the introduction of infection. This can be achieved by applying good flock disease security, including not sharing rams or mixing sheep with other flocks, and maintaining appropriate segregation at shows and sales. Quarantining and blood sampling of breeding replacements, or purchasing only from established MV virus-free sources is desirable, but may only be economically viable for pedigree flocks. In Britain, where the infection is essentially maintained in commercial flocks [3], strict segregation from pedigree flocks is essential. Several countries operate compulsory or voluntary monitoring and control schemes based on systematic flock serological examinations.

If flock infection is detected sufficiently early, when seroprevalence is low, a simple test-and-cull programme at 3–6-monthly intervals may be successful at eliminating infection, but it is only likely to be economically feasible in valuable pedigree flocks. It is essential that lambs born to seropositive ewes are also removed. Once endemic infection is firmly established, the options are limited. In many infected commercial flocks operating under favourable conditions, reasonably effective control can be maintained by increasing the flock-replacement rate and adopting a strict culling policy of not keeping ewes beyond 4–5 years of age [3]. A somewhat impractical option is to snatch lambs at birth for artificial rearing. A further alternative, which was found to be unworkable, is to build a new flock based on lambs taken from ewe lambs (considered less likely to be excreting virus). Attempts to build up MV-free flocks from heavily infected flocks have generally been unsuccessful under commercial conditions. This failure has resulted from inadequate separation between infected and clean flocks and underestimating the

extent of horizontal transmission. In most cases where MV virus infection is firmly established, the only feasible option is complete flock replacement with MV virus-free stock if available.

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31

Maedi-visna

1.29

G.C. Pritchard and I. McConnell

Maedi-visna (MV) is the composite name for an important multisystemic disease of adult sheep caused by infection with a retrovirus of the lentivirus subfamily. Maedi and visna are Icelandic words describing two apparently different clinical syndromes that affected Icelandic sheep in the1940s and 1950s. Maedi, or 'laboured breathing', was a fatal, progressive pneumonia of mature sheep; visna, or 'wasting', was a meningo-encephalitis which caused progressive paralysis and death. The disease appeared

in Iceland in the years following the importation of Karakul sheep from Germany in 1933. Its viral aetiology was established in the late 1950s, when it also became clear that maedi and visna were caused by essentially the same virus. The disease was eventually eliminated from Iceland in 1965 following phased de-stocking of the sheep-rearing areas and repopulation with sheep from unaffected parts of the country. Iceland is the only country that has eradicated maedi-visna successfully. In North America, where MV infection is widespread, the associated disease is termed ovine progressive pneumonia, or Montana sheep disease. In the Netherlands, the disease is known historically as *zwoergerziekte* and in France as *la bouhite*. Although MV has been reported from most of the major sheep-producing countries of the world, it has not been seen in Australia and New Zealand. However, the very closely related caprine arthritis encephalitis (CAE) virus infection occurs in both countries. Comprehensive up-to-date information about the prevalence of MV and CAE in many European countries is lacking but they appear to occur widely in the Mediterranean region, particularly Spain, Greece and Italy [1]. The term 'small ruminant lentivirus' (SRLV) is increasingly used to describe both MV in sheep and CAE in goats [1, 2].

In Britain, MV virus infection was first detected in the 1970s in exotic sheep (or their progeny) imported from continental Europe, or in indigenous breeds that had been in contact with exotic sheep. By 1983, it was demonstrated in commercial flocks of indigenous sheep [3] and serological surveys in the 1990s revealed infection in about 1.5 per cent of flocks. Despite concerns, confirmed clinical disease (mainly maedi), as opposed to serological evidence of subclinical infection, was recorded in only about 20–25 different indigenous flocks in Britain from on-going national surveillance activities up to the end of 2005.

CAUSE

MV virus is a non-oncogenic, exogenous, retrovirus belonging to the lentivirus subfamily, which includes human, simian, feline and bovine immunodeficiency viruses, equine infectious anaemia (EIA) virus and CAE virus. Retroviruses are RNA viruses that replicate via a DNA provirus that becomes integrated or closely linked to genomic DNA in the virus target cells. There is a very close relationship between MV and CAE viruses and cross-species transmission is possible, especially after feeding infected milk from one species to the other; both viruses are now commonly classified together as small ruminant lentiviruses (SRLV).

Lentiviruses are so-called because they are slow viral infections that persist for the lifetime of the individual and are progressively fatal with no immune recovery [apart from the vector-borne lentiviral infection of horses (EIA) in which some animals recover and become immune]. Antigenic variation in MV virus is well documented and in all lentiviral infections there is extensive mutation of the gene encoding envelope glycoproteins that allows mutated viruses to escape immune elimination. There has been intense interest in this group of viruses since the discovery that human AIDS was caused by infection with the lentiviruses of HIV-1 and HIV-2. The hallmark of lentiviral disease is a progressive multisystem chronic active inflammatory process occurring predominantly, but not exclusively, in the lungs and mammary gland, due to the action of the virus on cells of the macrophage monocyte series. Arthritis and degenerative joint disease also occur due to virus-induced pathology. The immunodeficiency state, which arises in some lentiviral diseases (such as HIV), is due to selective targeting and destruction of CD4 T cells. Additional lymphocyte subsets are targeted in feline immunodeficiency (FIV) and thus in cats the immunodeficiency state is severe. The primate lentiviruses of simian immunodeficiency virus (SIV) also resemble AIDS-like diseases in a range of primate species. Importantly, however, selective destruction of lymphocyte subsets is not a feature of MV infection since T cells are not targeted. Therefore, severe immunodeficiency does not occur and effects are restricted to cells of the monocyte macrophage series. Lentiviruses are an important group of viruses where there is no suitable laboratory animal model. Studies of naturally occurring lentiviruses, such as MV virus, are therefore of considerable importance to comparative medicine, with MV providing a unique insight into the chronic inflammatory process characteristic of all lentiviruses, uncoupled from T cell immunodeficiency. Although MV virus is commonly recognized as a prototype AIDS virus, it is important to stress that it does not infect other species such as humans.

CLINICAL SIGNS

The incubation period is protracted and most infection is subclinical. Clinical signs develop insidiously, epitomizing slow virus infection. The interval between the first introduction of infection and its overt manifestation can be as long as 10 years, by which time a high proportion of the flock is likely to be seropositive. Disease is not evident until at least 2 years of age, or more usually, 4–5 years. Maedi is the most common clinical manifestation: affected animals remain bright, alert and continue to eat but exhibit gradual loss of condition; tachypnoea and respiratory distress develop slowly and may initially only be evident on exertion. Pyrexia is not a feature unless secondary bacterial infection is present; auscultation of the thorax is generally unrewarding. Coughing and nasal discharge are occasional features; flared nostrils, neck extension and open-mouth breathing occur in more advanced cases; wool loss may be seen as debilitation progresses. Maedi is clinically much less obvious in large sheltered lowland flocks on a high plane of nutrition (as in parts of Britain), where mildly affected sheep may pass unnoticed for several months and can survive for a year or more until culled or destroyed on humane grounds. In some flocks the disease appears to persist virtually unrecognized almost indefinitely or its features are attributed to other causes.

Although maedi is untreatable and ultimately fatal, mortality rates due to uncomplicated disease are generally very low – less than 2 per cent in a heavily infected flock studied in the East Anglia region of England. Deaths are commonly associated with secondary bacterial infection, particularly mannheimiosis (formerly pasteurellosis), and there may also be an increased predisposition to other conditions such as pregnancy toxaemia. Mortality rates can be higher under harsher climatic and environmental conditions, such as those encountered in an infected flock in the north-east of Scotland [4], in which ewe mortality was 14 per cent, with associated losses in lambs due to poor viability and insufficient colostrum. However, the extent to which losses are due directly to MV virus infection or to secondary and opportunistic infections is not clear.

Visna, the nervous form of the disease, is also characterized by weight loss. It occurs much less commonly and is usually seen only sporadically in heavily infected flocks, often several years after the initial recognition of maedi. Both conditions may be present to varying degrees in the same animal or they can occur independently. Somewhat unusually, visna (rather than maedi) was the first clinical presentation of MV virus infection in two unrelated commercial flocks in Britain in 2003 [5]. Clinically, this suggested the possible emergence of an apparently neurotropic strain of virus, as was seen in Iceland during the last century. Clinical signs of visna relate to lesions localized in either the brain stem or spinal cord. Gait abnormalities of the hind limbs are the main initial feature, particularly unilateral dragging of the foot due to paresis of the affected limb, resulting in scraping of the hoof. Knuckling over and stumbling soon follow, leading to ataxia, general incoordination, hindlimb paralysis, recumbency and death. Other features include circling, progressive head tilt, fine tremor of the lips, dazed appearance, hyperaesthesia, separation from the rest of the flock and aimless wandering. Visual impairment and blindness, features not recognized until fairly recently, can also occur. Unlike maedi, visna cases usually progress relatively rapidly, resulting in death or euthanasia within a few weeks.

A slowly progressive, indurative mastitis [6] is a common feature in some heavily infected flocks but this has not been widely reported in Britain. This can be detected only by careful palpation of the udder; there are no gross changes to the character of the milk. The main consequence seems to be delayed weight gains in unweaned lambs, particularly twins and triplets. Arthritis is a rare manifestation of MV virus infection. Carpal joints are mainly affected, and joint enlargement is not usually associated with acute lameness.

PATHOLOGY

Maedi is the manifestation of MV virus infection most likely to be suspected from gross pathology. In cases uncomplicated by secondary infections, the lungs have a uniform dense rubbery texture and a swollen voluminous uncollapsed appearance; chest wall/rib impressions are sometimes evident. Pulmonary oedema, consolidation and abscessation are absent in uncomplicated cases. Despite usually weighing at least 1–2 kg, which is two to four times the normal weight (see Figure 31.1 in the colour plate section), the lungs remain aerated and invariably float in water until cases are very well advanced. Lung colour varies

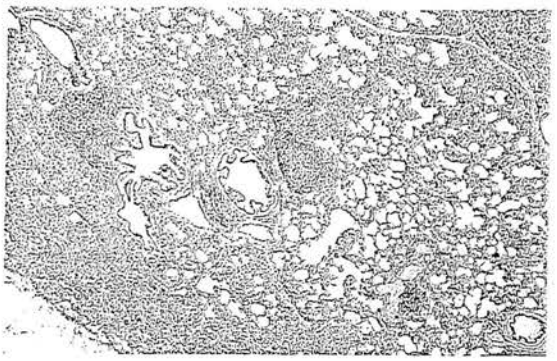

Figure 31.3: Histology of maedi lung, showing lymphoid infiltration in the septa and lymphoid nodules.

from uniform shades of dull red or pink (which can appear superficially similar to non-specific congestion) to a mottled pinkish-brown, variegated with firm greyish granular focal spots up to 5 mm in diameter. These lesions, which are visible on the surface and on cut section, are distributed throughout all lung lobes where they can extend to hard, grey confluent areas (see Figure 31.2 in the colour plate section), depending on the age of the lesions and degree of lymphoproliferative change. Associated lymph nodes are invariably grossly enlarged; the caudal mediastinal may reach up to 10 times normal size. In some affected flocks, uncomplicated maedi is the norm, whereas, in other flocks, cases usually present as mixed infection with lungworm, *Mannheimia haemolytica* pneumonia (pasteurellosis), purulent bronchopneumonia or, more notably [7], ovine pulmonary adenocarcinoma (OPA) – formerly sheep pulmonary adenomatosis (SPA). In such cases underlying maedi lesions can easily be masked and may not be detectable at routine necropsy unless suspected from the flock history. Visna lesions are located in either the spinal cord or brain and are not usually grossly apparent. When the udder is affected, the mammary glands are grossly small and firm to cut. Arthritis associated with MV virus infection produces gross thickening and sometimes a red/tan discoloration of the synovial membrane with joint enlargement, particularly of the carpus. Histological lesions [8] comprise immunologically mediated chronic active inflammatory changes, with lymphoid infiltration (Figure 31.3) and proliferation in one or more target organs including lung, central nervous system, mammary glands and joints. Main organ-specific changes consist of smooth muscle hyperplasia (lungs), demyelination (central nervous system), fibrosis (mammary gland) and proliferation of synovial membrane, and degenerative changes to the articular cartilage (joints). In advanced mixed pulmonary infections, particularly involving OPA, attribution of specific histological lesions can be problematical [3, 7].

DIAGNOSIS

Most infected sheep within a flock show few overt signs of disease but remain clinically silent virus carriers. For control purposes, it is essential that such animals are identified as soon as possible after infection. Antibody detection tests are routinely used for diagnosis and, since virus infection persists, the presence of antibody signifies current infection. The time period from infection to seroconversion is variously estimated at about 6 months [1] or from 3 weeks to several months [2] but it may take several years and some infected animals never seroconvert. It is shorter in more heavily infected flocks than those with low seroprevalence.

The agar gel immunodiffusion test (AGIDT) has been the classical gold standard test for the past 25 years but it is gradually being replaced by a range of enzyme-linked immunosorbent assay (ELISA) methods, which employ purified virus, recombinant proteins or synthetic peptides as antigens. ELISA shows good concordance with AGIDT [9]. It is increasingly recognized through flock testing regimes to be more sensitive than AGIDT and the Office International des Epizooties (OIE) has now accepted it as an alternative test for MV (AGIDT remains the prescribed test for international trade). It can detect seroconversion earlier and is particularly suitable for screening large numbers of serum samples in eradication and accreditation schemes [10]. The sensitivity of commercially available ELISAs appears to vary, however (this may be due to viral genomic heterogeneity), and ideally the antigen used for ELISA should contain epitopes present in the virus strains circulating in the population being investigated, if known [2]. In the MV accreditation scheme (Sheep and Goat Health Scheme) in Britain operated by the Scottish Agricultural College (SAC) (see 'Control and prevention' later), it is their policy to use the AGIDT for preliminary flock screening and monitoring of status, while ELISA is preferred

for within-flock eradication purposes (B.A. Synge, personal communication). ELISA has been used successfully for detecting MV antibodies in milk, which offers the potential for low-cost screening of milking sheep flocks (and goat herds for CAEV) and as a useful tool in national eradication schemes. Western blot, where available, is becoming established as a routine standard for confirming suspect ELISA results. Polymerase chain reaction (PCR) technology can be used for cases of special interest, such as when infection is suspected in a seronegative animal or in very young lambs which have received colostrum from infected ewes.

MV virus infection should always be suspected in flocks with a history of progressive weight loss, emaciation and chronic pneumonia in older ewes, or unusual nervous signs, especially where breeding replacements are purchased from sources not monitored for MV virus infection. However, maedi (although less likely with visna) can easily remain unrecognized in a flock for several years, particularly if mildly affected ewes are culled for other reasons before overt signs develop. Also, unless there is a serious flock problem, veterinary surgeons may not be consulted and, for economic reasons, necropsies are rarely undertaken routinely on older ewes. Clinically, maedi needs to be differentiated primarily from OPA, but atypical pneumonia, lungworm infection and chronic suppurative pneumonias also need to be considered. Histological examinations are essential to confirm the diagnosis. However, it should be emphasized that there can be difficulties in reaching a definitive diagnosis in the presence of secondary infection. Furthermore, the isolation, for example, of *M. haemolytica* from the lungs does not exclude the possibility of underlying MV virus infection and associated pathology. Gross examination of the lungs can provide a useful guide, particularly checking whether the weight (excluding visible trachea) exceeds 1 kg. Although clearly non-specific, this approach offers a simple preliminary screening test, particularly for lungs not exhibiting obvious consolidation or gross pathology. If maedi is suspected in home-bred sheep, the overall within-flock seroprevalence is likely to be at least about 60 per cent, with a higher prevalence in the older sheep. Hence, in most infected flocks, blood sampling just a few aged poorer ewes should be sufficient to detect at least one seropositive animal.

Visna is unlikely to be the main initial presentation of MV virus infection in a flock with no previous history, although primary visna was seen in two flocks in Britain referred to earlier [5] where the emergence of an apparently neurotropic strain of virus was suspected. Scrapie is a major differential diagnosis for visna and, significantly, some recent cases of visna seen in the UK were initially reported to the authorities as suspected scrapie. Cases also need to be differentiated from space-occupying brain and vestibular lesions. It may be necessary to differentiate visna from periparturient metabolic conditions, cerebrocortical necrosis and cerebral listeriosis, particularly if the flock has a previous history of these diseases. Definitive confirmation requires brain and spinal cord histopathological examinations, supported by serology.

EPIDEMIOLOGY AND TRANSMISSION

Following primary exposure, the virus establishes a persistent infection (by latent carriage in monocytes and macrophages) in peripheral blood. Viral expression is closely dependent on maturation of monocyte to macrophage. Variations in immune response, genetic susceptibility and virus phenotype appear to dictate what target organ system is involved and the progress of lesion development. Lesions develop over a protracted period and sheep may be relatively old in relation to their productive lifespan before overt signs appear. Despite mounting virus-specific humoral and cellular immune responses, infected sheep do not develop a protective immunity.

The main route of transmission is from an infected ewe to its offspring via colostrum (including pooled colostrum banks) and milk. Aerosol transmission between animals of all ages in close contact and over distances of several metres appears to be an almost equally significant route of spread both within and between flocks, particularly under intensive housing or grazing conditions or where concurrent OPA, which has a synergistic effect on transmission [7], is also present. Breed susceptibility and management practices also play significant roles. If naïve animals are introduced into a heavily infected flock, up to 50 per cent may seroconvert within 9 months. In the presence of OPA, seroconversion rates of 90 per cent within 18 months have been recorded: one such infected flock in England, for example, showed an

increase in seroprevalence from 4 to 93 per cent within 4 years [7]. In all endemically infected flocks, there is an increase in seroprevalence with age, from about 25 per cent seropositive at 2 years to over 90 per cent at 5 years or more.

Placentas contaminated with maternal blood, contaminated milking equipment and poor personal biosecurity practices by operatives may also contribute to the spread of infection [2]. Semen has been demonstrated to contain virus but its role in viral transmission is not known. The significance of intrauterine viral transmission remains unclear but it is generally thought that it is of minor importance compared with other routes [2].

ECONOMIC ASPECTS

The economic effects of clinical and subclinical MV virus infection are poorly understood and difficult to quantify because of the complex interaction between the virus, its host and the environment [2]. The disease develops very slowly and only about 30 per cent of infected animals ever develop clinical signs. Flock seroprevalence, genetic susceptibility, husbandry and management practices, environmental influences, intercurrent disease, culling policy and the relative value of cull ewes compared with breeding replacements are all important factors. The potential cost is inevitably much greater in pedigree flocks selling breeding stock than in commercial flocks engaged in finished-lamb production. The effects on ewe mortality, milk production and quality, lambing per cent, lamb birth weight, viability and weaning weight are all very variable. Some studies have described a weight shortfall of up to 5 kg per lamb at weaning due to decreased milk production from indurative mastitis [6], but this has not been confirmed by other workers. The effect of age is a major confounding factor when comparing the productive performance of MV-positive and MV-negative ewes because older ewes generally have a higher lambing percentage and heavier lamb birth weights regardless of MV status. Under extreme conditions, losses of 10–20 per cent of gross margin per ewe have been described in some affected flocks [4], whereas, under sheltered lowland conditions [7], the only measurable economic effect of MV virus infection is the need to cull ewes a year earlier than usual.

CONTROL AND PREVENTION

In common with other viruses of the Lentivirus subfamily, clearance of virus is never seen following either natural or experimental infection. The reasons for this include: rapid mutation of the virus envelope glycoproteins to give antigenically different subgroups of the virus; a failure to make IgG2 antibody essential for cell-mediated immunity and an inherent capacity of the virus to 'go-to-ground' as a latent proviral infection in cells of the monocyte macrophage lineage [11]. Latently infected cells do not express any viral antigens for recognition by the immune system and hence cannot be eradicated. However, they act as a reservoir of infected cells which can switch on production of fully infectious virus when they differentiate in response to inflammation. This may be why maedi is more evident in situations of poor husbandry and in association with secondary infections.

Several research groups are pursuing the possibility of controlling MV virus infection by vaccination. However, vaccination against MV can be a 'double-edged sword' and there are good experimental data to show that using whole inactivated virus will make the disease worse through the development of immunopathological lesions. To overcome this problem, novel vaccines based on recombinant viral proteins are being developed. Preliminary results show that the choice of viral antigen is critical in determining the outcome of vaccination since immunization with certain recombinant viral antigens enhances immunopathology rather than immunity.

Control programmes adopted for SRLV infection by different countries need to take into account national or regional characteristics and achievable objectives: merely reducing seroprevalence will lead to a useful decline in the incidence of clinical disease but reducing low seroprevalence to serologically negative is required to eradicate infection [2]. The potential for virus transmission between sheep and goats proved to be a significant risk factor which hindered final progress in the otherwise highly successful CAEV eradication programme in Switzerland [12]. For uninfected flocks, the first priority is to prevent the introduction of infection. This can be achieved by applying good flock biosecurity practices, including not sharing rams or mixing sheep with other flocks and maintaining appropriate segregation at

shows and sales. Quarantining and blood sampling of breeding replacements, or purchasing only from established MV virus-free sources is desirable, but may be economically viable only for pedigree flocks. In Britain, where the infection appears to be maintained within commercial flocks, strict segregation from pedigree flocks is essential. Several countries operate compulsory or voluntary monitoring, eradication and control schemes based on systematic flock serological examinations. If flock infection is detected sufficiently early, when seroprevalence is low, a simple test-and-cull programme at 3–6-monthly intervals may be successful at eliminating infection, but it is only likely to be economically feasible in valuable pedigree flocks. It is essential that lambs born to seropositive ewes are also removed. In Britain, a MV accreditation scheme is currently operated by the SAC veterinary services in partnership with practising veterinary surgeons.

Once infection is firmly established in a flock, the options are limited. Attempts to produce MV-free flocks from heavily infected flocks by snatching lambs at birth for artificial rearing or by adopting segregation and serological testing approaches have generally been unsuccessful or impractical under commercial conditions. Underestimating the significance of horizontal transmission and having inadequate separation between infected and uninfected sheep are important issues. However, a recent report [13] described the successful containment of an infected cohort of purchased sheep for two breeding seasons using rigorously enforced biosecurity practices. In many endemically infected commercial flocks operating under favourable environmental conditions, reasonably effective control can be achieved by increasing the flock replacement rate and adopting a strict culling policy for suspect clinical cases and not keeping ewes beyond 4–5 years of age. This has the effect of keeping flock seroprevalence down, thereby reducing the incidence of clinical disease. Complete flock replacement with assured MV virus-free stock, if available, is the other main option but is unlikely to be economically viable for most commercial flocks.

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Review article

Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes

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Abstract – Small ruminant lentiviruses (SRLV = maedi-visna in sheep and caprine arthritis encephalitis in goats) are distributed throughout most countries of the world, particularly Europe. Laboratories from 16 European countries established collaborations within the framework of a COST (CO-operation in the field of Scientific and Technical Research) action sponsored by the European Union in order to (i) better organize their research programmes on SRLVs and (ii) to coordinate efforts to combat these two diseases. After five years, a consensus conference – the first one in the veterinary medicine field – concluded the work of this network of laboratories by reviewing the present position and discussing three important questions in the field of SRLVs: routes of transmission, consequences of infection and potential role of eradication programmes at either a European or local level, according to the situation in each country or region. This paper brings together existing information regarding these questions and identifies areas for future research.

Maedi-visna / caprine arthritis-encephalitis virus / lentivirus / small ruminants / European consensus conference

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1. INTRODUCTION

Infections caused by small ruminant lentiviruses (SRLVs), comprising maedi-visna virus (MVV) initially isolated in sheep and caprine arthritis encephalitis virus (CAEV) initially isolated in goats, are widespread in many countries [10, 17, 26, 30, 32, 37, 40, 44, 67, 69, 97, 106, 108, 115, 127, 133, 138, 148]; SRLVs are responsible for significant economic losses that have led to the development of control programmes in Europe and elsewhere. A European Co-operation in the field of Scientific and Technical Research (COST) action was initiated in 1998 to coordinate research in 16 European countries. This review is a report of the discussions held during the final meeting of this COST[a] action; it was organized as a consensus conference for the purpose of identifying aspects of SRLV infections that are generally accepted and to discuss those that are open to debate due to a lack of scientific evidence or to regional differences.

Three questions were addressed: (i) What routes of transmission for infection by SRLVs need to mainly be considered? (ii) What are the consequences of SRLV infections? (iii) Should a control policy be implemented on a European or a local scale?

2. THE ROUTES OF TRANSMISSION

Precise knowledge of the routes of transmission is a critical component of control programmes [120].

Colostrum and milk are considered of prime importance in the transmission of

[a] http://ue.eu.int/cost

SRLVs from mother to offspring [35, 46, 55, 83, 87, 95, 115, 131]. This is also supported by the apparent success of eradication programmes in which offspring are removed at birth and reared on bovine colostrum and milk [29, 64, 115]. Particularly in sheep, contact between ewe and lamb also represents an important risk factor in viral transmission to the newborn [9, 66]. This may be explained by aerosol transmission from the respiratory tract, the lungs being a major target organ in this species. Aerosol transmission between animals of all ages in close contact and over distances of up to several meters appears to be a significant route of spread both within and between flocks and herds, particularly under intensive housing or grazing conditions [46].

The significance of intrauterine viral transmission continues to be unclear [28, 33, 34]. Published evidence suggests that intrauterine transmission may occur in up to 10% of fetuses born to infected dams [15]. The role of this route of vertical transmission is difficult to assess because the results can vary depending on the methodology employed and on the immune response to the virus. If PCR is used to demonstrate virus in the caesarean-derived foetus, the nucleotide sequences of the amplicon should be compared to the virus infecting the dam. If serology is used, the long and variable time to seroconversion may make it difficult to establish that transmission has been via the intrauterine route. Moreover, any contact with infected animals must be excluded. However, the success of eradication programmes based on removal at birth and rearing on bovine colostrum and milk suggests that intrauterine viral transmission is generally of minor importance [65].

Placentas contaminated by maternal blood may also represent a source of infection.

Semen has been demonstrated to contain virus but its role in viral transmission has not been studied [41, 100, 111, 141, 147]. However, virus-infected rams and bucks can infect females by other routes.

Transmission by contaminated milking machines or buckets is considered to be a risk factor [2, 84].

Humans may also contribute to the spread of infection by not changing clothing, boots and equipment when dealing with infected and uninfected flocks [55]. Animals such as dogs or cats are unlikely to play a role in viral transmission.

2.1. Methods of detection

A wide array of techniques is used to detect SRLV infections. These are based on the detection of either antibody or virus [75].

Serological tests include agar gel immunodiffusion (AGID), ELISAs, Western blot and radioimmunoprecipitation.

Tests for virus include isolation in cell culture and detection by cytopathic effect or antigen staining, and genome detection by PCR or RT-PCR. In situ hybridization is useful in histopathological studies [68, 122].

Although now there is no universally accepted gold standard to determine the sensitivity and specificity of the tests used for detecting SRLV infection, the success of control programmes indicates that the available tests are useful for reducing the prevalence of infection. Radioimmunoprecipitation and Western blot are considered good verification methods by most laboratories when using optimized antigen and technical protocols. Radioimmunoprecipitation is technically demanding and rarely used. Western blotting is established more widely as a routine standard for confirming ELISAs [12, 61, 137].

2.1.1. Serological tests

Some aspects specific for SRLV infections influence the outcome of serological tests. Estimates of the mean time from infection to seroconversion range from three weeks to several months although some animals may remain seronegative [70, 113, 132]. The mean time to seroconversion is

shorter in heavily infected flocks than in those with a low seroprevalence. In addition, animals with a low antibody titre may transiently become seronegative [59, 61, 78].

Genome analyses have shown that SRLVs are highly heterogeneous [6, 24, 86, 89, 98, 99, 139, 154]. This is also a notable feature of MVV and CAEV strains. Individual proteins of different SRLV strains have immunodominant linear epitopes, some of which are largely or strictly strain-specific [11, 56, 119]. Strain-specific epitopes are found not only in the more variable envelope proteins but also in the more conserved Gag-encoded proteins [56]. Current findings suggest that the immune reaction to these epitopes may have a significant impact on the sensitivity of serological tests. It is therefore advisable to use tests that can detect antibodies to antigens of strains present in the animal population under investigation.

2.1.1.1. Agar gel immunodiffusion

This test has been the classical method for the detection of antibodies to lentiviruses in domestic animals [1, 31, 39, 57, 76, 117]. It is considered to have a good specificity but is generally less sensitive than ELISA [114, 144]. It is technically simple but its interpretation, being somewhat subjective, requires experience [117, 137, 145].

2.1.1.2. ELISA

ELISAs are suitable for screening large numbers of samples and are more sensitive than AGID. An additional advantage is the quantitative readout, which permits computer-based analysis of raw data. A large number of different antigens is used to detect antiviral antibodies by ELISA. These include whole virus, recombinant proteins, and peptides containing immunodominant epitopes [13, 19, 22, 42, 48, 60, 62, 63, 72, 80–82, 90, 103, 110, 112, 114, 117, 126, 145, 151, 155]. Biphasic tests are preferable because they have a lower frequency of non-specific reactions. Such reactions may be caused by antibodies to cellular components in whole virus ELISAs, by glutathione-S-transferase, or by bacterial constituents in the case of recombinant proteins.

Recently, synthetic peptides have been used in ELISAs to detect antiviral antibodies [81]. Preliminary results look promising but knowledge of the SRLV strains present in the population being tested, as well as the selection of appropriate peptides are essential for the development of reliable tests.

2.1.1.3. Western Blot

Western Blotting is more sensitive than ELISA and is used routinely as a confirmatory test for sera that give indeterminate results in ELISA [14]. Currently, there is no standardized protocol for performance or interpretation of Western Blotting in SRLV diagnostics (e.g. reaction to one versus two or several bands, intensity of bands).

2.1.1.4. Other serological methods

Radioimmunoprecipitation and radioimmunoassay are largely limited to experimental work and these methods are not considered as routine diagnostic procedures [51, 77].

2.1.1.5. Recommendations on serology

The most sensitive and specific test should be used for SRLV serology. To facilitate large sample throughput and objective interpretation, it is advisable to use a proven ELISA. Due to the heterogeneity of viral strains, care should be taken when selecting the antigen. Specifically, the antigen should contain epitopes present in the virus strains circulating in the population being investigated. The results of the flock test should be considered when interpreting the results of individual animals [3]. Where validated,

milk may be used instead of serum for antibody detection [72, 94, 155].

2.1.2. Virus detection

Critical aspects of viral detection are (i) the body part and tissue sampled; (ii) the viral load at the time of sampling; (iii) the biological properties and genetic composition of the virus.

The most common material collected for virus detection is blood. The low viral load in blood is a major challenge for virus detection. In addition, as shown in goats experimentally infected with CAEV and in sheep infected with MVV, the viral load may fluctuate over time and may differ between individual animals [70, 156].

2.1.2.1. Virus isolation in cultured cells

This is the classical method of demonstrating the presence of a virus. The best results are obtained by co-culturing the test sample (ex: peripheral blood mononuclear cells or monocytes) with SRLV-free susceptible cells such as goat synovial membrane cells for CAEV and sheep chorioid plexus cells or skin fibroblasts for MVV [124]. Microscopic examination for the appearance of a cytopathic effect (presence of syncytia) in these sensitive cells is commonly used to confirm the presence of virus. Due to the existence of SRLVs with a low cytopathic potential, it is advisable to stain the cells for the presence of viral antigen. This may be combined with the measurement of reverse transcriptase activity and PCR [76].

2.1.2.2. PCR

Theoretically, this technique has the advantage of detecting infection in animals before seroconversion [8, 23, 68, 146, 152, 153]. For years, numerous groups have attempted to establish PCR as a diagnostic method for SRLV infection, with variable results, such as the finding of amplicons in some seronegative animals and vice versa [20]. The former may represent animals before seroconversion [68,113], or long-term seronegative carriers, while the latter suggests that the PCR protocols may have lacked sensitivity. The reasons for this may be due to a combination of low viral load and of the use of primers that failed to detect all strains of SRLVs. To overcome the latter problem, efforts should be made to determine the sequences of as many SRLV strains as possible. This is important for improving the performance of PCR as a diagnostic test and is a prerequisite for the establishment of molecular techniques to study the epidemiology of infection [85, 118, 125, 154].

2.1.2.3. Recommendations on virus detection

At present, virus detection by co-culture and PCR should not be considered as alternatives to serology, but as complementary tests for use in selected cases [47]. However, PCR protocols of use for a wide spectrum of strains and geographical areas are currently being developed. The sensitivity of virus isolation techniques by co-culture should always be confirmed by including a field strain at limiting dilution in parallel to the field sample being examined. The sensitivity of PCR can be enhanced by using nested or semi-nested methods for sequence amplification. Appropriate positive and negative controls should be included and strict control of contamination is essential. If necessary, the best control method is to sequence the amplicons obtained in positive test samples and to demonstrate that the sequence obtained is different from SRLV used in the laboratory.

2.2. The risk of live animal trading

Live animals trading is considered a major risk factor in the spread of SRLV infection between herds [25].

2.3. The role of derived products in viral transmission

As outlined in previous sections of this document, milk and colostrum as well as semen have been shown to contain virus. The placenta has also been considered in the context of the intrauterine transmission of SRLVs [2, 50]. A recent study has demonstrated the presence of CAEV-infected cells in the goat genital tract which suggests the potential role of this tract for vertical transmission of CAEV from doe to embryo or fetus [49]. Data on the virus content of ovocytes are not available.

Cheese is unlikely to be involved in viral transmission.

3. THE CONSEQUENCES OF SRLV INFECTION

"Maedi-visna" (MV) refers to pneumonia (Icelandic "maedi") and encephalitis ("visna"), which together with mastitis characterize lentiviral infection in sheep [129, 130]. The introduction of lentivirus-infected Karakul rams from Germany to Iceland in 1933 not only led to an epizootic in the indigenous breed of sheep in Iceland but culminated in the first isolation of lentiviruses and a description of the clinical signs of infection [101]. Knowledge of the viral cause of caprine arthritis-encephalitis (CAE) in goats is more recent. The disease was known to goat owners as "big knees" (i.e. enlargement of the carpal joints) long before the first isolation of the virus in the USA in 1980 from arthritic goats that had previously suffered from encephalitis [27]. In contrast to the epizootic nature of the outbreak of MV in Iceland and due to vertical transmission, CAE was originally believed to be a hereditary disease in the heavily infected regions of central Europe.

Knowledge of animal welfare and of economic consequences may determine if, and by what means, control or eradication of lentiviral infection is a priority in sheep and goat farming.

3.1. Economic consequences

The general consensus was that economic losses due to SRLV infection are significant. However, analysis of the literature indicated that information on such losses is incomplete and in some instances contradictory. The reasons for this are complex and involve the interaction of SRLVs with their hosts as well as management factors [136]. Factors influencing economic losses are the following: (i) clinical disease caused by SRLV infection develops slowly; (ii) only about 30% of infected animals develop clinical disease; (iii) disease signs and economic losses are related to seroprevalence, with no signs evident in herds with low prevalence of infection; (iv) genetic factors influence the extent of disease (in sheep, susceptibility to disease may be influenced by breed whereas in goats this is associated with certain major histocompatibility haplotypes present in all breeds investigated); (v) certain management practices such as milk production, or housing and keeping animals in crowded conditions increase the rate of viral transmission and hence seroprevalence [73]; (vi) disease signs may be influenced by concurrent infections which may vary with different geographic areas and management practices. For example, co-infection by maedi visna and Jaagsiekte retrovirus, which causes ovine pulmonary adenocarcinoma, a contagious lung tumor, results in aggravated disease [52, 149].

Analysis of the literature showed that SRLV infection influences the following production-related parameters.

3.1.1. Milk production

Milk production can be decreased by an estimated 10% – due to indurative mastitis, which is often a feature of SRLV infection [79, 135, 136]. Precise data are unavailable because standardized procedures to assess

milk production are less common in small ruminants than in dairy cattle. It should be noted that reduced milk production is influenced by husbandry practices [109]. The lower weight gain in offspring born to infected dams and demonstrated in most studies, indicates that losses have an impact on the productivity of the following generation [54].

3.1.2. Birth weight

There is evidence [54] or no evidence [7, 136] that SRLV infection decreases the birth weight. Low birth weight is known to negatively affect the development of lambs and kids, thus lowering productivity, particularly in heavily infected flocks [74].

3.1.3. Weight gain

Infection with SRLVs decreases the weight gain of lambs (presumably due to depressed milk yield from indurative mastitis [105]) [38, 45, 54]. Losses may range from 0.3 to 3.0 kg per lamb at weaning [7, 74, 104, 105].

3.1.4. Mortality rates

Mortality due to SRLV infection is low in enzootic areas, but is strongly influenced by concurrent disease, husbandry, nutrition and environmental factors. Observations made during the epizootic of MV in Iceland show that mortality may reach 20–30% in newly infected animals [128, 129]. SRLV infection decreases the average life span of animals due to decreased productivity. The effect of premature culling, typically a year earlier than uninfected animals, is generally underestimated [5]. It also affects productivity indirectly by decreasing the average number of offspring born to each generation. Related to the decrease in milk production is an increase in lamb mortality.

Alternatively feeding with bovine colostrum may increase lamb mortality due to haemolytic anaemia if the source cow is not well chosen.

An element that has not received much attention in the past is the impact of SRLV infection on the quality of production. Infection may negatively affect quality by increasing somatic cell counts in the milk. As consumers increasingly demand products from animals with the best health status possible, animals persistently infected with SRLVs may not meet this requirement.

3.2. Animal welfare consequences

The term "animal welfare" is used in relation to aspects of SRLV infection that directly affect the quality of life of the animals. Factors that primarily affect productivity without causing obvious pain or discomfort are not included in this definition. It is obvious that this distinction is difficult and may be subject to cultural differences and individual opinions. However, the consensus was reached that SRLV infection has a significant impact on animal welfare because the quality of life of clinically affected animals is reduced due to pain and disability.

Originally, MVV and CAEV were considered two distinct yet closely related viruses which infect sheep and goats, respectively. As will be seen below, these viruses are not strictly host-specific. It is the consensus that MVV and CAEV share the same target organs. Both viruses affect the mammary gland equally [143]. However, their predilection for some other organs is different. Lung and brain are classical targets for MVV [93], whereas joints appear to be the most commonly affected organs in CAEV infection. In contrast to MV, encephalitis is typically – although rarely – observed in young kids. Although weight loss and ill thrift are features of SRLV infection, actual emaciation is less common. When considering SRLV infection, it is important to emphasize that usually only a minority of infected animals develop clinical signs. There was consensus that immunosuppression is

not a feature of SRLV infection probably because, in contrast to the immunodeficiency-causing lentiviruses, SRLVs do not infect lymphocytes [16, 53]. Genetic factors of both the host (breed- and family-associated disease susceptibility) and the virus (virulence) influence the outcome of SRLV infection [36, 123].

3.3. Virulence of SRLVs

"Virulence" refers to the ability of a pathogen to cause disease. Information on the virulence of SRLVs is scant. It was noted in Iceland that certain strains of MVV are neurotropic and this was recently found to be correlated with a change in the LTR region of the viral genome [4]. Lentiviruses are genetically unstable, with a high rate of genome evolution typical of RNA viruses and subject to genetic recombination with viruses of identical or closely related species. However, there is no evidence from clinical and epidemiological data that SRLVs may evolve rapidly in vivo, nor is there evidence for recombination between MVV and CAEV in vivo. In fact, evolution of SRLVs in infected animals is approximately 10 times slower than that of HIV in humans. This may be because the viral load of SRLVs in infected animals is lower than that of immunodeficiency-causing lentiviruses. Therefore, the latter should not be regarded as models for SRLVs in this regard [91]. There is also no evidence that the higher disease incidence observed in heavily SRLV-infected flocks may be related to a higher virulence, because reducing the seroprevalence decreases the prevalence of disease.

3.4. The risk of the transmission of SRLVs to other species

Early reports suggested that MVV and CAEV could be transmitted experimentally to goats and sheep, respectively, but concluded that such a transfer was unlikely under natural conditions. However, in the light of several recent reports this conclusion cannot be upheld. Using phylogenetic sequence analyses, several reports clearly demonstrate that MVV can infect goats, and CAEV can infect sheep [18, 150]. It is unclear how infection crosses the species barrier [21, 71, 102, 116, 142]. The most likely risk factors are ingestion of virus-contaminated ovine colostrum and milk by goats and vice versa, as well as a close contact between the species in overstocked barns. It is unclear at what rate these viruses spread within the "heterologous" host population, but there was consensus that SRLV infection across the species barrier must be taken into account in control programmes. Specifically, the same regulations should apply to both MV and CAE, and control programmes that only target sheep or goats alone are no longer acceptable.

There is serological evidence of SRLV infection in wild ruminants such as moufflon, ibex and chamois [92]. The prevalence of SRLV infection is difficult to determine in these species. It is also unclear whether the viruses responsible for the SRLV-specific antibodies in these species originated from domesticated small ruminants, or form a cluster of their own. Preliminary evidence suggests that SRLVs in these species may be different from MVV and CAEV (Chebloune Y., personal communication). To date, no such viruses have been isolated from sheep and goats, which suggests that "exotic" SRLVs are not a cause of concern for domestic small ruminants. Moufflon and ibex are the closest relatives of sheep and goats respectively, which may explain the existence of SRLVs in these species [58]. There is no evidence that, under natural conditions, SRLVs can infect species such as cattle that are not closely related to sheep and goats.

Recently, the zoonotic potential of viruses of domestic animals has been intensively scrutinized particularly because of the zoonotic origins of HIV and new variant Creutzfeldt-Jacob disease [96]. There was a clear consensus that transmission of these

agents must not be confused with SRLVs. This consensus was based on the following arguments: (i) there is no evidence that diseases caused by SRLV in sheep and goats are new; (ii) although small ruminants have been domesticated for several thousand years and have lived in close contact with man, there is no epidemiological evidence, either clinical or serological, for SRLV infection in humans. A recent study has shown that in some goats infected with CAEV, antibodies can recognize linear epitopes on HIV gp120 [88].

In summary, it was the view of the conference that, based on the present knowledge, SRLV do not present a risk to man.

4. THE NEED FOR A CONTROL POLICY TO BE IMPLEMENTED ON A EUROPEAN OR LOCAL SCALE

Due to the increased role of free trade of live animals, embryos and semen in the EU, this issue is of key importance in national or regional control programmes. Several parameters must be considered if the best strategy is to be chosen, as indicated below.

4.1. The size and distribution of small ruminant populations in Europe

In December 2000, the sheep population of the EU was 96.5 million and the goat population 11.5 million. The population varied markedly in different countries. For instance, there were nearly 30 million sheep in the UK and 24 million in Spain, whereas the number in Germany was 2.15 million. Nearly half of the 11.5 million goats were in Greece and only 77 000 in England.

4.2. The incidence of SRLVs in different regions

In contrast to the census data, information on the prevalence of SRLVs in the sheep and goat population of Europe is less updated. In some countries, information dates from 2000 whereas in others the latest figures are from 1978. Assuming an average small ruminant generation time of four years, the latter information reflects the prevalence in animals six generations ago. Furthermore, in view of differences in sensitivity and specificity of diagnostic tests and varying sampling methods used, the data on the prevalence of SRLVs in different countries cannot be directly compared at present. However, the data clearly show that no European country can be considered to be free of SRLV infection as defined by the Office International des Epizooties (< 1% of herds infected, probability 99%). A notable exception is Iceland which eradicated MV following the epizootic initiated by the importation of MVV-infected rams.

4.3. Transmission between different regions

Transmission of SRLVs has occurred repeatedly between different regions. The export of European breeds of goats and sheep has resulted in the spread of SRLVs to various parts of the world [25, 140]. In contrast, certain indigenous goat breeds that have had no contact with imported goat breeds, such as Toggenburg and Saanen in Europe or Criollo in Mexico [140], are free of CAE. Phylogenetic evidence for the introduction of SRLVs via live animal trade is provided by the similarity of the South African sheep lentivirus with European strains such as the Scottish EV1. This reflects the historical role played by imported European sheep in the establishment of the South African sheep industry. The best known example of importation of disease via live animal trade is Iceland. SRLVs were also transmitted via live animal trade from Denmark to Norway, Scotland to Canada [44], England to Hungary [138], Holland to France and Sweden to Finland.

4.4. The results of the various eradication schemes

Control programmes have been initiated in many countries [120]. The European pioneer in the control of SRLVs was the Netherlands, where the eradication of MV in breeding flocks was initiated in the early 1980's [65]. This successful programme provided much information on the methods that could be applied to control SRLVs, not only in sheep but also in goats. Similar programmes were introduced in France, Italy, Germany, Spain, Finland and Switzerland [107, 134]. The removal of serologically positive animals, as well as the separation of lambs and kids of seropositive dams immediately at birth for rearing on serologically negative or bovine colostrum have been important features of successful CAE eradication programmes [9, 121]. The separation of seronegative from seropositive flocks and the strict control of live animal trade are other important measures adopted. The prevention of contact is also necessary during transhumance. This husbandry practice, in which livestock are moved to another climatic region such as grazing areas in the mountains in summer, plays an important role in some European countries.

All programmes were initiated voluntarily and highlighted the crucial role played by motivated sheep and goat owners as well as their organizations [43]. Official health authorities were more reluctant to introduce SRLV controls. The main reason was the cost and the lack of appropriate legislation. In Switzerland, CAE eradication started voluntarily in 1984 when it was realized that the seroprevalence was 60 to 80%. Federal, and in some instances, regional authorities supported the efforts by funding research projects for studying the epidemiology and improving diagnostic methods. Since 1998 "CAE-free" status has been legally recognized. Seroprevalence in the goat population has decreased to around 1% and disease is no longer seen.

5. FINAL RECOMMENDATIONS

5.1. Recommendations on the policy of SRLV control

SRLV infections are a concern for all European countries, irrespective of the current prevalence. Those countries with a very low prevalence aim to maintain this status or even become officially free of infection, while others aim to improve the situation by reducing the seroprevalence.

The consensus reached was the following. Europe should embark on a control programme with the aim of eventually eradicating the virus. The control programmes implemented in the different countries should take into account the national and regional differences and aim to maintain the gene pool of sheep and goat breeds in Europe. The stages of SRLV control leading to eradication are the following: (1) determine the prevalence of SRLVs by surveys and other sources of data; (2) reduce high seroprevalence to low seroprevalence thus decreasing the prevalence of disease; (3) reduce low seroprevalence to serologically negative thus eradicating disease; (4) consolidate the serologically negative status and eradicate the virus.

Each participating country should establish a national SRLV reference laboratory. The main tasks of these laboratories should be to: (1) advise on the national strategy for SRLV control adapted to the epidemiological situation and to the regional conditions; (2) decide on the diagnostic tests for the regional laboratories; (3) decide on the status of results that cannot be resolved by the regional laboratories; (4) organize national ring tests to bring the regional laboratories to the same level of quality; (5) advise the national and regional authorities responsible for animal health on all matters relevant to SRLV control, including the information policy for the goat and sheep farming industry; (6) participate in ring tests organized by the conference of the national reference laboratories within Europe; (7) participate in

the European conference of the national reference laboratories (see below).

The individual programmes should include the following points: (1) MV and CAE should not be treated as separate entities but combined; (2) SRLV-infected and -free flocks should not be kept on the same farm; (3) animal traffic should be regulated; (4) testing should be performed by accredited laboratories.

Individual countries should draft legislation supporting the eradication.

The EU should support the eradication programme by: (1) sponsoring research aimed at improving knowledge on pathogenesis, epidemiology, prophylaxis and diagnostics of SRLV infection; (2) sponsoring periodic meetings of the national SRLV reference laboratories (the conference of national SRLV reference laboratories); thus (3) facilitating exchange of knowledge and harmonization of diagnostic procedures which are essential for open trade in Europe.

5.2. The public and control of SRLV infections

Sheep and goat owners are mostly unaware of the role of SRLVs in animal welfare and economics of sheep and goat farming. Countries are encouraged to pursue an active information policy through pre-existing animal health information channels. Researchers should support this by advising the authorities on the pertinent aspects of SRLV infection and by lecturing and publishing in periodicals produced for sheep and goat farmers. Ideally, articles should be co-authored by farming leaders. The style should be easy to read and stimulate interest and any statements that might suggest a zoonotic dimension of SRLV infection should be avoided.

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Dandy-Walker malformation in lambs

SIR, – During the past two years we have received reports of multiple cases of hydrocephalus in lambs in three unrelated flocks.

Subsequent pathological examinations have revealed that these were actually cases of Dandy-Walker syndrome. This is a rare congenital brain and skull malformation of unknown aetiology that is well recognised in humans. A detailed report is in preparation.

In the meantime, with the lambing season currently underway, practitioners encountering suspected outbreaks are requested to contact their local veterinary investigation centre.

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Multiple cases of Dandy-Walker malformation in three sheep flocks

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Veterinary Record (1994) **135,** 163-164

THE Dandy-Walker syndrome is a rare congenital brain and skull malformation of unknown aetiology that is well recognised in man (Hart and others 1972). It comprises hydrocephalus, cystic enlargement of the roof of the posterior aspect of the fourth ventricle and agenesis or hypoplasia of the cerebellar vermis. A similar condition has been described in dogs and sheep (Pass and others 1981) and in calves (Jeffrey and others 1990). In marked contrast to these sporadic cases featured in the literature, this present communication describes distinct outbreaks in three apparently unrelated flocks of sheep – all of pedigree Suffolks – located in different parts of England and Scotland.

The largest outbreak (farm A) was in a 140-ewe flock which began lambing in early February 1992. A total of 17 lambs were born with enlarged domed skulls (Fig 1) from the first 60 ewes to lamb over a 17-day period. One further, much less severe, case occurred during the ensuing week but lambs born subsequently were all normal. Most of the affected lambs were born dead: the few born alive died a few hours later. Cases included twins in which either both lambs or only one was affected – the unaffected twin subsequently grew normally. The inevitably high incidence of dystocia frequently necessitated the splitting or crushing of the enlarged heads to enable delivery to take place. Further inquiries into the history of this flock revealed that several similarly affected lambs had been born in 1990 and 1991.

The second outbreak (farm B) occurred in January and February 1993 and was reported in a letter to *The Veterinary Record* as multiple cases of hydrocephalus (Miller 1993). However, subsequent histopathological examination of a brain from this outbreak confirmed that this was also an outbreak of the Dandy-Walker syndrome. Farm B was a mixed holding which included 22 pedigree Suffolk ewes, 40 blackface ewes and a few Texels. Lambing began in early January 1993. Sixteen lambs from the Suffolks exhibited the domed skulls characteristic of the Dandy-Walker syndrome, resulting in dystocia problems as described for farm A. No cases occurred in lambs from the other breeds. As with the previous flock, cases among twins and triplets occurred in varying combinations with no set pattern.

The third incident (farm C) also occurred in January 1993 and involved a 60-ewe pedigree Suffolk flock in which three ewes produced affected lambs including cases in which only one of a pair of twins was affected. The ewes involved were from a group of 20 all served exclusively by the same purchased ram. Further inquiries revealed that he had sired three similarly affected lambs during the previous season (1991-1992) which was his first year of extensive use. In that lambing season, one case suspected during routine scanning at mid-pregnancy was subsequently confirmed at term when dystocia necessitated a caesarean section.

Tissues and body fluids taken from affected lambs on farms A and C were tested for evidence of infection with border disease virus: virus isolation, antigen detection and serological examinations all gave negative results. Pathological investigations were undertaken on the brains of two affected lambs from farm A and one each from farms B and C. All brains had severe dilation of the ventricular systems including the lateral ventricles, third ventricle and the fourth ventricle. The cerebellum in each case was abnormal with no grossly visible vermis (Fig 2). The lateral cerebellar hemispheres were joined normally to the cerebellar peduncles. The left and right hemispheric lobules were juxtaposed mid-line but were not otherwise connected. Substantial fragments of a attenuated membrane were attached to the rostral and caudal poles of the cerebellar lobules and to the anterior and posterior medullary velum. This membrane had probably bounded a cyst-like cavity of the intact brain within the calvaria. Histologically the cerebellum of one lamb had an irregular internal granule cell layer with some loss of neurons; the other two brains had frequent corpora amylacea in the cerebellum, medulla or cerebral cortex. Hydrocephalus with agenesis of the cerebellar vermis are features considered to be consistent with Dandy-Walker-like malformations.

The affected farms were visited in an attempt to determine whether there were any common epidemiological factors involved in the outbreaks. The flocks all lambed early – in January or February – but were otherwise unremarkable, adopting conventional systems of husbandry and disease control including the use of anthelmintics, insecticide dips and vaccines. Whereas there appeared to be a genetic component to cases on farm C, no such relationship was detected in the other two flocks: affected lambs were born to apparently unrelated rams and their dams were from several different sources. Full tracing of parentage was not possible, however. On farm A, the flock had been subdivided into f

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FIG 1: Affected lamb showing the typical domed skull associated with the Dandy-Walker syndrome

FIG 2: Transverse section of brain showing agenesis of the cerebellar vermis

groups before tupping, each in a different field with a different sire. The breeding records indicated that the birth of Dandy-Walker lambs was associated with only two of these fields and their corresponding rams. A careful search of all four fields revealed several recognised poisonous plants but there was no evidence to incriminate any of the species recorded by Humphreys (1988) as being associated with teratogenic effects.

The aetiological agent involved in these outbreaks could not be established from these investigations. The fact that cases were confined to Suffolks may have been coincidental but it was also possible that a breed predisposition or genetic cause was involved. Exposure to an unidentified teratogen during early pregnancy could not be excluded.

Acknowledgements. – The authors thank the flock owners and veterinary surgeons involved in these outbreaks for their cooperation and assistance with these investigations. Virological examinations were performed by Penrith Veterinary Investigation Centre.

Addendum

Earlier this year a short letter by the authors (*VR*, February 5, p 51) alerted practitioners to this condition and requested notification of suspected outbreaks to the local veterinary investigation centre. Reports of further suspected outbreaks in England, Scotland and Wales were subsequently received – all in pedigree Suffolks or Suffolk crosses. Pathological examinations on a total of 12 brains submitted from seven affected farms confirmed the diagnosis of Dandy-Walker syndrome. This additional information, which was not available when this communication was submitted for publication, now suggests that this is an inherited con-

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RHOMBENCEPHALIC MALFORMATIONS AND CONGENITAL HYDROCEPHALUS IN SUFFOLK LAMBS

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Abstract
(Materials, Methods, Results, Discussion)

Congenital hydrocephalus is emerging as a significant cause of lamb mortality in flocks of Suffolk sheep in the UK. The incidence is not known but cases have been confirmed in 12 flocks. Skulls and brains from 6 hydrocephalic neonatal Suffolk lambs were radiographed and dissected, and compared with 2 control neonatal Suffolk lambs. Standardised measurements of cranial vault, rhombencephalon and their relative positions were obtained. In 3 lambs, severe hydrocephalus was associated with marked dilatation of the entire ventricular system, including fourth ventricle, cystic dilatation of the posterior fossa, agenesis or severe hypoplasia of the cerebellar vermis, and elongation of the medulla between the caudal cerebellar peduncles and obex. Fragments of membrane stretched between the cerebellar hemispheres and in one lamb contained nodules of cerebellum. Three lambs had moderate hydrocephalus accompanied by tentorial herniation of the cerebellar vermis, elongation of the medulla oblongata and enlarged retrocerebellar subarachnoid space. It is concluded that severity of hydrocephalus is related to the extent of the rhombencephalic anomalies. In lambs with severe hydrocephalus, malformations include those of the Dandy-Walker malformation (cystic enlargement of the posterior fossa, dilatation of the fourth ventricle and agenesis or dysgenesis of the cerebellar vermis), plus consistent elongation of the medulla oblongata. The combination of rhombencephalic malformations in lambs with moderate hydrocephalus appears to be a previously unrecognised developmental disorder.

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Ryegrass staggers

SIR, – The Veterinary Investigation Service's disease surveillance reports for August (*VR*, September 30, p 336) and September (*VR*, October 21, p 421) referred to outbreaks of ryegrass staggers in cattle and sheep. There have been reports from various parts of Britain including Kent, Essex, Somerset, Hampshire, Wiltshire, Worcestershire, Gloucestershire, Nottinghamshire, Yorkshire, Shropshire and Gwent. A significant upsurge in cases coincided with the 1976 drought although during the intervening years the condition was encountered sporadically by the veterinary investigation centres (VICs), invariably following dry spells. The purpose of this letter is to remind veterinary surgeons in practice of the main features of this unusual condition. Much of the information concerning recent outbreaks was kindly provided by colleagues at other VICs.

Most of this year's outbreaks involved sheep – either ewes or fattening lambs – but there were three reports of the condition in cattle, where the age range was from six months to adult. Morbidity was difficult to estimate because signs were not evident in all animals at once and mainly developed when they were disturbed. Generally, 15 to 80 per cent of animals in a group were affected to some extent. Clinical signs included affected animals lagging behind the others, hindlimb ataxia and incoordination, a wide-based stance, stiff-limbed action, tremors and collapse. Occasionally, sheep exhibited 'rabbit hops'. A statuesque posture such as 'dog sitting' was sometimes adopted; other cases developed lateral or posterior recumbency with legs outstretched, usually lasting for only a few minutes. There was almost invariably no mortality although, in one incident, five lambs drowned in a dyke. Bloat was another potential problem if recumbency was prolonged. The worst affected animals took up to a week or more to recover but most outbreaks ceased soon after the stock were removed from the affected pasture.

It is now well established that ryegrass staggers is a neurotoxicosis of animals grazing perennial ryegrass (*Lolium perenne*) infected with a seed-borne endophytic fungus *Acremonium lolii*. Endophyte-infected seed confers some protection to the sward against insect pests and for this reason in New Zealand its presence is encouraged despite the endemic occurrence of ryegrass staggers. Although commercial ryegrass seed presently used by farmers in the United Kingdom is largely endophyte-free, the infection is common and widespread in long-established permanent pastures even when ryegrass is only a minor component, as was the case with several of the recent outbreaks.

Ryegrass staggers occurs seasonally, predominantly in late summer and autumn when grass is under drought stress. The lack of herbage forces animals to graze down to ground level thus ingesting a high proportion of stem-base material where concentrations of the causal alkaloid lolitrem B are highest. Interestingly, most of the recent cases in Britain occurred at the end of August or in early September, coinciding with heavy dews and rainfall at the end of the drought period. There were fewer reports of the condition occurring during the drought itself. Presumably the leafy regrowth of pasture was attractive to stock, leading to increased ingestion of neurotoxin.

Diagnosis of ryegrass staggers is based mainly on clinical history although the presence of endophyte infection in plants can be detected by microscopic examination of stained strips of epidermis removed from the stem-base of the sward. This examination is available at VICs. Brain histopathology may also be helpful particularly for differential diagnosis.

When ryegrass staggers is suspected it is recommended that stock are moved to another pasture; however, it should be noted that the neurotoxin can persist in hay and possibly in silage. VICs are always interested in receiving reports of outbreaks, and assisting veterinary practitioners in investigations where appropriate.

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'Kangaroo gait' in ewes

SIR, – We wish to report histologically confirmed cases of 'kangaroo gait' in three unrelated flocks in different parts of England in 2006. The Veterinary Laboratories Agency (VLA) is also aware of several anecdotal reports of the condition this year from veterinary surgeons in general practice. 'Kangaroo gait' was first described in Britain in 1984 by Duffell and others (1986) and followed earlier reports from New Zealand (Moffat 1978). Although uncommon, there have been occasional accounts of the disease during the past 20 years, including Clements and others (2002), but the underlying aetiology still remains obscure.

'Kangaroo gait' is a disorder of ewes typically characterised by knuckling of both front fetlocks combined with an unusual propulsive method of locomotion using the hindlimbs. It has been associated with a bilateral peripheral neuropathy of the radial nerves, although this lesion may not always be present (Barlow and Greig 1986, Clements and others 2002). Cases have mainly been reported in lactating ewes several weeks after lambing, but the condition has also been described in pregnant ewes. Most affected animals appear to recover by weaning. They are bright and alert but reluctant to move. Without veterinary examination and peripheral nerve histopathology it is difficult to know whether cases suspected by the flock manager are classical 'kangaroo gait' or an unrelated condition.

Flock 1 (in Suffolk) comprised 1300 Scottish mules on permanent lowland pasture, part of which was managed under a stewardship scheme to conserve traditional grasses. The owner reported eight suspect cases in lactating ewes of varying ages, one to two months after lambing, between the beginning of April and the middle of May. Apart from one that deteriorated and was euthanased and another submitted for postmortem examination, all cases recovered within a few days following removal from the pasture and treatment with minerals and antibiotics. This suggested that they may not all have been typical 'kangaroo gait' associated with radial neuropathy.

In flock 2 (Somerset), five typical cases occurred from 100 Suffolk ewes of varying ages between March and May on permanent pasture. Four recovered by weaning and one was euthanased on welfare grounds because of illthrift and persistence of clinical signs of kangaroo gait.

In flock 3 (Staffordshire), eight of 120 Texel ewes on permanent pasture were affected by bilateral forelimb knuckling and crossover, including one young ewe in which only one forelimb was affected. The first case was observed a week before lambing and the others developed up to three weeks after lambing in March. All affected ewes improved but none was considered to have recovered completely two months later.

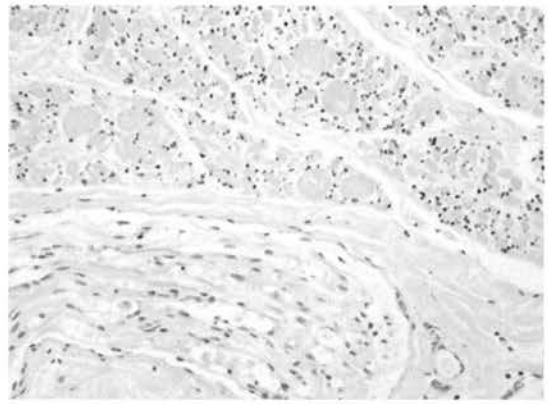

FIG 1: Denervation atrophy in the triceps brachii muscle and Wallerian degeneration in the adjacent branch of the radial nerve

Postmortem and detailed histological examinations (including brain, spinal cord and selected peripheral nerves) on an affected ewe from each flock revealed only radial neuropathy characterised by Wallerian degeneration in all cases (Fig 1) accompanied by mild/marked subperineurial loose fibroblastic/myxoid proliferation in two sheep. Further examinations in two ewes found denervation atrophy of the triceps brachii and extensor carpi radialis muscles similar to that reported by Duffell and others (1986) (Fig 1) and no significant lesions in the median, ulnar, sciatic and suprascapular nerves apart from the suprascapular nerve in one ewe, in which changes similar to those in the radial nerve were present. There were no significant lesions in the brain or spinal cord; immunohistochemical examinations for scrapie and atypical scrapie carried out on sections of obex and cerebellum from all three ewes showed no evidence of disease-specific prion protein.

In all three flocks, clinical signs of 'kangaroo gait' were observed for the first time in 2006. Ewes and lambs were turned out shortly after lambing in each flock. In flock 1, the only significant difference in management in 2006 compared with previous years was that ad libitum silage, fodder beet and proprietary concentrates were additionally provided from the beginning of March because of prevailing cold weather with a shortage of grass, heavy frosts and a cold wind. Similar weather conditions and lack of grass were noted in flock 2 but there were no significant management changes. In flock 3, the farmer also thought that body condition and fleece quality were poorer than in previous years because of slow growth of early grass and lack of grazing.

Apart from the lack of grazing, follow-up inquiries did not indicate any obvious epidemiological similarities between the three affected flocks. A toxic or metabolic cause seems unlikely to cause preferential involvement of the radial nerve. O'Toole and others (1989) suggested that severe bilateral compression of a proximal radial nerve site could explain the radial neuropathy in 'kangaroo gait'. Rooney (1963) noted that hyperextension and abduction of the forelimb in horses caused marked tension in the radial nerve, pressing the nerve firmly against the caudomedial aspect of the shoulder joint possibly resulting in spontaneous radial paralysis. The nature of the subperineurial loose fibroblastic/myxoid proliferation observed in two ewes in the present study is reminiscent of the composition of Renaut bodies, and it is of interest that O'Toole and others (1989) found numerous Renaut bodies in one of their cases of 'kangaroo gait'. Although the clinical significance of Renaut body formation is debated, it is notable that they formed at the site of maximum compression of the peripheral nerve in rats (Ortman and others 1983).

We would welcome comments and observations from colleagues on the occurrence of 'kangaroo gait' and possible predisposing environmental factors or management changes coinciding with the appearance of the condition in flocks for the first time this year.

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Generalised caseous lymphadenitis

SIR, – Caseous lymphadenitis (CLA) is currently a disease of much concern among sheep veterinarians (Baird 1997, Rizvi and others 1997, Winter 1997). The most commonly reported signs have been single or multiple superficial abscesses, usually on the head. Systemic CLA does not appear to have been reported in the UK. We would like to describe two cases of CLA with significant internal spread.

At the Royal Veterinary College a pedigree Suffolk ram was presented with a submandibular swelling of at least two weeks duration. A needle aspirate contained green 'pistachio-coloured' pus, which on culture yielded *Corynebacterium pseudotuberculosis*, the causative agent of CLA. The animal was euthanased. Abscesses were found in both submandibular lymph nodes, both prescapular nodes, in the bronchial and mediastinal lymph nodes, and in the lung parenchyma. Culture of the lung again yielded *C pseudotuberculosis*.

Despite this ram being in apparently good health, it exhibited multiple systemic abscessation. The prescapular lymph nodes were difficult to palpate, even postmortem. Thus the swelling could have easily been mistaken for a 'routine' sheep abscess and ignored or, worse still, lanced and expressed. This would have greatly increased the risk of contaminating the environment – *C pseudotuberculosis* may last up to eight months in soil (Brown and Olander 1987).

To avoid such contamination, the ram was euthanased. On farms with no previous history, even in cases where infection only appears superficial, euthanasia is the best option. This is because treatment of CLA, even in superficial cases, is unrewarding. For example, at the Royal Veterinary College we treated a ewe with large bilateral prescapular abscesses by lancing, flushing (with povidone-iodine and peroxide) and high dose penicillin. After eight weeks, although the swellings had greatly reduced in size, *C pseudotuberculosis* was found at necropsy in both lymph nodes (though nowhere else in the body).

At Cambridge veterinary school, CLA was found in another pedigree ram that had died suddenly. On postmortem examination it was found to have pituitary and internal lymph node abscesses containing *C pseudotuberculosis*. In the same flock, a ram had a poll abscess from which *C pseudotuberculosis* was isolated.

Internal abscessation can also be found in animals with no external clinical signs. At the Royal Veterinary College we isolated *C pseudotuberculosis* from inguinal lymph node abscesses in two apparently normal Welsh Mountain ewes, bought at market for a postmortem class.

CLA is a potentially serious and insidious disease, with zoonotic implications, the true incidence and severity of which is probably greatly underestimated. Veterinary surgeons connected with the sheep industry, particularly pedigree flocks, should be aware of the condition and its implications.

CLA may now be endemic in many areas of Britain (Rizvi and others 1997, Smith and others 1997); in the south east we are aware of at least five infected flocks, all of which buy and sell stock. We are also informed by shepherds that healed abscesses on the heads and necks of pedigree sheep at shows are not uncommon.

Shearers seem generally unaware of the potential problems of CLA. We know of one farm, with no history (as yet) of the disease, whose sheep were sheared immediately after those of a farm with CLA, without disinfection; and of another that had CLA abscesses in 20 ewe-lambs six weeks after shearing. Strict hygiene precautions should always be taken before, during and after shearing.

Vigilance is paramount in preventing the spread of this disease. All purchased sheep should be carefully examined for head abscesses (especially healed ones) and enlarged lymph nodes. Suspect sheep should be rejected; however, it is important to realise that CLA abscesses are not always external and could therefore be missed. Rejection of the whole batch where one sheep is suspect may be necessary.

Examination before shearing is important. Ideally, suspect sheep should be shorn last, in an area away from the rest of the flock. Bacterial culture is the only appropriate confirmatory test as no serological methods have as yet been shown to be reliable in the UK.

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Ulcerative vulvitis and balanitis in sheep flocks

G. C. PRITCHARD, S. F. E. SCHOLES, A. P. FOSTER, E. S. E. MITCHELL, J. LAWES, G. IBATA, M. BANKS

Outbreaks of ulcerative vulvitis and balanitis occurred in three commercial sheep flocks in England and Wales. Between 29 and 44 per cent of the ewes were affected; most of the lesions resolved in three weeks. Pathogens such as mycoplasmas, which have previously been associated with these conditions, were not detected despite using improved laboratory techniques. In one of the flocks, ovine herpesvirus type 2 was detected by PCR in the blood of two acutely affected ewes, from the vulval ulcers of one of them, and from the penis of an affected ram.

THERE have been several reports of ulcerative balanitis and vulvitis occurring in sheep flocks in the UK and elsewhere. Greig (2007) divided them into four main entities: venereal parapoxvirus (orf) infection; enzootic posthitis (pizzle rot) caused by *Corynebacterium renale* or other diptheroid organisms; a mycoplasma-associated vulvovaginitis (related to *Mycoplasma mycoides* subspecies *mycoides*); and a condition of unknown aetiology. Other organisms variously isolated from the lesions have included *Streptococcus zooepidemicus* (Dunn 1996), *Histophilus ovis, Actinomyces pyogenes* and the mycoplasmas, *Mycoplasma fermentans* and *Mycoplasma bovigenitalium* (formerly *Mycoplasma* ovine group 11) (Nicholas and others 1998, Ayling and others 2004). This paper describes three outbreaks that were investigated during the autumn and winter of 2006, including a particularly severe outbreak in a lowland flock in East Anglia from which ovine herpesvirus type 2 (OHV-2) was detected in blood and vulval and penile lesions.

MATERIALS AND METHODS

Case histories

Flock 1 The outbreak began in late October 2006 in a long established 450 ewe commercial lowland flock of Suffolk mules and Texel crosses in coastal Suffolk. The sheep were grazing on 100 acres of permanent marshland pasture in an isolated location, and had no direct contact with other livestock, apart possibly from feral deer. A herd of 420 suckler cows was kept on another part of the farm, and the sheep sometimes grazed on the same pasture after the cattle, but the only time they may have been in direct contact was when they were housed during lambing. About 25 to 30 per cent of the breeding flock was replaced each year (by shearlings) but the only animals added during the previous four months were five shearling Suffolk rams purchased from a single flock in south-east England through a livestock dealer. These arrived at the beginning of October and were immediately mixed with the six resident rams and subsequently put in with the ewes without going through a quarantine period.

After the rams had been serving the ewes for three weeks, one of the purchased rams was observed by the owner to have a bleeding and swollen penis and was promptly separated from the group and not used again. Ten days later the owner noticed that several ewes had developed swollen vulvas, which were bleeding. Worried about possible affects on reproductive performance, he gathered the entire flock and found gross lesions on the vulva of 190 (42 per cent) of the ewes, but the other rams were unaffected. The affected group of ewes was segregated into a separate field in an attempt to limit the spread of the problem, pending veterinary advice.

At the veterinary examination on the following day, the affected ewes had discrete friable haemorrhagic crater-like ulcerative lesions on their vulvas, which bled readily with digital pressure (Fig 1). The lesions were restricted to the skin side of the mucocutaneous junction of the severely swollen turgid external vulval lips and did not extend to the mucous membrane. The lesions were from about 0·5 cm in diameter and in severe cases they coalesced into larger confluent haemorrhagic areas up to 4 to 5 cm in diameter. As healing progressed the lesions became overlain with reddish-grey crusts (Fig 2a). In 5 to 10 per cent of the ewes the skin of the ventral aspect of the tail-base (where it made contact with the vulva) was similarly affected with erosive and ulcerative lesions (Fig 2b), some of which had become infested with maggots (Fig 2c). The docked tail stumps of a few ewes had similar lesions. There were no gross lesions elsewhere (particular attention was given to the mouth and feet) and the sheep were otherwise clinically normal. The purchased ram was the only one of the 11 rams with any gross lesions, which consisted of healing ulcers 1 to 2 cm in diameter on the glans penis. Because there was no evidence at that stage that the lesions were significantly affecting the flock's reproductive performance, the affected and unaffected groups of ewes were recombined, but the most severely affected ewes were colour marked so that their fertility could be assessed later. No antimicrobial or other treatment was administered.

The entire flock (including those in the previously unaffected group) was examined individually three weeks later. No active ulcerated lesions were present and there was no sign of any fresh lesions, suggesting that mixing the two groups had not spread the problem, although by then the main service period was over. Most of the ewes appeared to have recovered completely, apart from some with slight scarring and minor distortion of their vulvas, which, according to the owner, was still evident in several of them when they lambed in March 2007. In a few cases there was raised granulation tissue over the healing lesions. The lesions on the affected ram were limited to a single very small (0·5 cm) granulating ulcerative lesion on the glans and another similar one near the base of the penis. Although mating activity had appeared unaffected, when the flock was ultrasound scanned 8·1 per cent of the ewes were not pregnant, compared with 3·6 per cent in the previous year. The owner commented that most of the not pregnant ewes had been marked as having been severely affected during the outbreak. Unfortunately, the flock later experienced an outbreak of enzootic abortion and it was not possible to assess the full effect of the vulvitis on its reproductive performance. The lambing percentage (born live) fell to 150 per cent from about 180 per cent in previous years.

Flock 2 This outbreak involved a group of ewes within a flock of 720 sheep in south-west Wales. During November 2006, varying degrees of penile prolapse with deep ulceration

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FIG 1: Swollen haemorrhagic ulcerated vulva of an acutely infected ewe in flock 1

and associated granulation tissue (Fig 3), were observed in three of the four Welsh mountain rams within four weeks of their being introduced to a group of 208 ewes. About 60 (29 per cent) of the ewes developed circular punctuate lesions on the skin of the vulva and base of the tail similar to those described in flock 1. The three affected rams were removed from the group as soon as the lesions were detected. The affected group of ewes was divided into two groups and a bought-in ram was introduced to 140 of them and the unaffected ram was left with the remainder. No lesions developed on the bought-in ram. The two rams remained with the ewes for a longer period than was normal practice in the flock in an attempt to improve the conception rate. No records of the reproductive performance of the affected group were available but the flock owner reported their lambing performance to be excellent.

Flock 3 This was a small cross-bred flock in Worcestershire in which vulval lesions affected 25 of 57 ewes in November 2006. The lesions developed on the vulval lips and ventral aspect of the tail base as 1 to 2 mm encrusted focal papules, with oval to circular crusting lesions up to 2 cm in diameter (Fig 4). Cases were first observed one week after the introduction of a purchased Texel ram, which was found to have three small papular lesions on the ventral aspect of its prepuce. The lesions on the ewes resolved within about two weeks.

Laboratory investigations

Blood samples in EDTA and tissue scrapes or deep swabs were collected from several affected sheep in each flock. Samples from the lesions were cultured for aerobic and anaerobic bacteria by the methods routinely used by the Veterinary Laboratories Agency (VLA) and they were also examined for mycoplasmas by using Eaton's broth and denaturing gradient gel electrophoresis (DGGE) (McAuliffe and others 2005), which detects all sheep mycoplasmas and ureaplasmas; samples were examined by electron microscopy for evidence of poxvirus infection. Attempts were made to isolate viruses from the EDTA blood samples and tissue swabs on sheep choroid plexus cells, and a specific OHV PCR (Li and others 1995) was applied to them. Tissue was not available for histopathology from the acute cases in flock 1 but at the second visit a typical healing granulating ulcerative vulval lesion was excised, fixed in formal saline and examined histologically. The prolapsed penis of a severely affected ram from flock 2 was also examined histologically.

RESULTS

In flock 1, bacteriological cultures from six animals all yielded mixed growths of *A pyogenes* and *Prevotella* species bacteria; *Pasteurella multocida* was also isolated from four animals and

FIG 2: Flock 1. (a) Healing vulval lesions overlain with crusty scabs in a ewe. (b) Healing vulval lesions with circumscribed ulcer at the base of the tail of a ewe. (c) Haemorrhagic healing vulval ulcer and large maggot infested ulcerative lesion at the base of the tail of a ewe

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FIG 3: Prolapsed penis of a ram in flock 2 with ulceration and granulation tissue

FIG 4: Encrusted healing vulval ulcers in a ewe in flock 3

an unidentified *Corynebacteria* species (not *renale*) was isolated from one other. In flock 2, *Streptococcus dysgalactiae* and *Staphylococcus aureus* were isolated from vulval lesions and *Proteus* species and *Streptococcus viridans* were isolated from the ram. No potentially significant bacteria were identified from flock 3 apart from *S aureus*, which was isolated in mixed growth from vulval lesions.

There was no evidence of either mycoplasma or poxvirus in samples taken from any of the flocks. No cytopathic effects were observed in the attempted virus isolations, but in flock 1, OHV-2 was detected by PCR in EDTA blood samples from two ewes with early lesions, a lesion swab from the ram and a lesion swab from one of the EDTA-positive ewes. The virus was not detected from more chronically affected ewes in flock 1 or from resolving lesions in either of the other two flocks. Histological examination of the healing lesions from flock 1 revealed a deep ulcer underlain by a dense layer of inflamed granulation tissue and bordered by intact hyperplastic vulval epidermis. Histological examination of the penis of the ram in flock 2 revealed a severe chronic suppurative, necrotising and haemorrhagic balanoposthitis with necrosis associated with *Fusobacterium*-like bacteria and extensive colonisation by chains of coccoid bacteria.

DISCUSSION

The clinical signs observed in these three outbreaks suggested that they were slightly different stages of the same condition, namely the vulvitis and balanitis of unknown aetiology described by Greig (2007). In all three outbreaks the within-flock prevalence was much greater than the 15 per cent described by Dunn (1996). The history of each flock strongly suggested that the infection was introduced by rams, although the possibility that it began in some of the ewes and initially went unnoticed by the stockman could not be excluded. In flock 2 the putative index case was a home-bred ram, but in the other two flocks purchased rams were implicated. This emphasises the need for flock managers to examine and isolate purchased breeding replacement rams before they are used. The initial clinical sign in the ewes in flock 1 was the appearance of fresh blood around the base of the tail. It seems likely that in extensively reared hill flocks, particularly where the tails are kept intact, the condition could easily resolve without being detected or pass unnoticed until wool is trimmed from around the perineal area in preparation for lambing. In the hill flock (flock 2) it was the penile prolapse in the rams rather than the vulval lesions in the ewes that first drew attention to the condition.

In flock 1, the findings at the first veterinary inspection suggested that the outbreak was clinically very severe and extensive. However, this may have been because it was observed at an earlier stage of the infection than in the other two flocks, in which the lesions were beginning to resolve when they were investigated. In flock 1 only one ram (the apparent index case) was clinically affected, whereas in flock 2 three of the four rams were affected. This fact, and the apparent lack of further spread of the condition among the unaffected group of ewes in flock 1 after they had been recombined with the affected ewes, was probably attributable to the prompt detection and removal of the affected ram, thereby preventing further venereal spread. Similarly, after the three affected rams had been removed from flock 2, the condition did not spread to the replacement ram. It may be concluded from these observations that the period of infectivity of this condition is probably short and limited to the acute phase.

Despite undertaking a comprehensive range of laboratory tests incorporating technology unavailable to previous authors, none of the organisms formerly linked to vulvitis and balanitis in sheep were detected. No mycoplasmas, which have frequently been implicated in this condition (Ayling and others 2004, Kidanemariam and others 2005) were found despite using the very sensitive PCR/DGGE test that incorporates a 16S ribosomal DNA primer and can detect all mycoplasmas and ureaplasmas, including any new and unknown species (McAuliffe and others 2005).

The only positive laboratory finding was the detection of OHV-2 in flock 1. Examinations for herpesviruses were initially undertaken because the lesions showed some similarities to conditions in other species caused by alphaherpesviruses, such as caprine herpesvirus type 1 infection in goats and infectious pustular vulvovaginitis due to bovine herpesvirus type 1 infection in cattle (Radostits and others 2007). OHV-2 is a gammaherpesvirus responsible for malignant catarrhal fever (MCF) in many ruminants, but in sheep, the natural host, it is ubiquitous and essentially apathogenic (Reid 1992), although disease has recently been induced experimentally in sheep after its intranasal inoculation (Li and others 2005).

In flock 1 OHV-2 was detected in samples from the ram and from two of three ewes with very early lesions, but not from ewes with more advanced lesions, and it was not detected in sheep in the other two flocks, which had resolving lesions when they were sampled. Unfortunately, because these were essentially clinical investigations, no blood samples were collected for PCR from unaffected animals, and they should clearly be sampled in any future studies of the possible association of the condition with OHV-2. The observations suggest that OHV-2 may have been shed from the vulva only during the very early stages of the condition. It is recognised that OHV-2 can be detected in epithelial tissues and the B lymphocytes of healthy

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sheep (Baxter and others 1997) and may simply be carried to the site of inflammation within lymphocytes. There is no information in the literature about the excretion of OHV-2 from the external female genitalia, but the virus has been detected in nasal secretions from sheep of all ages (Li and others 2001, Kim and others 2003) and in semen (Ackermann 2006). These and other authors have shown that the shedding of cell-free virus in nasal secretions peaks between six and nine months of age and is present in 24-hour spikes of excretion; thereafter, nasal excretion is limited to sporadic episodes of lower level excretion (Li and others 2004). In this investigation no attempts were made to determine whether the OHV-2 was cell-free or within latently infected cells (Kim and others 2003).

The epithelial lesions associated with OHV-2 infection in cattle are characterised by the degeneration of individual epithelial cells and the necrosis associated with the intra-epithelial infiltration of lymphocytes (Liggitt and DeMartini 1980). The authors are not aware of any published descriptions of vulval lesions in cattle with OHV-2 associated MCF. In a MCF-like disease in sheep induced by the experimental intranasal inoculation of OHV-2, epithelial lesions developed in the respiratory and upper alimentary tracts, again associated with the degeneration of individual and groups of epithelial cells, necrosis and infiltrates of mixed mononuclear cells (Li and others 2005). The lesions observed in the vulval biopsy therefore differed from those reported in association with OHV-2 infection.

To recapitulate, OHV-2 is considered to be ubiquitous in sheep flocks in the UK (Reid 1992), although there have been no recent prevalence surveys, and the data presented here are insufficient to suggest a causal relationship between OHV-2 and vulvovaginitis in sheep. This paper records new observations and revises the clinical description of this enigmatic disease of unknown aetiology. The observations suggest that the disease may be more prevalent than published reports suggest, because it is a short-lived condition and could easily pass unnoticed, particularly in large hill flocks. The early investigation of similar outbreaks, using the latest laboratory techniques, should be encouraged.

There are inevitably unanswered questions. Despite in depth investigations there was no evidence of any of the aetiological agents implicated by other authors in vulvitis and balanitis in sheep. The possible role of OHV-2 remains unclear, but even if the OHV-2 was derived from latent virus within lymphocytes attracted to the site of inflammation, it could still represent a previously uncharacterised horizontal transmission route among sheep, in addition to the routes established through nasal secretions and semen (Hussy and others 2002, Kim and others 2003).

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Correction

Ulcerative vulvitis and balanitis in sheep flocks by G. C. Pritchard, S. F. E. Scholes, A. P. Foster, E. S. E. Mitchell, J. Lawes, G. Ibata, M. Banks (*VR*, July 19, 2008, vol 163, pp 86-89). The Discussion states that 'The authors are not aware of any published descriptions of vulval lesions in cattle with OHV-2 associated MCF.' Following a recent publicaton, this should have read: 'Superficial lesions may occur in the vagina and vulva similar to those of the oral cavity and skin in cattle with MCF (Brown and others 2007).' The authors regret the error.

BROWN, C. C., BAKER, D. C. & BARKER, I. K. (2007) Alimentary system. In Pathology of Domestic Animals. 5th edn. Vol 2. Ed M. G. Maxie. pp 152-158

Alopecia in calves associated with milk substitute feeding

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Outbreaks of alopecia with unusually high morbidity occurred among calves reared on milk substitutes on two unrelated farms in Suffolk. On one farm alopecia occurred for three consecutive years; during the winter of 1981-82 there were also clinical signs of muscular dystrophy among the same calves. On the second farm calves with alopecia also showed signs of muscular dystrophy. The apparent relationship between alopecia and milk substitute feeding is discussed together with the possible involvement of vitamin E.

ALOPECIA of varying severity is not uncommon in calves reared on milk substitute. The incidence is usually sporadic, affecting only individual animals in a group. However, veterinary investigation centres have occasionally encountered incidents involving from 20 to nearly 100 per cent of calves in a batch (J. Kemp, R. G. Holmes and A. L. Duncan 1982, personal communication)

Commercial skimmed milk preparations for feeding to calves usually contain added vegetable or animal fat which is incorporated by homogenisation or by blending with the aid of an emulsifying agent (Roy 1980). Alopecia has often been attributed to the nature of the fat or to incorrect emulsification (Dirksen and Hofmann 1974, Grunder 1976, Roy 1980). However, most proprietary milk substitutes in current use appear to be based on beef tallow and coconut oil, neither of which has been implicated as causing alopecia providing the method of incorporating them is satisfactory.

Two unrelated outbreaks of alopecia among calves receiving milk substitute on separate farms in Suffolk are described here. The outbreaks had several features in common and posed unresolved questions which appear to merit further investigation.

Case histories

Farm A

In the winter of 1979-80, alopecia affected about half of 100 home bred Friesian calves on a calf rearing unit (farm A) in Suffolk. The problem was mild and gave no cause for concern. The following winter (1980-81) nearly all the calves were affected.

The calves were reared on a conventional system in single or double pens. They received colostrum or whole milk for six days, followed by proprietary milk substitute fed warm, twice daily, until weaning at six weeks. Hay, proprietary calf pellets and water were freely available.

Alopecia usually developed within two to three weeks of introduction of the milk substitute. The distribution of lesions was as described by Dirksen and Hofmann (1974). Hair loss was especially noticeable on the muzzle, around the eyes and the base of the ears. It was also very apparent on the hindlimbs.

The condition showed no response to multivitamin injections but the hair started to regrow within one or two weeks of weaning. The owner tried different milk formulations in an attempt to prevent the condition. Alopecia occurred with six different brands of commercially available high fat (15 to 20 per cent) milk powder and also with the one low fat preparation that was used. One brand of high fat milk substitute appeared not to give rise to alopecia.

As the calving season began the next winter (1981-82) alopecia was seen again, even with the previously successful brand of milk powder. However, this time it was accompanied by muscular weakness, stiffness, tachycardia and other clinical features similar to those associated with muscular dystrophy caused by vitamin E deficiency. The animals were treated with a single dose of a selenium-vitamin E preparation (Dystosel; Intervet Laboratories). The muscular condition appeared to respond to this and the hair began to regrow. Thereafter, selenium-vitamin E was given to all calves at about one week of age; no further cases of either syndrome occurred.

Blood samples from affected calves showed normal selenium levels as indicated by erythrocyte glutathione peroxidase activities. They were not examined for vitamin E content during the acute phase of the disease but samples taken during the recovery phase, after treatment, showed fairly low plasma vitamin E (alpha tocopherol) concentrations, ie, 0·7 to 1·5 µg/ml. (Blood serum levels of vitamin E below 1 µg/ml indicate an inadequate dietary intake; levels above 1·5 µg/ml suggest the intake is adequate, P. H. Anderson, personal communication.)

Farm B

During the same winter (1981-82), a similar incident developed on another farm in Suffolk (farm B). The calves, Friesians, were purchased at three days of age from a local dairy farm and reared on a system similar to that described above. The owner has previously used a low fat (10 per cent) milk powder but had recently changed to a higher fat preparation (18 per cent).

At about three weeks of age alopecia was first noted in five of the 12 calves. When subsequently taken out of the pens for examination, clinical signs suggestive of muscular dystrophy, including hyperextension of the fetlocks and a stiff gait, were also noted in these five calves and in three others in the batch. The alopecia was again as described by Dirksen and Hofmann (1974). The first signs included scurfiness of the skin, followed by sharply defined areas of hair loss, particularly on the head and limbs (Fig 1). Skin biopsy failed to show any pathological change. No ectoparasites or dermatophytes were found in direct examinations of skin scrapings.

At the onset of the problem, blood samples from the affected calves showed an adequate selenium status, but plasma vitamin E levels were 0·5, 0·8, 0·9 and 1·3 µg/ml, which are low for the time of the year. Blood samples taken two weeks later from other affected calves showed even lower vitamin E concentrations, ie, 0·2, 0·3 and 0·4 µg/ml. The owner reverted to the low fat milk powder used previously and both the muscular dystrophy and the alopecia showed a rapid improvement.

Four younger Hereford cross Friesian calves from a different source, which had just begun to show symptoms of alopecia and muscular dystrophy, also quickly responded to the change in milk powder. The details of the case histories from the two farms are summarised in Table 1.

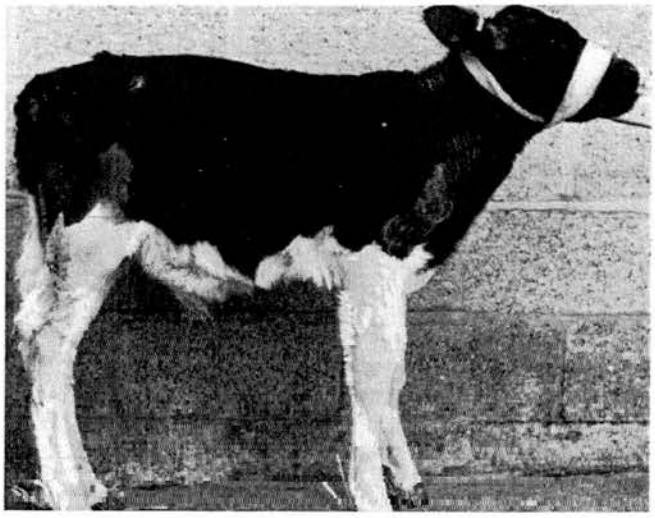

FIG 1: Calf from farm B showing alopecia

Field trial

A small field trial involving 12 calves was subsequently set up on farm B in an attempt to compare directly the effect of using either the high fat or low fat milk substitute. It was not successful in that neither alopecia nor muscular dystrophy occurred in either group.

The average vitamin E level of these calves at one week of age was 1·2 µg/ml, but at weaning had fallen to 0·7µg/ml. This confirms the observation that calves on proprietary milk replacers are frequently surviving on suboptimal levels of vitamin E (P. H. Anderson, personal communication).

Discussion

The clinical features of the incidents described above appeared to be associated with nutritional factors related to the various milk substitute preparations used. However, it seems that one or more predisposing factors were also involved since the conditions were not reproducible to order. Moreover, the same preparations were in use on many other farms in the area with no reported problems.

The relationship between the alopecia and muscular dystrophy is clearly questionable. Their occurrence in the same calves does not necessarily imply a common aetiology. However, hair did begin to regrow when vitamin E was given as a treatment for the muscular dystrophy on farm A. We have no reports of the use of vitamin E in the treatment of alopecia in the absence of muscular dystrophy.

TABLE 1: Summary of findings

Farm	Year	Clinical signs	Vitamin E levels	Successful therapy
A	1979-80	Mild alopecia	Not examined	None attempted
	1980-81	Alopecia	Not examined	Alopecia did not occur with one brand of high fat milk powder
	1981-82	Alopecia; muscular dystrophy	Fairly low during recovery	Parenteral vitamin E-selenium
B	1981-82	Alopecia; muscular dystrophy	Very low	Change from high to low fat milk powder

Nutritional myopathy associated with low levels of vitamin E, as a result of diets containing large amounts of polyunsaturated fatty acids, has been described by several authors (Roy 1980). This did not appear to be the explanation in the cases of muscular dystrophy described above. There was no obvious relationship between the development of the clinical features described and the declared levels of vitamin E in the milk powders. Neither could prolonged storage before use be implicated.

Lister and Emmons (1976) noted more severe alopecia in calves fed high fat (22·2 per cent) than those fed low fat (6·2 per cent) milk replacer. Maidment (1981) noted that partial alopecia occurred more in colostrum fed calves than in those receiving a reconstituted milk powder containing 17 per cent fat. The present case histories suggested that alopecia and muscular dystrophy were probably more likely to occur with high fat diets.

Dirksen and Hofmann (1974) and Grunder (1976) advised that husbandry and feeding should be changed in cases of alopecia of unknown cause, and that vitamins, particularly B, C and E, should be administered. However, the possible role of vitamins in the aetiology of alopecia in calves is unclear. Besides the low levels of vitamin E in our cases, blood samples from some of the calves examined also showed low levels of vitamin A although no conclusions could be reached.

With the present lack of knowledge regarding nutritional alopecia in calves, therapy must inevitably be somewhat empirical. Our findings suggest that the milk substitute should be changed, perhaps to a low fat preparation, and that vitamin E and possibly vitamin A could be administered. However, since the hair usually appears to regrow soon after weaning, whatever course of action is adopted, it may be difficult to evaluate the efficacy of therapy.

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Papers and Articles

Severe disease in a dairy herd associated with acute infection with bovine virus diarrhoea virus, *Leptospira hardjo* and *Coxiella burnetii*

G. C. Pritchard, E. D. Borland, L. Wood, D. G. Pritchard

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Between November 1984 and February 1985, a serious outbreak of pyrexia, diarrhoea, agalactia, metritis and severe weight loss affected most of the recently calved cows in a 183-cow dairy herd in Norfolk. Fifteen cows died and 20 were culled. Forty cows aborted during or after the outbreak, and many of them produced mummified fetuses; 18 calves were stillborn and three others died soon after birth. Necropsy of three affected cows revealed ulceration of the gastrointestinal tract similar to that seen in cases of mucosal disease. Bovine virus diarrhoea virus was isolated from the intestines of one cow that died soon after the onset of illness. The virus was also isolated from the blood of four acutely ill cows and seroconversion was demonstrated in three of those that survived. The virus was isolated from three aborted fetuses, a stillborn calf and a live neonatal calf. Body fluids from two aborted fetuses were seropositive for the virus as were sera from all the aborting cows tested. In addition to widespread seroconversion to bovine virus diarrhoea virus during the outbreak, there was serological evidence of recent infection with *Leptospira interrogans* serovar *hardjo* and *Coxiella burnetii* in a high proportion of cows. It was concluded that this was primarily an acute outbreak of bovine virus diarrhoea but its unprecedented clinical severity was probably associated with the concurrent introduction of *L hardjo* and *C burnetii* into an immunologically naive herd during the main calving period. Epidemiological analysis suggested that the source of the virus and *L hardjo* was down-calving heifers returning from communal marsh grazing.

INFECTION with bovine virus diarrhoea virus (BVDV) is widespread in cattle herds in Britain (Harkness and others 1978) and normally causes only a mild or subclinical illness (Duffell and Harkness 1985). Transplacental infection during the first half of pregnancy can result in fetal death with subsequent abortion or mummification or the birth of a live, weak or congenitally malformed calf (Duffell and Harkness 1985). Some infected calves which may be apparently normal at birth are persistently infected with BVDV and some of them subsequently die of mucosal disease (Roeder and Drew 1984). The trigger factor for the onset of mucosal disease in cattle already persistently infected with a non-cytopathogenic strain of BVDV is thought to be superinfection with an antigenically related cytopathogenic strain of BVDV (Brownlie and others 1984).

Although most BVDV infection in seronegative immunocompetent cattle ('acute BVD') is subclinical, disease outbreaks resulting in fever, inappetence and diarrhoea have been described (Baker 1987). Barber and others (1985) reported an outbreak in a dairy herd in Scotland which caused pyrexia, diarrhoea, a fall in milk yield and abortion together with persistent viraemia – with a high incidence of pneumonia – in calves born to infected dams. This paper describes an outbreak of BVDV-associated disease similar to that described by Barber and others (1985) but with a hitherto unprecedented clinical severity, resulting in a high mortality in adult cows and extensive fetal losses.

Herd details

The disease occurred in a self-contained Friesian/Holstein dairy herd in Norfolk. At the beginning of the outbreak the herd comprised 183 cows, 64 dry cows and down-calving heifers and 121 young stock, managed on two premises (A and B) about two kilometres apart. The milking cows were winter-housed in conventional cubicle accommodation on farm A and the dry cows and in-calf heifers were kept in adjacent strawed yards. The cows calved mainly during the autumn and winter. Winter feeding consisted of proprietary concentrates, sugar-beet pulp, brewer's grains and grass silage.

The calves were all reared from a few days of age on farm B. Male calves were sold but female calves were retained as heifer replacements and grazed on nearby communal marshes (C, D). The heifers returned from the marshes to farm A to calve in early autumn.

Disease outbreak in the cows

At the beginning of November 1984, several recently calved cows on farm A developed profuse watery diarrhoea, a fever of 40 to 42°C, anorexia and agalactia. No external lesions were evident. Clinical signs, including the high fever, persisted in the affected animals for a week or more unless they died sooner. There was no significant response to antibiotic or supportive therapy. There was considerable dehydration and weight loss and when recovery occurred it was very protracted.

In the ensuing weeks during the herd's main calving period, further similar cases of acute enteric disease were seen – invariably during the two or three weeks after parturition. By early December 1984, more than 60 per cent of the adult cows had been affected. During the later stages of the outbreak, new cases were clinically milder than those observed earlier; the fever and scour were less marked but metritis and an unattributable generalised lameness frequently complicated the

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TABLE 1: Haematological, virological and serological findings from eight cows affected with acute enteric disease

Cow	Initial blood samples: Days since onset of illness	Leucocytes ($\times 10^9$/litre)	Lympho-cytes (%)	Neutro-phils (%)	Eosino-phils (%)	Mono-cytes (%)	BVDV isolated	Second blood samples: BVDV titre (SNT)	*L hardjo* titre (MAT)	BVDV isolated	BVDV titre (SNT)	*L hardjo* titre (MAT)
21*	2	NT	NT	NT	NT	NT	+	< 1/10	1/400	–	1/60	1/100
75*	2	NT	NT	NT	NT	NT	+	< 1/10	1/100	Died †	–	–
64*	4	3·2	66	34	0	0	+	< 1/10	1/400	–	1/60	1/100
52*	2	2·3	47	50	2	1	+	< 1/10	1/100	–	1/80	1/100
91*	4	1·5	61	38	0	1	–	1/60	1/100	–	1/960	1/100
58	17	9·1	55	40	4	1	–	1/60	1/100	–	NT	1/100
282	18	4·3	43	52	4	1	–	1/320	1/100	Died †	–	–
170	27	10·5	33	59	8	0	–	1/320	1/100	–	NT	1/100
Normal haemato-logical values (Doxey 1971)		4-10	45-75	15-45	0-20	2-7						

SNT Serum neutralisation test, MAT Microscopic agglutination test, NT Not tested. * Acute cases. † See Table 2 for post mortem findings

clinical picture. The outbreak finally came to an end in February 1985 by which time the illness had spread to some dry cows and in-calf heifers in the yard adjoining the cubicle house. Cattle on farm B remained clinically normal throughout the outbreak.

A total of 15 cows died, either soon after the onset of clinical signs or after several weeks' illness; 20 cows were culled, primarily because of emaciation and infertility.

Reproductivity losses

Fetopathy followed in the wake of the illness in the cows. The first abortion (at less than 271 days gestation) occurred about a week after the beginning of the clinical outbreak and by August 1985, 40 cows had aborted mainly in mid or late pregnancy. About 50 per cent of the fetuses were mummified. A further 18 calves were stillborn at or close to term and three calves died soon after birth.

Investigations into the cause of the disease

Laboratory investigations commenced in November 1984 and continued for about 12 months. During this time, post mortem material was examined either on the farm, at the knacker's yard or at the veterinary investigation centre. To simplify the presentation of the data, the methods employed and results have been grouped together.

Initial bacteriological examinations

Using standard procedures (Ministry of Agriculture, Fisheries and Food 1984) 14 faeces samples or rectal swabs collected from scouring cows at the beginning of the outbreak were examined for salmonellas and campylobacters. No significant bacteria were isolated. Milk samples from four acutely ill cows with agalactia were screened for mastitis pathogens including mycoplasmas; *Staphylococcus aureus* was isolated from one. Leptospires were not detected in urine collected from two sick cows; cultures were not undertaken.

Initial examinations on blood samples from affected cows

Clotted and EDTA blood samples were collected from five recently affected and three convalescent cows on November 20, 1984. Two of the cows died within two days and the survivors were re-sampled two to six weeks later to compare antibody titres.

The serum samples were examined for the presence of antibodies to BVDV using the serum neutralisation test to the NADL strain of virus (Edwards and Roeder 1983), infectious bovine rhinotracheitis virus (IBRV) using the serum neutralisation test (Frerichs and others 1982), bovine malignant catarrhal fever virus (MCFV) using an indirect fluorescent antibody labelling technique (Rossiter and others 1978, Rossiter 1983), and 18 serogroups of *Leptospira interrogans* by a microscopic agglutination test (Pritchard 1986).

TABLE 2: Post mortem findings on three cows affected with acute enteric disease

Cow (date examined)	Gross post mortem findings	Microbiological results	Comments
282 * (November 21)	Enteritis and abomasal ulceration. Lungs emphysematous and contained several large encapsulated abscesses Liver fatty	No significant bacteria isolated Virus isolation not attempted	Calved October 7; ill for 18 days before death. (Examined at knacker's yard: only limited scrutiny possible)
75 * (November 22)	Multiple subcutaneous haemorrhages throughout carcase. Pulmonary emphysema. Extensive ulceration of hard palate, abomasum and small intestine. Severe haemorrhagic gastroenteritis	No significant bacteria isolated. BVDV isolated from small intestine	Calved November 7; died two days after onset of illness. Good bodily condition
80 (December 2)	Numerous healing ulcers and erosions in oesophagus. Moderate haemorrhagic enteritis with localised ulceration Chronic endometritis, tracheitis and pulmonary emphysema	*Corynebacterium pyogenes* isolated from uterus and *Acholeplasma laidlawii* from tracheal exudate. No viruses isolated	Calved November 13; ill for a week before death. Dehydrated and emaciated

* See Table 1 for antemortem laboratory findings

In addition, the samples were examined for the presence of BVDV using immunoperoxidase techniques (Meyling 1983) and were further examined for BVDV and other cytopathic agents (including rinderpest), in secondary calf kidney cell cultures using flying coverslips and a direct immunofluorescent labelling system. Total and differential leucocyte counts were performed on the EDTA blood samples using standard procedures.

As shown in Table 1, BVDV was isolated from four of the five acute cases but not from the convalescing animals. Haematological examinations showed a fairly marked leucopenia in the early cases but normal leucocyte counts, with slight neutrophilia, in the chronic group.

The convalescent cows were all seropositive ($\geq$ 1/10) to BVDV. Three of the surviving acute cases seroconverted to BVDV and were no longer viraemic. The other acute case (cow 91) showed a rise in BVDV titre from 1/60 to 1/960. All the cows were seropositive ($\geq$ 1/100) to *L hardjo*, of which two (21 and 64) had titres which fell from 1/400 to 1/100. There was no evidence of infection with IBRV or MCFV.

Necropsy of affected cows

Three affected cows were necropsied during November and December 1984; two of these (282 and 75) had been blood sampled shortly before death (Table 1). Histological, bacteriological (including cultures for mycoplasma and chlamydia) and virological examinations were carried out on selected viscera.

The cows had different degrees of acute or chronic ulceration of the gastrointestinal tract similar to that seen in cases of mucosal disease (Table 2). Histological examinations of viscera from cows 75 and 80 showed moderate haemosiderosis in the spleen, fatty change (cow 75) and centrilobular degeneration (cow 80) in the livers and mild multifocal chronic interstitial nephritis (cow 80). These non-specific changes were consistent with the duration of illness in the cows concerned.

BVDV was isolated from the intestines of cow 75 which had died during the acute stage of illness. With the exception of *Corynebacterium pyogenes* from the uterus of cow 80, no significant bacteria were isolated.

Mycotoxin analysis of feed

Samples of grass silage, sugar-beet pulp and dairy nuts fed at the beginning of the outbreak were examined for the presence of mycotoxins using chemical analysis, dialysis and two dimensional thin layer chromatography (Patterson and Roberts 1979). There was no evidence of aflatoxins B1, B2, G1 and G2, ochratoxin A, zearalenone, T-2 toxin, diacetoxyscirpenol or sterigmatocystin.

Serological examination of blood samples from aborting cows

Blood samples collected between November 1984 and August 1985 from 17 aborting cows on the day of abortion and submitted to the veterinary investigation centre for statutory (BS7) abortion investigation were examined for the presence of antibodies to *Brucella abortus* using the Rose Bengal plate test (Brinley-Morgan and others 1978), BVDV, and *L interrogans*. Some of the serum samples were also examined for antibodies to IBRV and other possible infectious causes of abortion namely *Coxiella burnetii* with the complement fixation test (Slavin 1952), *Chlamydia psittaci* by complement fixation test (Stamp and others 1952) and *Toxoplasma gondii* using the dye test (Sabin and Feldman 1948) and the latex agglutination test (Eiken Chemical Company).

The cows were all seropositive for BVDV with titres ranging from 1/30 to 1/320. Seven were seropositive for *L hardjo* at 1/100 and one was also seropositive for *L icterohaemorrhagiae* at 1/100. Four of 11 cows tested were seropositive ($\geq$ 1/10) for *C burnetii* with titres up to 1/80. Six of 13 cows tested for IBRV were seropositive but all with fairly low titres ($\leq$ 1/12). There was no serological evidence of infection with *T gondii* or *C psittaci*.

Examination of fetuses and stillborn calves

Twenty-three aborted fetuses or stillborn calves were necropsied between November 1984 and August 1985. Routine bacteriological examinations (MAFF 1984) including microscopic examination of stained smears from cotyledons and stomach contents for *C burnetii* and fungal hyphae, and fluorescent antibody testing of fetal kidneys for *L hardjo* (Ellis 1981) were performed. Isolation of BVDV was attempted from pooled fetal viscera. Body fluids, taken from the thoracic cavity of non-mummified fetuses, were examined for serological evidence of *L hardjo*, *C burnetii* and *T gondii* using methods described previously and to BVDV using an indirect immunofluorescence procedure for BVDV antibody. Tibias taken from three non-mummified fetuses (including a set of twins) were examined radiologically to determine developmental age and growth arrest lines (Done and others 1980).

Nine of the fetuses were severely mummified and weighed only a few kilograms. The remainder were fresh aborted fetuses or stillborn calves weighing between 10 and 50 kg, with estimated gestational ages ranging from about 180 to 280 days. The radiological examinations revealed developmental ages of 248 days (twin), 239 days (twin) and 193 days at expulsion. There were distinct growth arrest lines which indicated a crisis – from which the fetuses had recovered – at 191, 193 and 155 days, respectively. The only significant findings from the microbiological examinations were the isolation of BVDV from three fetuses that were aborted during the last two months of pregnancy and from a stillborn calf; BVDV antibodies were detected in two other fetuses that were aborted at six to seven months gestation.

Detection of BVDV in the herd

On December 12, 1984 the entire herd which then comprised 360 animals, including 167 young stock at farm B, was blood sampled and tested for BVDV by virus isolation and immunoperoxidase techniques. As many calves as possible that were born after December 12 were similarly tested by three months of age. This excluded the majority of male calves because they were sold soon after birth.

BVDV was not detected in any of the samples taken from the herd test but it was isolated in April 1985 from one of 56 calves sampled subsequently.

Further serological investigations

Having established that BVDV, *L hardjo* and possibly *C burnetii* were involved in the outbreak, further serological investigations were undertaken to determine the extent and source of infection with these pathogens.

Herd Profile.— At the end of November 1984, 30 randomly selected cows on farm A were blood sampled to ascertain the seroprevalence of BVDV, *L hardjo* and *C burnetii*. Fifteen (50 per cent) of the samples were seropositive for BVDV all with titres of 1/40 or more, 16 (53 per cent) were positive for *L hardjo* (one at 1/400, the remainder at 1/100) and five (17 per cent) were seropositive for *C burnetii* with titres of 1/20, 1/40, 1/160, 1/160 and 1/320.

Seroconversion.— Systematic resampling of many acute clinical cases was not feasible because of the constraints associated with the management of a commercial dairy herd. However, some surviving cows from the herd profile and others blood sampled in November and December 1984, including those that aborted, were resampled at least six weeks later in January 1985. The paired sera were examined for evidence of seroconversion or rising (four-fold) titres to BVDV, *L hardjo* and *C burnetii*.

Of 87 cows retested for BVDV, 79 (91 per cent) were seropositive and 45 (52 per cent) seroconverted or showed rising titres to BVDV between samplings, as did 20 (30 per cent) of 67 tested for *L hardjo* and 32 (52 per cent) of 62 tested for *C burnetii*.

Source of infections.— The ages and potential exposures of the 167 young stock on farm B were obtained at the herd blood sampling on December 12. The rearing of calves born between 1982 and 1984 had resulted in six possible exposures in 1984, namely farm B (housing), farm B (pasture), marsh C, marsh D, farm A (running with the bull) and farm A (at entry into the dairy herd). Birth cohorts were subsequently analysed according to these exposures and the prevalence of antibodies to BVDV, *L hardjo* and *C burnetii* established.

The relationship of the exposures to the seroprevalence in December 1984 is shown in Table 3. High prevalence of antibodies to *L hardjo* and BVDV were seen in cattle exposed to marsh grazing before entering the dairy herd, whereas antibodies were absent or virtually absent from calves that had not been on the marshes. Cohorts born between October and December 1984 showed increasing seroprevalences to these infections, presumably due to maternally derived antibodies. Antibodies to *C burnetii* were confined to calves born between October and December 1984 indicating that this infection had also been recently introduced to the dairy herd but apparently not via the heifers returning from the marshes.

Discussion

The exposure chart analysis of the prevalence of antibodies to BVDV, *L hardjo* and *C burnetii* showed that these infections entered the herd concurrently in the autumn of 1984. The evidence implicating BVDV as the primary pathogen in the ensuing disease outbreak in the cows and in the fetal losses, was conclusive. Not only was BVDV isolated from acutely affected cows but seroconversion and intra uterine infection were convincingly demonstrated in survivors. BVDV was isolated from the alimentary tract (and from the blood two days previously) of a cow that was necropsied after it died during the acute stage of the illness. There was no evidence of persistent viraemia in any of the cows in the herd. The overall findings therefore clearly indicated that the disease in the cows was associated with acute BVDV infection and not mucosal disease.

The clinical findings were very similar to those described by Barber and others (1985) although the present outbreak was much more severe and extensive. Barber and others (1985) also found gastrointestinal ulceration in a cow that died during the outbreak although they did not isolate BVDV from the carcase.

The role played by *L hardjo* was unclear. Like BVDV infection, *L hardjo* can cause fever, a decrease in milk yield and abortion. The fact that two acutely ill cows sampled at the beginning of the outbreak had falling titres to *L hardjo* suggested recent infection, as did the detection of another animal with a titre of 1/400 from the herd profile carried out a week later. Furthermore, seroconversion or rising titres to *L hardjo* were detected in 30 per cent of the herd between December 1984 and January 1985. However, the large rises in antibody titre commonly seen with the milk drop syndrome (Higgins and others 1980) were not demonstrated and none of the blood samples from aborting cows had high titres.

Serological examinations for *C burnetii* were initially carried out only as part of the differential diagnosis for infectious causes of abortion and it was difficult to know whether this organism contributed significantly to the severity of the clinical disease or to the fetal losses. There were, however, high antibody titres to *C burnetii* suggestive of a recent infection, and over 50 per cent of the herd seroconverted or showed rising titres during the outbreak. Neither *C burnetii* nor *L hardjo* were demonstrated in fetal material.

C burnetii infection appears to be fairly common in Britain. G. C. Pritchard and D. G. Pritchard (unpublished data) found serological evidence of infection in 22 (8·5 per cent) of 260 herds in East Anglia surveyed in 1985-86. Although *C burnetii* infection (Q fever) is an important zoonosis, there is little evidence that it causes clinical disease in British herds (Little 1983). However, reports from elsewhere of *C burnetii* causing abortion and metritis in cattle (Goyon 1981, Dopfer and others 1986) suggest that it has the potential to be involved in the disease syndrome reported here.

Strains of BVDV of different virulence related to cytopathogenicity in vitro have been described, but fatal disease in seronegative immunocompetent adult cattle has not been reported in experimental studies (Baker 1987). All the BVDV isolates from the present study were non-cytopathogenic on first passage. Because of concern over the zoonotic implications (Aitken 1987) of handling material potentially infected with *C burnetii* and because of other constraints, further cytopathogenicity studies and experimental infection of cattle were considered inappropriate.

From the outset, intensive antibiotic and supportive therapy was administered in an attempt to combat the disease. Improvements in hygiene and husbandry at calving were also adopted. Once it became apparent that *L hardjo* was present in the herd, vaccination using a commercially available inactivated vaccine (Leptavoid H; Wellcome) was commen-

TABLE 3: Exposure chart and antibody prevalence for BVDV, *L hardjo* and *C burnetii* in young stock sampled on December 12, 1984

Birth cohort (month/year of birth)	Number sampled	Exposures during 1984						Prevalence of antibody (Number of cohort seropositive)		
		Farm B		Marsh C	Marsh D	Farm A (bull)	Farm A (dairy herd)	BVDV	*L hardjo*	*C burnetii*
		Housed	Pasture							
December 1984	7	+	−	−	−	−	−	6	1	1
November 1984	16	+	−	−	−	−	−	8	1	4
October 1984	22	+	−	−	−	−	−	18	0	2
September 1984	4	+	−	−	−	−	−	1	0	0
February-May 1984	11	+	+	−	−	−	−	0	0	0
December 1983-January 1984	33	+	+	−	−	−	−	7	0	0
October-December 1983	29	+	+	−	−	−	−	0	0	0
December 1982-September 1983	35	−	−	+	−	+	−	18	12	0
June-November 1982	5	−	−	−	+	−	−	3	3	0
February-June 1982	3	−	−	−	+	−	+	3	2	0
November 1981-January 1982	2	−	−	−	+	−	+	2	1	0
Total	167									

ced but without significant effect. During the later stages of the outbreak, levamisole (Nilverm injection; Wellcome) was administered routinely at calving in an attempt to stimulate the cell mediated immune system (Amery and Horrig 1984) and to counteract the immunosuppression observed in the acutely affected cows. This appeared to be of some value and although it was not possible to carry out a controlled trial the findings appeared to support those of Saperstein and others (1983) who reported a higher mean leucocyte count in calves experimentally infected with BVDV and given levamisole. This may partly explain why new cases seen in the later stages of the outbreak were clinically less severe.

Several authors including Malmquist (1968) and Edwards and others (1986) have commented on the immunosuppressive effect of BVDV infection. Baker (1987) suggested it gave BVDV the potential to cause severe disease. The immunosuppression has been attributed to a decrease in the number and percentage of circulating lymphocytes and an impairment of the normal protective function of neutrophils and macrophages (Reggiardio and Kaeberle 1981, Bolin and others 1985). The immunosuppressive role of BVDV might explain (Palfi and Molnar 1987) the lack of very high titres to *L hardjo* seen in this outbreak. There was nothing to suggest that the fatty liver syndrome – another potential cause of post calving immunosuppression (Reid and Roberts 1983) – was involved.

The probable explanation for the unprecedented clinical severity of this acute outbreak of bovine virus diarrhoea was its multifactorial aetiology, involving the stress of calving and concurrent infection with *L hardjo* and probably *C burnetii*. The virulence of the latter two organisms might have been potentiated by the immunosuppressive activity of BVDV. Other bacterial pathogens such as *C pyogenes* could also have been involved, particularly in those cows with endometritis (Hartigan and others 1974). Diseases which have been shown to act concurrently or synergistically with BVDV, including malignant catarrhal fever (Sharpe and others 1987), salmonellosis (Wray and Roeder 1987) and pasteurellosis (Barber and others 1985) were not involved in this outbreak.

The total cost to the farmer from this disease outbreak was more than £50,000. In the light of the findings and those of Barber and others (1985) it may be necessary to reassess the clinical significance of acute outbreaks of bovine virus diarrhoea in herds which are particularly susceptible. Owners should be encouraged to improve herd security and to monitor their herds and breeding replacements for major infectious diseases. This is particularly relevant in parts of Britain, such as East Anglia, where a declining cattle population and the isolation of many farms are likely to result in many herds being immunologically naive to common infections.

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Disease in single suckled beef herds

From Mr G. C. Pritchard and Mr M. E. Bardsley

SIR, — We wish to alert colleagues to potential disease problems in newly established single-suckled beef herds and to record the death – apparently from acute (ie, post natal) bovine virus diarrhoea (BVD) virus infection – of a bull soon after joining one such herd.

During the past few years there have been increasing financial incentives for farmers to engage in low-cost suckled calf production. Suckler herds can utilise permanent pasture on arable farms and are particularly well suited to environmentally sensitive areas such as the Norfolk Broads and Suffolk river valleys. This has led to an increase in the number of single suckled beef herds, including those managed by farmers with little previous experience of suckler cattle or even of livestock. Some of these new herds have been hastily assembled from numerous sources with widely varying disease status.

These factors inevitably increase the likelihood of such herds experiencing serious disease outbreaks. For example, one recently formed 180 cow suckler herd in East Anglia has already suffered severe losses from rotaviral diarrhoea and parasitic bronchitis.

Of particular interest was the death of a four-year-old pedigree Limousin bull four weeks after entering this herd. It came from an established high health status herd and was tested negative for BVD virus (and BVD antibodies) immediately prior to sale. After about three weeks, the bull became depressed and developed a persistent diarrhoea and muzzle erosions. Despite intensive supportive therapy it rapidly deteriorated and died.

At post mortem examination there was extensive ulceration and haemorrhage throughout much of the alimentary tract, including the oesophagus. No salmonellas or other significant bacteria were isolated and neither BVD virus, infectious bovine rhinotracheitis virus nor other cytopathic agents were detected in cell cultures. Examination of paired sera at the Central Veterinary Laboratory, Weybridge using ELISA clearly demonstrated seroconversion to BVD virus.

Although circumstantial, the evidence strongly indicated that this immunologically naive animal died from acute BVD virus infection. The findings were similar to those described by Pritchard and others (1989) for an acute BVD outbreak in a dairy herd although in this present case there was no evidence of concurrent infection with other pathogenic agents.

We should not underestimate the potential clinical significance of acute BVD virus infection. Practitioners should also be aware of potential disease problems in newly established suckler herds. In addition to husbandry advice, these are likely to require close veterinary supervision. Routine vaccination against rotavirus, infectious bovine rhinotracheitis and *Leptospira hardjo* should be considered.

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EPIDEMIOLOGY OF BHV1 INFECTION IN CATTLE BREEDING HERDS IN NORFOLK

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Bovine herpesvirus 1 (BHV1) has a worldwide geographical distribution and is responsible for a broad spectrum of disease manifestations including infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/infectious pustular balanoposthitis (IPV/IPB) (Wyler et al, 1989); infection can also be mild and inapparent (Kahrs, 1977). In common with other alphaherpesviruses, BHV1 is characterised by the ability to establish latent infection, mainly in ganglion cells (Studdert, 1989).

The concept of BHV1 latency dictates that after recovering from acute infection, cattle probably remain a potential source of virus for life. Although many studies have shown that reactivation and shedding of latent virus can be induced experimentally, particularly by synthetic corticosteroid injections (Sheffy & Davies, 1972), the extent to which this occurs naturally under field conditions, leading to new infection, is not known. The cross-sectional studies described here utilise the results of whole-herd serological screening tests undertaken in breeding herds in Norfolk to examine the epidemiology of subclinical BHV1 infection with particular reference to the rate of virus transmission amongst susceptible cattle.

MATERIALS AND METHODS

Cattle Health Scheme herds

In April 1987, the State Veterinary Service introduced the Cattle Health Scheme (CHS) for Great Britain (ADAS, 1987). This superseded the Enzootic Bovine Leucosis (EBL) Attested Herd Scheme (Roberts & Bushnell, 1982) and also offered an IBR monitoring programme which gave farmers the opportunity to carry out serological testing for BHV1 and to eradicate the infection from their herds where appropriate.

Sixty-three breeding herds in Norfolk joined the CHS to become EBL attested during the four year period between March 1987 and March 1991. A preliminary questionnaire was completed at the initial visit to each farm, at which the author was accompanied by the owner's veterinary surgeon. Details recorded included: herd size (number of cattle aged two years or more), herd type (dairy or beef suckler), breed, status (pedigree or non-pedigree/commercial), replacement policy (main sources and numbers purchased during the previous 10 years), general management practices and disease history.

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Owners were encouraged to have some, or all, of their cattle examined for serological evidence of BHV1 infection when they were tested for EBL, provided the herd had no recent clinical history of IBR (or IPV/IPB) or IBR vaccination (which would have made it virtually impossible to interpret serological findings). Seven herds were thereby excluded from the investigations: in three the owner declined to undertake any serological testing for BHV1 and four had a history of IBR and had vaccinated during the previous two years. One dairy herd that had experienced epidemic IBR in 1981 and been vaccinated with "Tracherine" (Smith Kline Animal Health) was included because there was no history of revaccination or clinical recrudescence.

The 23 dairy herds in the study population had all been established for at least 20 years and were of pedigree status (20 herds were Holstein/Friesian and three were Ayrshire). Most of the 33 suckler herds had been assembled during the previous 10 years and all but one contained solely or predominantly pedigree cattle. The main suckler breeds represented were: Simmental (10 herds), Charolais (four herds), Blonde d'Aquitaine (four herds), Limousin (three herds), Salers (two herds), and one herd each of: Aberdeen Angus, South Devon, Bazardais, Longhorn, British White, Murray Grey, Highland, Belgian Blue, Shorthorn and Hereford. The systems of husbandry and management were essentially conventional but, being high quality pedigree herds, the sale of breeding stock and regular exhibition at agricultural shows featured prominently in most enterprises.

Serological testing

All cattle, including calves, were blood sampled in 22 herds (10 dairy, 12 suckler): the actual number of calves sampled in these herds depended on the time of year of the test in relation to the calving pattern. Adult cattle only (≥two years) were tested in 31 herds (10 dairy, 21 suckler) and in four dairy herds testing was limited to a sufficient number of adult cattle to detect a 10% level of reactors with a 95% confidence (Cannon & Roe, 1982).

An enzyme-linked immunosorbent assay (ELISA) technique (Edwards et al, 1986) was used to examine sera for BHV1 antibodies. When the CHS was first introduced, cattle were deemed seropositive ("reactors") at optical density (OD) values of >0.1 using a serum dilution of 1/50, but an inconclusive reactor (IR) category (OD 0.10 - 0.19), which required resampling, was subsequently introduced in April 1989 to cope with some inconsistent results. In 1990, the 24 hour serum neutralisation test (SNT) (Edwards et al, 1986) was adopted as the decisive test for IRs that could not be clarified by ELISA: SNT titres of ≥1/2 were classed as positive. Reagent concentrations were altered in October 1989 resulting in a change in the OD range for IRs to 0.15 - 0.25, although the sensitivity of the test remained the same.

Collation and analysis of data

The OD values for each animal tested were expressed as either seropositive or seronegative and recorded with their ear tag or freeze

brand number, sex, age, source (homebred or purchased) and any individual vaccination history (if known).

Herds were coded D (Dairy) or S (Suckler) and numbered sequentially according to the date of the blood test. The serological results were tabulated and also displayed graphically to show the distribution of BHV1 antibodies according to herd identity, herd type (D or S), age and whether cattle were homebred or purchased. Herds were classified according to antibody prevalence and the apparent rate of seroconversion amongst susceptible cattle.

Results were analysed using standard statistical methods (Dunn, 1967); mean values, standard deviations (SD) and standard errors (SE) were calculated where appropriate. Differences in antibody prevalence between dairy and suckler herds were compared using the chi-square test. Correlation coefficients (r) were used to examine the association between the number of reactors and: herd size, number of cattle purchased in the previous 10 years and number of purchased cattle currently in the herd. The association between reactors and purchase was further examined by fitting a generalised linear model (binomial error, logit link) with Genstat 5 (1987), the linear predictor consisting of terms herds and homebred/purchase.

After the test results were known, herd managers were contacted by telephone to discuss the significance of the findings. In herds with a low seroprevalence, details of the movement history (eg communal marsh grazing, attendance at agricultural shows and temporary residence in other herds) of individual reactors and the length of time they had been in the herd were recorded. Where seropositive calves up to nine month old were known to have sucked seropositive dams, they were assumed to have acquired maternally derived (colostral) antibodies rather than infection.

RESULTS

Antibody prevalence according to type and size of herd

Sera from a total of 4,219 cattle (3,201 dairy, 1,018 suckler) aged ≥two years were examined for BHV1 antibodies: 639 (15.1%) were positive. Herd sizes and the number and proportion of reactors in dairy and suckler herds are shown in Tables 1 and 2. The proportion (14.6%) of reactors in dairy cattle was not significantly different to the proportion (16.8%) of reactors in sucklers (chi-square = 2.84, $p>0.5$). There was a significant correlation ($r=0.05$, $p<.05$) between the number of reactors and herd size in suckler herds but not in dairy herds.

There were no reactors in five (21.7%) of the 23 dairy herds and 11 (33.3%) of the 33 suckler herds (chi-square = 0.90, $p>.05$). Of the remaining dairy herds, 14 held 20% or fewer reactors but the other four (D3, D16, D20, D21) each contained more than 60% reactors. Fifteen suckler herds contained 20% or fewer reactors; only one herd (S27) had a seroprevalence of more than 60% but in two others (S19, S20) it exceeded 40%. The frequency distribution for the proportion of reactors in all herds is summarised in Fig. 1.

Table 1 - Prevalence of BHV1 reactors in dairy herds

Herd	Month/Year of test	Cattle aged ≥2 years No	+	%
D1	3/87	227	0	0
D2	3/87	*60 (95)	5	8.3
D3	4/87	136	97	71.3
D4	5/87	*26 (136)	5	19.2
D5	10/87	254	14	5.5
D6	10/87	189	0	0
D7	1/88	133	18	13.5
D8	4/88	154	6	3.9
D9	7/88	123	6	4.9
D10	11/88	114	0	0
D11	5/89	117	1	0.9
D12	11/89	*30 (161)	0	0
■D13	12/89	117	12	10.3
D14	1/90	140	2	1.4
D15	3/90	176	7	4.0
D16	3/90	*28 (200)	25	89.3
D17	3/90	260	19	7.3
D18	5/90	121	7	5.8
D19	9/90	52	0	0
D20	2/91	134	84	62.6
D21	2/91	147	92	62.5
D22	2/91	196	39	19.9
D23	3/91	267	29	10.9
Totals	3/87 - 3/91	3201	468	14.6

No Number of cattle tested for BHV1 antibodies
\+ Number of reactors
% Percentage of reactors
* Statistical screening only (total number of cattle ≥2 years in herd)
■ IBR in 1981

Table 2 - Prevalence of BHV1 reactors in suckler herds

Herd	Month/Year of test	Cattle aged ≥2 years No	+	%
S1	7/87	24	2	8.3
S2	2/88	10	0	0
S3	2/88	18	1	5.5
S4	2/88	90	3	3.3
S5	3/88	56	6	10.7
S6	4/88	7	1	14.3
S7	11/88	16	1	6.3
S8	12/88	60	4	6.7
S9	1/89	20	0	0
S10	2/89	29	4	13.8
S11	5/89	25	0	0
S12	6/89	56	11	19.6
S13	7/89	25	2	8.3
S14	7/89	4	0	0
S15	7/89	5	0	0
S16	11/89	9	0	0
S17	12/89	27	6	22.2
S18	1/90	86	4	4.7
S19	2/90	76	42	55.3
S20	4/90	125	55	44.0
S21	5/90	18	0	0
S22	6/90	6	0	0
S23	9/90	10	3	30.0
S24	10/90	25	0	0
S25	11/90	12	0	0
S26	11/90	8	2	25.0
S27	11/90	16	10	62.5
S28	12/90	40	0	0
S29	12/90	13	5	38.5
S30	12/90	10	2	20.0
S31	12/90	22	2	9.1
S32	1/91	35	4	11.4
S33	1/91	35	1	2.9
Totals	7/87-1/91	1018	171	16.8

No Number of cattle tested for BHV1 antibodies
\+ Number of reactors
% Percentage of reactors

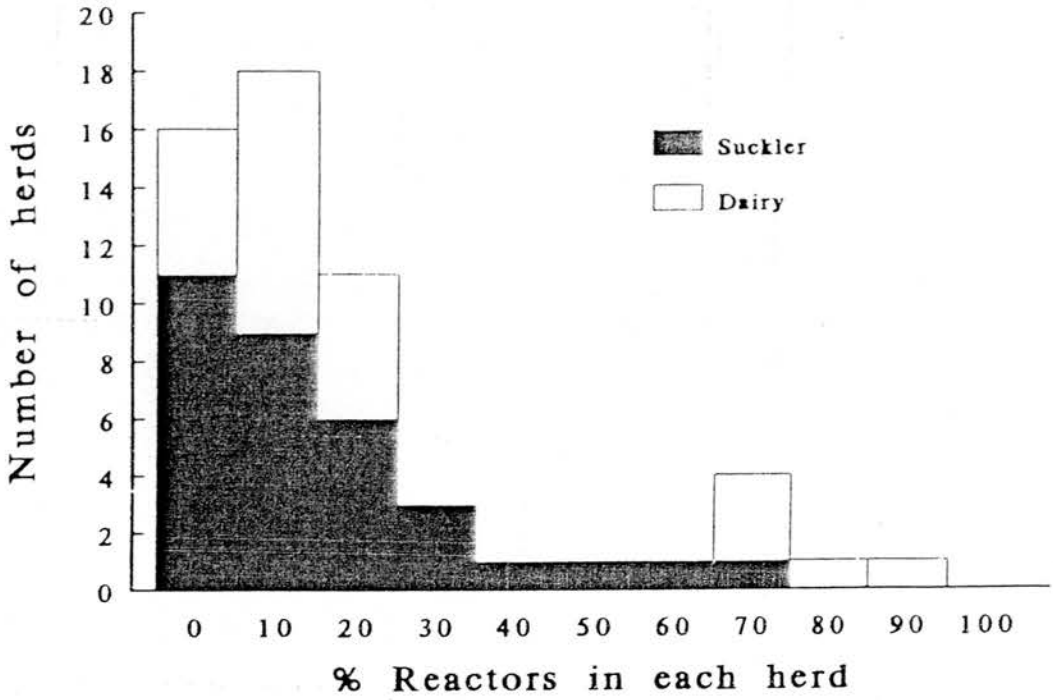

Fig. 1 - Frequency distribution of BHV1 reactors in CHS herds

Antibody prevalence according to source of origin

Breeding replacements were purchased from a wide geographical area, mainly outside Norfolk, extending from Scotland to the West Country and continental Europe. Only three herds (D1,D6,D9) had been completely closed during the previous 10 years (Tables 3, 4), with the mean number of purchases being 17.1 ± SD 17.5 in dairy herds and 22.7 ± SD 22.5 in suckler herds. The mean number of purchased cattle in dairy herds at the time of the test (7.2 ± SD 8.4) was less than half that in suckler herds (15.9 ± SD 20.1) but the latter included several recently established herds (notably S27, which had been formed a year previously from cattle purchased exclusively from S19). Significant correlations were noted in suckler herds, but not in dairy herds, between the number of reactors and the number of cattle purchased in the previous 10 years ($r = 0.42$ $p<.05$) and between the number of reactors and the number of purchased cattle in the herd at the time of the test ($r = 0.63$, $p<.01$). In dairy herds, the mean percentage of purchased reactors (41.3 ± SE 3.2) was significantly greater than that of homebred reactors (17.6 ± SE 0.7) ($p<.001$). In suckler herds this difference was less marked (13.9 ± SE 1.5, 9.9 ± SE 1.4 respectively) but still statistically significant ($p<.05$).

In two dairy herds (D8, D11) and 14 suckler herds (S1, S3, S6, S7, S8, S10, S13, S17, S26, S27, S29, S30, S31, S32) all reactors had been purchased; some comprised the original foundation stock, still in the herd after seven (S30) or eight (S6) years. Many of the purchased reactors in suckler herds, but not in dairy herds, were embryo transfer recipients known to have been vaccinated before introduction.

Table 3 - Prevalence of BHV1 reactors in dairy herds according to source

Herd	Purchased Cattle			Homebred Cattle	
	No in 10 years	No in herd	No of Reactors	No in Herd	No of Reactors
D1	0	0	0	227	0
*D2	25	5	3	55	2
D3	24	16	15	120	82
*D4	30	2	1	24	4
D5	2	2	1	252	13
D6	0	0	0	189	0
D7	60	34	7	99	11
D8	58	20	6	134	0
D9	0	0	0	123	6
D10	21	14	0	100	0
D11	10	10	1	107	0
*D12	6	4	0	26	0
D13	8	8	4	109	8
D14	8	8	1	132	1
D15	40	16	5	160	2
*D16	32	9	9	19	16
D17	2	1	1	259	18
D18	10	2	1	119	6
D19	10	0	0	52	0
D20	3	1	0	133	84
D21	25	4	4	143	88
D22	10	9	5	187	34
D23	9	0	0	267	29
Totals	393	165	64	3036	404

* Statistical screening only

No in 10 years: Total number of cattle purchased in the previous 10 years

No in herd : Total number of purchased cattle in the herd and included in the test

Table 4 - Prevalence of BHV1 reactors in suckler herds according to source

Herd	Purchased Cattle			Homebred Cattle	
	No in 10 years	No in herd	No of Reactors	No in Herd	No of Reactors
S1	20	12	2	12	0
S2	3	1	0	9	0
S3	18	3	1	15	0
S4	8	3	2	87	1
S5	30	23	4	33	2
S6	5	5	1	2	0
S7	36	6	1	10	0
S8	25	18	4	42	0
S9	6	6	0	14	0
S10	27	27	4	2	0
S11	8	4	0	21	0
S12	54	54	10	2	1
S13	25	25	2	0	0
S14	4	4	0	0	0
S15	3	3	0	2	0
S16	4	4	0	5	0
S17	24	12	6	15	0
S18	94	86	4	0	0
S19	65	47	21	29	21
S20	45	68	26	57	29
S21	18	18	0	0	0
S22	12	6	0	0	0
S23	4	4	2	6	1
S24	6	1	0	24	0
S25	4	3	0	9	0
S26	8	8	2	0	0
S27	16	16	10	0	0
S28	30	9	0	31	0
S29	13	13	5	0	0
S30	4	4	2	6	0
S31	70	4	2	18	0
S32	10	8	4	27	0
S33	50	20	0	15	1
Totals	749	525	115	493	56

No in 10 years: Total number of cattle purchased in the previous 10 years

No in herd : Total number of purchased cattle in the herd and included in the test

Antibody prevalence according to age

As shown in Tables 5 and 6 the mean antibody prevalence in dairy and suckler herds calculated from pooled data declined from about 6% in calves aged up to six months to virtually nil (only one reactor from 220 calves) by 12 months. Seroprevalence amongst yearlings, two-year-olds and three-year-olds was markedly lower in dairy herds than in suckler herds but since most of the latter reactors were limited to a few herds (particularly S19), statistical comparisons between herd types were considered inappropriate. From four years of age, mean seroprevalence increased at a similar rate in both types of herd and by 10 years, 45% of all cattle were reactors, including the eight (in herds D21,D22,S17,S30 and S32) that were 15 years or older.

All reactors detected amongst calves up to six months old and the single reactor aged nine months in D5 had received colostrum from known seropositive dams, with the exception of the reactor in S9 which had been purchased from a local market at two months of age. Single reactors were present amongst cattle less than two years old in three herds (D10, S9, S11) in which all older cattle were seronegative. Those in S9 and S11 were both purchases and that in D10 was a homebred heifer known to have broken in with a group of cattle of unknown disease status whilst grazing communal marshland about a month before the test.

As illustrated by the pooled data presented in Figs 2 and 3 the age-specific prevalence for individual herds revealed two distinct patterns of seroconversion which corresponded to the main antibody prevalence groupings noted earlier. In the four dairy herds (D3, D16, D20, D21) with a high prevalence (HP) of BHV1 antibodies (>60%) seroconversion appeared to commence amongst heifers within two or three months after they joined the main herd for calving; uncalved heifers on separate grazing were all seronegative. By four years of age, 75% of cattle in these herds were reactors. In the 14 dairy herds with a low prevalence (LP) of BHV1 antibodies (<20%) there was no such evidence of active infection amongst heifers after calving and less than 5% of cows in their second or third parity were seropositive. The proportion of reactors in LP dairy herds rose sharply after six years of age (Fig. 2); seroprevalence amongst cattle aged nine years or more was higher than average in D5, D7, D22 and D23 (Table 5).

Only three suckler herds (S19, S20, S27) showed marked increases in seroprevalence with age indicative of extensive virus circulation: these were also herds identified previously as having a high prevalence of BHV1 antibodies (>40%). Instead of commencing amongst recently calved heifers, seroconversion in the HP suckler herds appeared to start at between one and two years of age and to continue at a lower rate after calving than in HP dairy herds. The age-specific prevalence pattern in LP suckler herds (Fig. 3) was virtually identical to that recorded in LP dairy herds (Fig. 2).

Table 3 - Age-specific prevalence of BHV1 antibodies in dairy herds

Herd Ref	Age in years 0 - ½		>½ - <1		1		2		3		4		5		6		7		8		9		≥10	
	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+
D1	4	0	32	0	30	0	70	0	38	0	34	0	27	0	13	0	15	0	10	0	8	0	12	0
#D2*									17	0	5	0	12	0	6	2	6	0	8	1	6	2		
■D3					70	1	4	0	23	5	23	10	32	31	23	22	9	9	6	6	3	3	13	11
#D4*							6	0	5	1	1	0	3	0	2	1	4	2	1	0	3	0	1	1
#D5			1	1	1	0	71	0	42	0	40	0	40	1	27	0	16	1	5	1	8	8	5	3
D6	19	0	28	0	68	0	13	0	48	0	32	0	30	0	28	0	16	0	9	0	3	0	10	0
#D7							24	0	17	0	21	1	27	2	21	0	4	0	2	1	8	7	9	7
#D8					25	0	30	0	20	0	14	0	22	0	32	1	28	5	6	0			2	0
#D9	7	0			46	0	17	0	20	0	18	1	15	0	16	0	9	0	13	4	4	0	11	1
D10	23	0	6	0	13	1	18	0	17	0	26	0	7	0	8	0	10	0	8	0	6	0	14	0
#D11	12	0	9	0	32	0	21	0	32	0	22	1	6	0	9	0	6	0	2	0	9	0	10	0
D12*									10	0	3	0	5	0	1	0	4	0	2	0	3	0	2	0
#D13	21	2			13	0	19	0	24	2	19	0	19	1	15	0	11	3	3	0	1	0	6	6
#D14	6	0			8	0	21	0	26	0	21	0	16	0	16	0	11	0	13	0	7	1	9	1
#D15							24	0	17	0	21	0	37	2	21	0	27	3	18	1	8	1	3	0
■D16*					2	0	2	0	13	13	3	3	2	2	1	1	3	2	1	1	1	1	2	2
#D17							54	0	24	0	39	0	41	0	37	1	12	0	21	3	12	2	20	13
#D18							7	0	28	0	24	0	22	0	14	0	7	1	2	0	10	2	7	4
D19	17	0	6	0	9	0	10	0	5	0	10	0	6	0	5	0	11	0	2	0	2	0	1	0
■D20					6	0	12	0	21	2	19	12	22	16	26	21	18	17	7	7	3	3	6	6
■D21							9	0	29	2	39	38	17	13	17	10	4	4	6	5	7	4	19	16
#D22					4	0	55	0	23	0	23	1	19	3	26	4	22	13	14	6	4	4	8	8
#D23	65	9	41	0	56	0	64	0	53	0	40	1	30	4	26	3	28	5	12	7	9	5	5	4
Totals	174	11	123	1	383	2	551	0	555	25	499	68	457	75	390	66	281	65	171	43	125	43	175	83
Reactors %	6.3		0.8		0.5		0		4.5		13.6		16.4		16.9		23.1		25.1		34.4		47.4	

* Statistical screening only ■ High prevalence herd # Low prevalence herd

Table 6a - Age-specific prevalence of BHV1 antibodies in suckler herds

Herd Ref	Age in years: 0 - ½		>½ - <1		1		2		3		4		5		6		7		8		9		≥10	
	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+
#S1	23	2	4	0	16	0	3	0	3	0	4	0	4	0	5	0			1	1	2	0	2	1
S2					15	0	5	0	1	0	3	0			1	0								
#S3									3	0	5	1	2	0	1	0	3	0	1	0			3	0
#S4	42	0	10	0	34	0	29	0	18	0	13	0	5	1	9	0	4	0	3	0	4	0	5	2
#S5			4	0	18	7	11	3	13	1	6	0	10	1	4	0	6	1	4	0			2	0
#S6	2	1			2	0	3	0	1	0	1	0					1	0					1	1
#S7	3	0	12	0			4	0	2	0	3	0	1	0	2	1	3	0	1	0				
#S8	2	0	14	0	22	0	22	0	10	0	9	0	6	0	8	0	1	0			1	1	3	3
S9	5	1			15	0	3	0	3	0	3	0	4	0	1	0	1	0	2	0	1	0	2	0
#S10					9	2	25	3	3	1													1	0
S11	2	0	13	0	3	1	4	0	4	0	4	0	3	0	3	0	2	0	2	0	1	0	2	0
#S12	11	1	9	0	23	0	11	0	10	1	5	2	6	1	3	1	2	0	2	1	2	0	15	5
#S13	21	2	3	0					19	0	4	1	1	0									1	1
S14	3	0							3	0			1	0										
S15					1	0	2	0							3	0								
S16	2	0	7	0	1	0	2	0	2	0	1	0	1	0			1	0	1	0	1	0		
#S17	6	0	12	0	7	0	4	0	6	0	3	1	4	0	1	0	2	1	1	1	1	1	5	2
#S18							23	0	2	1	31	0	30	3										

...continued/

Table 6b - Age-specific prevalence of BHV1 antibodies in suckler herds cont'd

Herd Ref	Age in years																							
	0 - ½		>½ - <1		1		2		3		4		5		6		7		8		9		≥10	
	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+	No	+
■S19					1	0	10	9	16	12	10	3	6	1	9	2	6	5	6	2	6	2	7	6
■S20					29	18	32	3	23	6	16	10	5	2	13	4	20	17	3	3	4	3	9	7
S21							18	0																
S22			6	0											6	0								
#S23							2	0	1	0			1	0	1	1					2	1	3	1
S24							7	0	8	0	1	0	4	0	1	0	2	0			1	0	1	0
S25			4	0			2	0	4	0			4	0			1	0					1	0
#S26	5	0			5	0	2	0					6	2										
■S27	5	1	4	0	4	2	6	4	4	3	2	0	2	2					1	0			1	1
S28							4	0	16	0	11	0	3	0	1	0	2	0			1	0	2	0
#S29							6	4	1	0	2	0	2	1	2	0								
#S30			1	0			3	0			1	0	1	0	1	0			2	0			2	2
#S31							13	0	9	2														
#S32					7	0	8	0	7	0	5	0	1	0	3	0	1	0	1	0	2	0	7	4
#S33							2	0	5	0	5	0	6	0	3	0	6	1			3	0	5	0
Totals	132	8	97	0	212	30	266	26	197	27	140	18	119	14	81	9	64	25	31	8	32	8	80	36
Reactors %	6.1		0		14.2		9.8		13.7		12.2		11.8		11.1		39.1		25.8		25.0		45.0	

■ High prevalence herd

Low prevalence herd

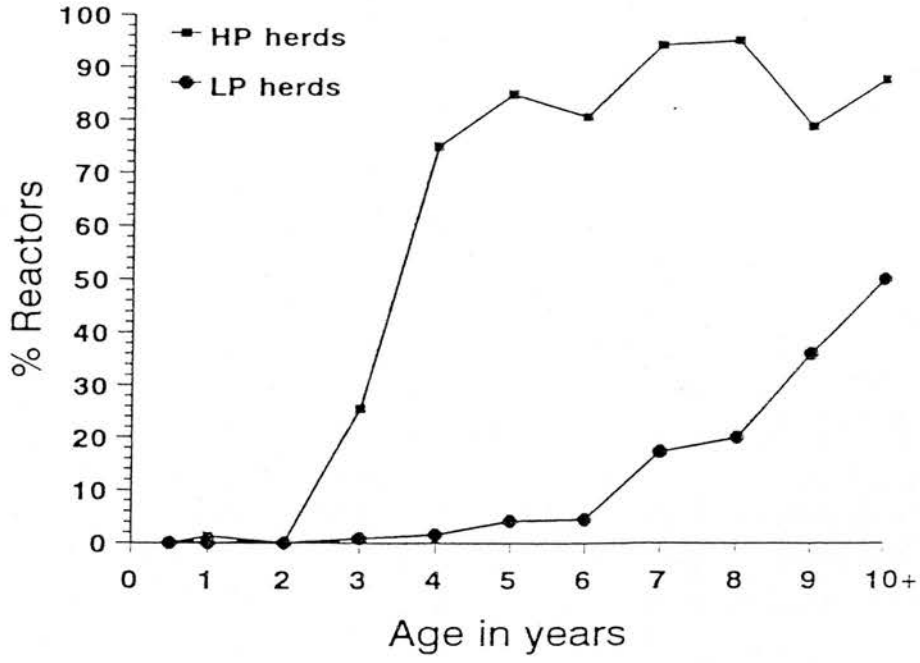

Fig. 5.2 - Seroconversion patterns in dairy herds according to antibody prevalence category

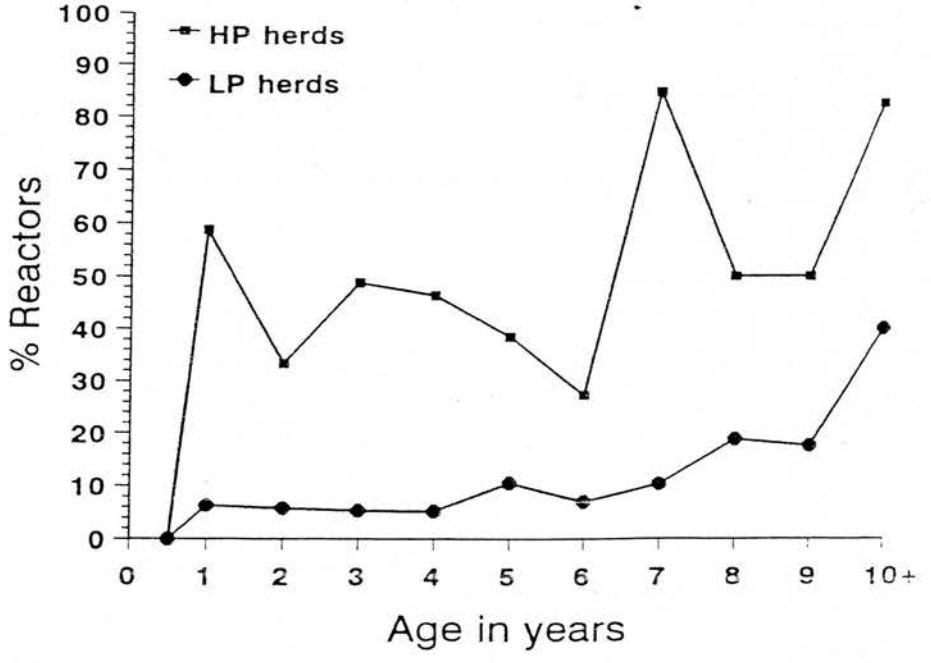

Fig. 5.3 - Seroconversion patterns in suckler herds according to antibody prevalence category

DISCUSSION

The 56 herds included in this study represented about 9% of all dairy and suckler herds containing 10 or more cows listed in the 1989 agricultural census for Norfolk (MAFF, 1990). The extent of BHV1 infection in this sample population was essentially unknown before investigations commenced: apart from D13 which had experienced an epidemic outbreak eight years previously, none of the herds had any clinical history of IBR. The large proportion of herds with a low prevalence of BHV1 antibodies appeared similar to the situation in Germany (Forschner, 1988) and Spain (Espuna et al, 1988). The latter authors lent further support to the present studies by also finding that herds tended to be either heavily or lightly infected, with few having intermediate seroprevalence.

The significant correlation between herd size and the number of reactors in suckler herds appeared mainly to reflect a tendency for larger suckler herds to contain more purchased cattle. On average, suckler herds contained more than twice as many purchased cattle as dairy herds. The four largest dairy herds (D1, D5, D17, D23) had purchased a total of only 13 replacements during the previous 10 years. The strong association between reactor status and purchase supported the generally accepted view (Gibbs & Rweyemamu, 1977; Pastoret et al, 1984) that BHV1 is most frequently introduced by the addition of latently infected carriers to susceptible herds.

Remarkably, purchased cattle - including many that had been in the herd for several years - were the only reactors detected in 16 of the 40 infected herds, suggesting that no virus transmission had occurred following their introduction. Similarly, in herds in which some homebred cattle were also reactors, anecdotal information often suggested sources of infection other than lateral spread from purchased animals. In S4, for example, the only homebred reactor was a bull that had been loaned to a herd in the south of England for four months before returning to Norfolk; in D13, six of the eight homebred reactors had been in the herd during the IBR outbreak recorded in 1981. No satisfactory explanation was found for the exclusively homebred reactors in D9 and S33 but it was assumed that they had acquired infection in unrecorded incidents, similar to that in D10, in which a heifer broke in with a group of other cattle.

Two of the homebred reactors in D18 were specifically identified as having attended several agricultural shows. However, several other large herds had exhibited cattle at six or seven major shows each year for the previous 15 years without any evidence of them acquiring infection from this source. Although detailed records were rarely available to make any systematic risk assessment, the present findings suggested that agricultural shows and similar livestock events did not pose any major threat, particularly when there was only a short period of close contact with other cattle.

Overall, field observations suggested that, in most herds, the risk of virus being transmitted from seropositive cattle (including vaccinates) to susceptible in-contacts was quite small. Although reexcretion of latent virus is undoubtedly of potential epidemiological importance, its role under natural conditions, in the absence of experimental provocation, appears to have been overestimated. Van Nieuwstadt & Verhoeff (1983) similarly found no evidence of virus transmission for at least three years in eight of 20 dairy herds in Holland and Baker et al (1989) detected only a very low rate of transmission of live intranasally administered vaccinal virus to co-mingled steers. In contrast, widespread virus circulation, manifested by a high rate of seroconversion, was clearly evident in the seven herds with the highest antibody prevalence. Presumably, these herds were initially infected via purchased replacements or similar sources but unlike in LP herds, the virus then appeared to have spread rapidly to susceptible cattle, amongst which it was maintained sufficiently to infect each year's input of heifers.

There were no discernible differences between HP and LP herds in environmental or intercurrent disease stress factors likely to cause viral reactivation. Edwards et al (1991) found that calves infected with BHV1 subtype 1 excreted more virus than those infected with subtype 2b strains and it appeared likely (S Edwards, personal communcation) that the apparent difference in virus behaviour between LP and HP herds was associated with different strains of virus, possibly beyond the sensitivity of simple restriction endonuclease fingerprinting techniques. This hypothesis was supported by the observation that herd S27, which was formed entirely from cattle in S19, had assumed the seroconversion pattern of its parent herd.

Corkish (1988) suggested that calves in suckler herds were more likely to acquire infection from their dams than those in dairy herds which were removed soon after birth. There was, however, no serological evidence from these studies to indicate that active infection had occurred amongst calves aged less than a year. Maternally derived antibodies invariably disappeared by about six months and although sera were examined from calves in only one (S27) of the three HP suckler herds, consistently seronegative findings from calves in other herds in this age range suggested that the likelihood of them acquiring infection through sucking seropositive dams was small.

Seroconversion occurred amongst 12 - 24 month old heifers in HP herds S20 and S27 but not in the HP dairy herds. This difference was attributed to the management practice commonly adopted in suckler herds, whereby maiden heifers were housed close to adult cattle during the winter. Similar findings, including seroconversion amongst yearlings, were noted in suckler herds in the north-west of Scotland (N. G. Brookes, personal communication). In dairy herds, replacement heifers were almost invariably maintained as completely separate populations, on marsh or upland grazing, until they joined the milking herd after calving. Hence, provided they did not acquire infection from outside sources, uncalved heifers from HP dairy herds were seronegative on

entering the milking herd. By comparing serological status with date of calving it appeared that most subsequent seroconversion in HP herds occurred two or three months after calving, coinciding with winter housing.

In LP herds the likelihood of cattle becoming reactors did not increase until after six years whereas most cattle in HP herds had seroconverted by this time. Regardless of prevalence category almost 50% of all cattle in infected herds, aged 10 years or more, were seropositive. The preponderance of reactors amongst older animals suggested that even the LP herds had been heavily infected in the past, but with regular routine culling, infection was gradually working its way out of these herds. Provided it is not reintroduced, self-elimination of BHV1 from LP herds appears to be inevitable in view of the lack of spread to susceptible herd members. Van Nieuwstadt & Verhoeff (1983) similarly suggested that BHV1 might disappear in the long-term from herds in which no recent virus circulation had occurred. Conversely, BHV1 infection was likely to be maintained indefinitely in HP herds unless there was a change in virus behaviour or in some environmental factor not apparent from these studies. There was no obvious explanation to account for the presence of eight seronegative cows from amongst the 57 aged 10 years or more in the seven HP herds.

Because of the high rate of virus circulation detected in HP herds, further enquiries were made to check whether there was any clinical history that could possibly be attributed to BHV1 infection. These revealed that in August 1989, four recently calved cows in D21 experienced pyrexia and milk drop; a further similar case occurred in October 1990. No BHV1, bovine virus diarrhoea virus or Leptospira hardjo antibodies had been detected in blood samples taken from these cows at the acute stage of illness but when they were retested for the CHS in February 1991 all five were found to have seroconverted to BHV1. Although not conclusive because of the long time interval between testing, this evidence suggested that the herd had experienced clinical recrudescence. Further evidence linking recrudescence and HP status, in the absence of a history of epidemic IBR, subsequently emerged from D3. At the time of the herd test in 1987 there was no clinical history of BHV1 infection but several recrudescences were subsequently confirmed by paired serology amongst second and third parity cows between 1989 and 1991.

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ELIMINATION OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS INFECTION FROM A HEAVILY INFECTED DAIRY HERD

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ABSTRACT

Infectious bovine rhinotracheitis virus (IBRV) is widespread in the cattle population of the United Kingdom and elsewhere in the world. The effects of IBRV infection vary from mild or inapparent illness to severe respiratory disease. In recent years there has been much interest in the elimination of IBRV infection, particularly in Europe, because of its significance to trade in live cattle, semen and embryos. Infection can be eliminated from herds with a low seroprevalence by a simple test and cull approach, combined with disease security precautions but this method is not feasible in heavily infected herds with extensive virus circulation. This paper describes the elimination of IBRV infection from such a herd under commercial conditions.

During the previous six months, four of 10 cows tested had seroconverted thereby confirming that IBRV infection was active in the herd. At the start of the trial a total of 106 (84.8 percent) of the 125 milking cows were seropositive (reactors). Uncalved homebred heifers were all seronegative. The 19 seronegative cows were managed separately from the cows in the reactor group. The latter were vaccinated with an inactivated polyvalent IBR vaccine to reduce the risk of them shedding latent virus and infecting seronegative cattle. Some contact was still possible between the groups, particularly during winter housing. As they calved, the heifers (which had been reared on separate premises), were gradually added to the seronegative group and reactors were culled. The serological status of the uninfected group was monitored regularly.

During the course of the trial three new reactors were detected and subsequently culled. The herd achieved seronegative status within 30 months. Although the relative benefits of vaccination and partial segregation could not be evaluated, the trial showed that elimination of IBRV infection was a feasible and economically viable proposition even in a heavily infected herd. The findings are discussed with reference to the epidemiology of IBRV infection.

KEYWORDS: Recrudescence, herpesvirus, IBR, vaccines, eradication

INTRODUCTION

Infectious bovine rhinotracheitis virus (IBRV) is responsible for a wide range of conditions varying from subclinical or mild infection to severe respiratory disease. Infectious bovine rhinotracheitis (IBR), was first recorded in USA in the 1950s and has since been identified throughout the world. In Britain, a serious respiratory form emerged after 1976 (Edwards 1988). The more common, clinically milder forms of IBR cause bilateral conjunctivitis with lacrimation and mucopurulent nasal discharge. However, in many cases IBRV infection is subclinical. Recrudescences can occur in endemically infected closed herds (Kahrs 1977).

As a typical herpesvirus, IBRV infection exhibits the phenomenon of latency whereby latent virus can be reactivated and reexcreted (Sheffey and Rodman 1973). Various stress factors including transportation, parturition and intercurrent disease have been associated with reactivation of either field or vaccinal virus. Latency dictates that after recovering from acute infection cattle remain a potential source of IBRV for life.

Although reactivation and shedding of latent virus can be induced experimentally, the extent to which this occurs naturally under field conditions appears to be variable. In a study in Norfolk, England, Pritchard (1992a) noted that in breeding herds in which more than 40% of adult cattle were seropositive, most incoming heifers seroconverted to IBRV within two

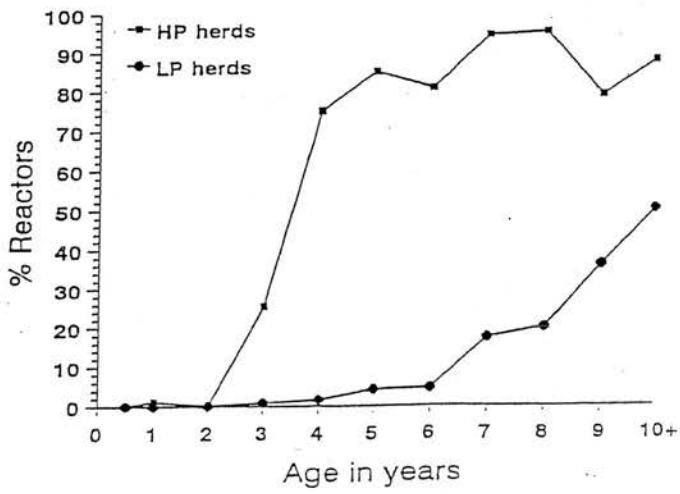

Fig 1: Seroconversion patterns in dairy herds according to antibody prevalence category (Pritchard 1992a)

years of first calving (Fig 1) but there was virtually no evidence of virus transmission in herds containing <20% serological reactors. Those reactors that were present in these low prevalence (LP) herds, were mainly restricted to purchased animals or older herd members. There was little or no serological evidence of infection in young stock, even in high prevalence (HP) herds. Maternally derived antibodies usually persisted until six months of age (exceptionally nine months). Calves in infected dairy herds remained seronegative provided they were kept apart from the cows and external sources of infection until they rejoined the adult herd after their first calving. The apparent difference between LP and HP herds was attributed to virus strain effects rather than extraneous stress factors.

Whereas potent live vaccines are available to prevent economic losses from IBR, their widespread adoption in countries such as the USA has caused problems with the export of live cattle, semen and embryos to parts of the world demanding seronegative status (this aspect can be overcome with the use of recently developed gene deletion marker vaccines). Inactivated vaccines have been used to boost the immunity of latently infected carriers to reduce viral excretion and thereby prevent spread to uninfected herd members: virus shedding is apparently suppressed if humoral antibody titres can be maintained at a high level by repeated vaccination (Forschner 1988).

This paper describes the elimination of IBRV infection from a heavily infected dairy herd under commercial conditions using a combination of test-and-cull, partial segregation and vaccination.

MATERIALS AND METHODS

Herd Details

The study was undertaken in an established 125-cow family-run Holstein/Friesian dairy herd in Suffolk, England. Cows were winter-housed in strawed yards and calved July to September. The herd experienced a classical outbreak of IBR with high morbidity, pyrexia, ocular-nasal discharge and milk drop in December 1984 following the purchase of two cows from a dispersal sale. The herd was subsequently vaccinated with a modified live virus vaccine (Tracherine, Smith-Kline Animal Health Ltd) and revaccinated in July 1985. After the IBR outbreak had resolved, sporadic recrudescences with pyrexia, milk drop and mild respiratory signs, were confirmed between 1986 and 1988 by serological examination of acute and convalescent blood samples. Recrudescences mainly affected a few second and third parity cows and indicated that IBRV was still circulating in the herd.

In June 1988, 31 (75.6%) of 41 randomly selected adult cattle proved to be seropositive (optical density ≥0.1) by ELISA (Edwards and others 1986). All cattle aged at least a year were subsequently tested in December 1988: 106 (84.8%) of the 125 milking cows then in the herd were seropositive but the younger cattle, including 10 uncalved two-year-olds, which had not yet entered the main herd, were seronegative (Table 1). Of 10 cows that were seronegative in June 1988 and retested in December 1988, four (aged between 2½ and four years) had seroconverted to IBRV, confirming recent virus circulation and HP status.

Table 1: Age-specific prevalence of IBRV antibodies before commencement of eradication programme

Age (years)	Cattle tested No	+	%
1	23	0	0
2	20	10#	50.0
3	26	19	73.1
4	25	23	92.0
5	17	17	}
6	8	8	}
7	7	7	} 100*
8	6	6	}
9	8	8	}
≥10	8	8	}

\# All recently calved
No Number of cattle tested
\+ Number of reactors
% Percentage of reactors
* All vaccinated in 1984/8

Elimination Strategy

Uncalved heifers and the 19 seronegative cows identified in December 1988 were to provide the basis for a new IBR-free milking herd to be established within about three years, without the need to purchase any seronegative breeding replacements. Between January 1989 and June 1991, a non-infected group, initially comprising the 19 seronegative cows, was managed separately from the infected group (initially consisting of 106 cows). Recently calved home-bred heifers were introduced into the negative group and infected cows were gradually culled as convenient. Instead of allowing 1987-born heifers to calve as two-year-olds in 1989, service was delayed in order to calve most as three-year-olds in 1990; 1988-born heifers also calved in 1990, but as two-year-olds. These managemental changes permitted as many seronegative cattle as possible to be introduced simultaneously, having first reduced the size of the infected group. Heifers were served exclusively by an IBRV-seronegative bull and kept strictly apart from the main herd until calving.

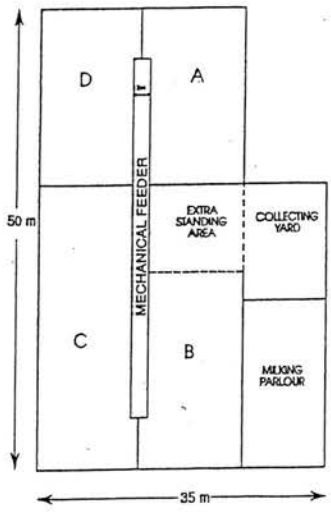

Fig 2: Layout of buildings to show groups of cattle

Seronegative cows were milked first and had no direct contact with the reactors either in the milking parlour or the collecting yard; separate equipment and utensils were used for infected and uninfected groups as far as practicable. The two groups were grazed separately and had no direct contact between the months of April and September. There was insufficient accommodation to house them totally apart during the winter but contact was minimised by dividing up the strawed yard (Fig 2). In 1989 and 1990, the small negative group occupied section A; in 1991 the expanded negative group went into sections B, C and D and the remaining reactors were put into section A. Some head-to-head contact was still possible between cattle in sections A and B through unenclosed metal gates which were moved to provide an extra standing area during milking; a mechanical feeder, about 2 m wide separated most of sections A and D.

In addition to segregation, the reactors were vaccinated with an inactivated polyvalent vaccine (Pneumovac Plus, C-Vet Ltd) in an attempt to reduce virus shedding during winter housing by following the approach adopted in Germany (Forschner, 1988). Seropositive cattle received two 2 ml doses of vaccine, intramuscularly, in January and February 1989 (about four weeks apart); those still in the infected group were revaccinated in November 1989 and September 1990. The seronegative group were blood tested every two to six months to monitor progress; any new reactors were moved into the positive group and vaccinated.

RESULTS

Most reactors were culled during 1990 and 1991 to coincide with the main influx of heifers; a few remaining reactors were sold shortly before the final test in June 1991 which confirmed that IBRV infection had been eliminated within two-and-a-half years. A total of three new reactors were detected during this period (Table 2) and were put into the positive group. In addition, an inconclusive reactor was detected in February 1989. This was initially moved into the infected group where it remained for several months before rejoining the uninfected cows after proving to be seronegative with the 24 hour serum neutralisation test (Edwards and others, 1986).

Table 2: Progressive elimination of IBRV infection from a heavily infected dairy herd

Date of blood test (Month/year)	NUMBER OF CATTLE		
	Positive group*	Negative group	New reactors
12/88	106	19	0
2/89	103	19	0
4/89	102	19	1
8/89	100	26	0
12/89	91	27	0
6/90	91	30	2
9/90	63	74	0
4/91	30	88	0
6/91	0	86	0

* Not retested after 12/88

DISCUSSION

An IBR Monitoring Scheme operated by the State Veterinary Service of Great Britain (Penton 1987) demonstrated that IBRV infection could be readily eliminated from LP herds using a simple test-and-removal approach because of the virtual absence of virus circulation in these herds. In HP herds, however, a more aggressive approach was necessary because of the high rate of virus transmission (Pritchard 1992b).

The inevitable constraints imposed on field trials performed under farm conditions meant that it was not possible to set up a control group for this study. However, the previously established history of extensive active infection provided a good basis for comparison. It was impossible to determine whether the successful elimination of IBRV infection was achieved by partial separation alone or whether vaccination had played a significant part. Although this vaccine had been used with some success in Germany (GD Bell, personal communication) it is no longer available in Britain and was generally considered to be of relatively low efficacy at least for the control of clinical disease (Frerichs and others, 1982).

The strategic use of inactivated vaccines to reduce virus shedding by latently infected carriers has formed an integral part of voluntary control schemes in several European countries. It is, however, not clear from the literature whether the apparently successful use of inactivated vaccine to stabilise virus transmission applies equally to herds in the HP category as to those containing only a few reactors. The latter LP herds would be expected to exhibit only minimal virus shedding even without vaccination. Significantly, in a small study at the Central Veterinary Laboratory,

Weybridge, the inactivated vaccine used here failed to reduce the titre of virus shed from experimentally infected calves following the administration of Dexamethasone (S Edwards, personal communication).

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ZUSAMMENFASSUNG

Das infektiöse bovine Rhinotracheitis-Virus (IBRV) ist im Rinderbestand Großbritanniens ebenso wie in anderen Teilen der Welt weit verbreitet. Die Auswirkung einer Infektion mit IBRV erstreckt sich von einer milden oder heimlich verlaufenden Erkrankung bis hin zu einer ernsten Erkrankung der Luftwege. Seit einigen Jahren besteht reges Interesse an einer Auzmerzung der IBRV-Infektionen, besonders in Europa, weil sie den Handel mit lebendem Vieh, mit Sperma und mit Embryonen beeinträchtigen. In Herden mit geringer Durchseuchung gemessen an der Seroreaktion kann die Infektion durch ein einfaches Vorgehen mittels Tests und Auslese bei gleichzeitig angewandten Vorsichtsmaßnahmen gegen Infizierung ausgemerzt werden, aber diese Methode läßt sich bei stark befallenen Herden mit weithin zirkulierendem Virus nicht durchführen. Die vorliegende Arbeit beschreibt, wie eine IBRV-Infektion aus einer solchen Herde unter kommerziellen Bedingungen ausgemerzt wurde.

Im vorangegangenen halben Jahr hatte sich gezeigt, daß das Serum in vier von zehn getesteten Kühen von negativ nach positiv umgeschlagen war, womit der Beweis erbracht war, daß eine Infektion mit IBRV in der Herde umging. Zu Beginn des Versuchs waren insgesamt 106 (84,8 Prozent) von 125 Milchkühen seropositiv (positive Reagenten).

Alle Färsen aus der heimischen Zucht, die noch nicht gekalbt hatten, waren seronegativ. Die 19 seronegativen Kühe wurden getrennt von den Kühen in der Gruppe der positiven Reagenten gehalten. Letztere wurden mit einem inaktivierten multivalenten IBR-Impfstoff geimpft, um die Gefahr zu vermindern, daß durch sie latente Viren verbreitet und seronegative Rinder angesteckt wurden. Kontakte zwischen beiden Gruppen konnten nicht völlig verhindert werden, vor allem nicht während der Winterstallhaltung. Nachdem die Färsen (die völlig getrennt von den übrigen Tieren aufgezogen worden waren) gekalbt hatten, wurden sie nach und nach der seronegativen Gruppe zugeführt, und positive Reagenten wurden ausgesondert. Der serologische Zustand der nicht infizierten Gruppe wurde regelmäßig überwacht.

Im Verlauf des Versuchs wurden drei Tiere gefunden, die zu positiven Reagenten geworden waren und danach ausgesondert wurden. Innerhalb von 30 Monaten erreichte die Herde einen seronegativen Zustand. Obwohl der relative Nutzen von Imfpung und teilweiser Absonderung nicht genau abgewogen werden konnte, zeigte der Versuch, daß ein Ausmerzen der Infektion mit IBRV auch in stark infizierten Herden durchführbar und wirtschaftlich vertretbar ist. Die Ergebnisse werden mit Berücksichtigung der Epidemiologie der Infektion mit IBRV besprochen.

RÉSUMÉ

Le virus de la rhino-trachéite bovine infectieuse (IBRV) est largement répandu dans la population bovine du Royaume-Uni et ailleurs dans le monde. Les effets de l'infection par IBRV varient, d'une maladie bénigne ou non apparente à une maladie respiratoire grave. Au cours de ces dernières années, on s'est beaucoup intéressé à l'élimination de l'infection par IBRV, particulièrement en Europe, à cause de ses répercussions sur le commerce des bovins vivants, de la semence et des embryons. L'infection peut être éliminée des troupeaux où la prévalence sérologique est basse, par une simple approche "épreuve et réforme", combinée avec des précautions de sécurité contre la maladie, mais il est impossible de mettre cette méthode en pratique dans les troupeaux fortement infectés où se produit une circulation importante du virus. Ce document décrit l'élimination de l'infection par IBRV d'un tel troupeau, dans des conditions commerciales.

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TO BSE OR NOT TO BSE?

The investigation of a "BSE-like" outbreak in Norfolk

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INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a neurological disorder first identified in British cattle in November 1986 (Wells and others 1987) and made notifiable in June 1988. Reports of suspected cases are investigated by the State Veterinary Service and disease confirmed by histopathological examination of the brain. This article describes a suspected outbreak affecting more than 50 per cent of dairy cows in one Norfolk herd. Although the clinical features were remarkably similar to BSE, subsequent investigations eliminated this as the cause in all but one animal.

BACKGROUND

The outbreak involved an established 140-cow pedigree Holstein/Friesian dairy herd in Norfolk. Breeding replacements were mainly homebred but about 50 in-calf heifers had been purchased since 1989 from various sources, including herds with histories of multiple confirmed BSE cases. Cows were winter-housed in cubicles and grazed on permanent pasture during the summer. Calving took place in groups in the autumn either at grass or in a yard. No attempt was made to disinfect between calvings, and placentae were left *in situ*. The herd was subdivided into high and low yielding groups for dietary management purposes and fed conventional rations including proprietary concentrate, sugar beet pulp, brewers grains, grass silage, rape seed meal, corn distillers meal and potatoes.

Between May 1989 and April 1992, 23 suspected cases of BSE were reported from this herd. Of these, one animal recovered and the remainder were slaughtered: 21 were confirmed as BSE. There were several cases of mucosal disease in January 1991 but the herd's recent disease history was otherwise unremarkable.

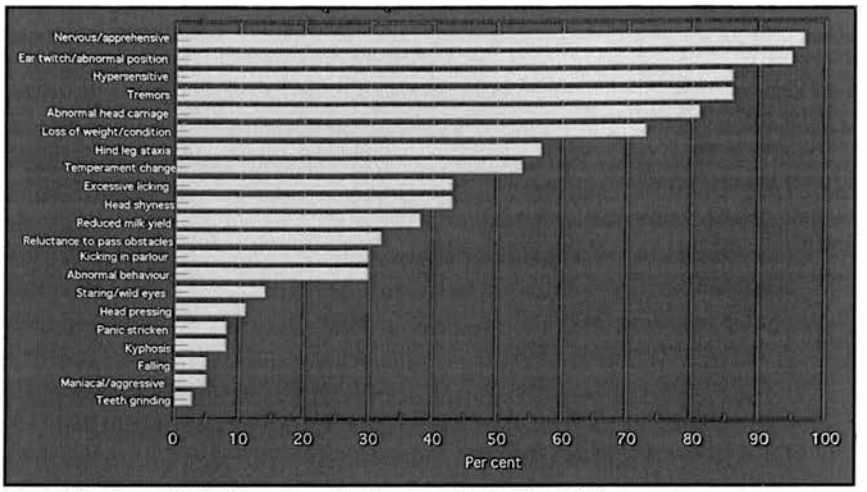

Figure 1. Frequency of clinical signs presented in 37 suspects placed under restriction

BSE-LIKE OUTBREAK IN 1992

On 11 May 1992 - three weeks after turnout - the farm manager reported 14 suspected cases of BSE. During the ensuing weeks, notifications were received of further cases and by late May more than 50 per cent of cows in the herd had developed some clinical signs suggestive of BSE. Blood samples taken by the owner's private veterinary surgeon showed no evidence of hypomagnesaemia or nervous acetonaemia. Statutory restrictions were placed on 37 animals, 12 of which (age range three to seven years) were slaughtered during May.

Affected cows were examined individually in a collecting yard; the main clinical signs exhibited are summarised in Figure 1.

The most prominent features were apprehension, ear twitching, hypersensitivity, tremors, abnormal head carriage and loss of condition. The manager commented that coughing and salivation were apparent amongst some cows but these signs were not observed during veterinary examinations. There was no evidence of pyrexia, inappetence or diarrhoea. Apart from cows that were slaughtered there were no deaths and all but one of the affected animals recovered, without any treatment, by the end of June. This one cow was eventually slaughtered as a BSE suspect in November 1992.

As the scale of the problem emerged, it appeared increasingly unlikely that BSE was involved and an investigation of other possible causes was initiated at the beginning of June in anticipation of negative BSE results.

INVESTIGATIONS

The main conditions considered in the differential diagnosis are listed in Table 1.

The age and parentage of affected cows was recorded and the farm environment was investigated for neurotoxic hazards. An assessment was made of feeding and management practices including pasture applications, water sources, access to poisonous plants, veterinary treatment and dietary additives. Blood samples collected from nine affected and three normal cows were subjected to metabolic profiles, enzyme tests and other biochemical and haematological examinations.

Samples of mouldy brewer's grain, sugar beet pulp and grass silage fed during the outbreak were removed for mycological examinations and mycotoxin screening (Patterson and Roberts 1979, Scudamore and Hetmanski 1992). Randomly selected samples of ryegrass swards taken from

Table 1. Differential diagnosis of BSE-like disease outbreak

BSE

Metabolic disorders:

- Nervous acetonaemia
- Hypomagnesaemia

Toxicities:

- Lead
- Mercury
- Nitrate
- Organophosphorus compounds
- 4 methyl-imidazole (bovine bonkers)
- Mycotoxins, especially tremorgens (ryegrass staggers)
- Algal/cyanobacterial neurotoxins
- Agrochemicals, eg metaldehyde
- Poisonous plants, eg ragwort, oxalate-containing plants

Infectious diseases:

- Listeriosis
- Rabies
- Louping Ill
- Aujeszky's disease

Genetic disorders:

- Weaver syndrome

Miscellaneous:

- Cerebrocortical necrosis (CCN)
- Cold cow syndrome

the pastures being grazed during the outbreak were examined microscopically (Lewis and Clements 1986) for the fungal endophyte *Acremonium loliae* responsible for the tremorgenic mycotoxicosis referred to as "ryegrass staggers" (Gallagher and others 1981). Water samples from a drinking trough containing heavy growths of algal material were examined microscopically and by mouse bioassay for cyanobacterial species associated with neurotoxicity (Gunn 1992). This trough occupied an unsheltered position in a field which was grazed at turnout and intermittently thereafter.

In seven of the 12 suspects slaughtered, histopathological examinations of the brain were confined to the obex (Wells and others 1989). For the remaining five cows examinations extended to whole brain histopathology and attempted detection of scrapie-associated fibrils (SAFs) from the cervical spinal cord (Scott and others 1990).

FINDINGS

With the exception of one case, histological examination of the obex sections failed to reveal spongiform change typical of BSE. The more detailed examinations of the whole brains showed scattered degenerative lesions particularly in the brain stem, including spheroid formation, glial nodules, mononuclear cell perivascular cuffs and occasional neuronal chromatolysis. In one animal there was bilateral vacuolation of the substantia nigra accompanied by milder white matter vacuolation in the medulla. The SAF tests all gave negative results.

The wide variation in age, origin and parentage of affected cows effectively eliminated hereditary factors. There was no circumstantial evidence to suggest agrochemical poisoning and the absence of ammoniated roughage feeding excluded 4 methyl-imidazole toxicity ('bovine bonkers') (Perdok and Leng 1987). Tests on blood samples eliminated lead, organophosphorus, metaldehyde and nitrate toxicities. Serum enzyme tests showed normal liver, kidney and muscle function. There was no evidence of cerebrocortical necrosis (CCN) with the erythrocyte transketolase test. Examinations for metabolic disorders and the main trace element deficiencies gave negative results. Haematological parameters were all within the normal range.

High counts (4.4×10^8 propugules/gm) of *Aspergillus fumigatus* were found in the brewer's grain but no mycotoxins were detected. Fungal endophytes were absent from the grass stems examined. The water trough yielded an abundance of filamentous green algae and diatoms but no cyanobacteria. The freeze-dried algal material did, however, produce marked neurotoxic effects with mouse bioassay.

DISCUSSION

Farmers occasionally report several cases of BSE on the same day in their herd and although unusual, the initial report of 14 suspect cases (which included some purchased animals) was not considered to be particularly remarkable in view of the herd's previous BSE history and the possible stress of recent turnout. However, the escalating nature of this outbreak soon raised doubts about the tentative diagnosis of BSE. The unavoidable delay (Matthews and Ryan 1992) of several weeks between slaughter and completion of histopathological examinations demanded that investigations to establish an alternative diagnosis should begin before BSE was definitely eliminated.

The clinical syndrome associated with BSE is readily recognised by experienced observers: currently about 85 per cent of compulsorily slaughtered suspects are confirmed as BSE by subsequent examinations. The clinical signs observed during this outbreak were virtually identical to those listed by Wilesmith and others (1992) for BSE. The close similarity with BSE was further highlighted by the histological confirmation of a single case of this disease in a cow showing signs clinically indistinguishable from the other affected stock.

The histological lesions in the brains examined in detail were mild and non-specific and failed to suggest any definitive diagnosis. There was, moreover, no similarity to the condition in BSE-negative suspects described by Jeffrey and Wilesmith (1992). Nervous acetonaemia, hypomagnesaemia and lead toxicity, which are usually considered as possible alternative diagnoses to BSE, were ruled out at an early stage of the investigations, as was organophosphorus toxicity. This last point was significant because of unsubstantiated claims in the popular farming press linking organophosphorus compounds with BSE.

There was a possible association with recent turnout - a feature also seen with the 'cold cow syndrome' - a condition of unknown aetiology whose clinical signs include staggering and muscle tremor (Rice and others 1983). However, other features of cold cow syndrome, including inappetence, diarrhoea and milk drop, were not apparent here.

No mycotoxins were found in the feed samples but it was possibly significant that high concentrations of *A. fumigatus* were detected in the brewer's grain fed during the outbreak. This fungus is known to produce several tremorgens but unfortunately further investigation was not possible on this occasion.

Although no toxin-producing cyanobacteria were demonstrated, neurological signs were induced in mice by the freeze-dried green algal material present in the water trough. Algal growth in this trough had probably been encouraged by the unusually hot and sunny weather during April and May. Whilst the effects of cyanobacteria (blue-green algae) are widely acknowledged, it should also be noted that there are reports in the literature of toxicity associated with green algae (Schwimmer and Schwimmer 1962).

A very similar outbreak to that described here occurred in a nearby 110-cow dairy herd in Norfolk at about the same time. Some 40 per cent of cows developed BSE-like signs during May, shortly after turnout. They recovered by mid-June but not before five cows had been slaughtered as suspect BSE cases, all of which subsequently proved negative on histopathology. Detailed investigations into this latter outbreak revealed only that the cows had been drinking from a shallow scum-covered lagoon containing potentially neurotoxic *Oscillatoria* sp. cyanobacteria but a causal association could not be proved.

Reports of numerous suspected BSE cases occurring in a single herd over a short period of time, should clearly be approached with caution. With the anticipated decline in the number of BSE cases after 1992 the prompt investigation of possible alternative causes of such outbreaks will become increasingly important. The precise aetiology of the two outbreaks seen in Norfolk could not be determined although circumstantial evidence strongly implicated a neurotoxicity. The findings concerning the role of green algae, cyanobacteria and tremorgenic mycotoxins remain somewhat inconclusive but their possible involvement should be considered when investigating similar outbreaks. Associations with unusually hot and sunny weather or with recent turn-out should also be borne in mind.

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Infectious pustular vulvovaginitis/infectious pustular balanoposthitis in cattle

SIR, – During the 1960s and early 1970s the venereal condition known as infectious pustular vulvovaginitis (IPV)/infectious pustular balanoposthitis (IPB) was not uncommon in Britain. However, with the introduction of stricter control measures in artificial insemination centres this condition has been very rarely reported here, and apparently elsewhere in Europe, during the past 10 to 15 years. In contrast, infectious bovine rhinotracheitis (IBR), which is the respiratory form of infection with the same virus, that is, bovine herpesvirus 1 (BHV1), has become very prevalent, with recent serological surveys suggesting that infection is present in at least 30 to 40 per cent of herds in Britain. The dearth of reports of IPV/IPB to veterinary investigation centres (VICs) has supported the belief that this condition is primarily of historical interest. Langford VIC did, however, identify a single case in a cow in 1992 and Penrith VIC confirmed a classical outbreak associated with the use of a shared bull in May 1996.

We wish to report a severe outbreak of IPV that occurred in January 1997 in a 150-cow dairy herd in southern England, associated with the use of two hire bulls, one of which, with hindsight, had shown suspicious clinical signs a few weeks after its arrival the previous month. During the first week of the outbreak 70 to 80 per cent of the cows exhibited pyrexia (up to 40·5°C), milk drop (about 210 litres per day for the herd), bilateral conjunctivitis with serous discharge and pustular vulvovaginitis. About 40 to 50 per cent also had a clear bilateral nasal discharge.

The genital tract lesions comprised white plaques and ulceration, progressing to sloughing of brown necrotic tissue as the ulcers coalesced. Nearly 50 per cent of cows had sore, reddened teats. There was also an increased rate of return to service. The herd was vaccinated intranasally with a live avirulent IBR vaccine (Inbovac IBR; Intervet UK) three days after the commencement of the outbreak and clinical signs resolved over a two-week period apart from the occasional recrudescence of milk drop in a few cows.

Only one of seven clotted blood samples from acutely affected cows was seropositive for BHV1 by ELISA, suggesting that the herd was predominantly immunologically naive at the commencement of the outbreak, apart from a few older cows which were unaffected. Nasal and conjunctival swabs from affected animals were positive for IBR with fluorescent microscopy. Both oculonasal and vaginal isolates were subsequently typed as type 2b at the Central Veterinary Laboratory by restriction endonuclease analysis (HindIII, HpaI and EcoRI). This is the same as the reference 'Oxford' isolate which is commonly associated with both IPV/IPB and typical low/medium virulence outbreaks of IBR (Edwards and others 1990). Although the herd had been vaccinated by this time, these isolates were clearly distinguishable by DNA analysis from the vaccinal virus.

This outbreak was unusual on two main counts: it appears to be one of very few documented outbreaks of IPV in recent years and, moreover, it comprised both respiratory and genital tract involvement. The latter appears to be a particularly uncommon occurrence and was last described in Britain by Collings and others (1972).

Clearly IPV/IPB is still present in Britain and the use of shared bulls poses a particular risk. This practice can also lead to the introduction of several other infections such as *Leptospira hardjo* and campylobacteriosis. Colleagues in general practice should advise their clients accordingly.

Advisory guidelines on the disease risks associated with bull hiring are available from the British Cattle Veterinary Association Office, The Green, Frampton-on-Severn, Gloucestershire GL2 7ER.

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Subclinical breakdown with infectious bovine rhinotracheitis virus infection in dairy herd of high health status

G. C. PRITCHARD, M. BANKS, R. E. VERNON

Infectious bovine rhinotracheitis (IBR) virus infection was detected by an antibody ELISA in the bulk milk of a large closed dairy herd of high health status in an area of low cattle density in East Anglia. The herd was managed under high standards of biosecurity and was known to have been serologically free of IBR virus for the previous 13 years. Although over 70 per cent of the cows had seroconverted to IBR virus no clinical signs were observed apart from a slight bilateral watery ocular discharge in a few cows, and their performance and productivity were unaffected. The causal virus, which was isolated after it had been reactivated with corticosteroid, had the DNA profile of a bovine herpesvirus type 1 strain normally associated with clinically severe respiratory disease. In spite of extensive enquiries and seroepidemiological investigations the source of the infection was not determined.

BOVINE herpesvirus type 1 (BHV-1) infection is widespread in Britain and elsewhere in the world. It causes a range of diseases in cattle, including classical infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/balanoposthitis (IPV/IPB). The clinical signs range from subclinical infections to mild or severe disease, depending on the strain of virus, the route of exposure, the immunological status of the host and environmental factors (Kahrs 1977). Although a clinically severe respiratory form of IBR emerged in Britain after 1976 (Edwards 1988), milder outbreaks with signs of bilateral conjunctivitis, lacrimation and a mucopurulent nasal discharge are more common. In many endemically infected herds, the effect of an IBR virus infection is largely subclinical although clinical signs can occur intermittently, even in closed herds (Kahrs 1977). IPV/IPB has been virtually absent from Britain since the 1970s, although an outbreak combined with IBR was reported by Pritchard and others (1997). Recently the eradication of IBR virus infection from several European Union (EU) member states (Austria, Denmark, Finland, Sweden and the Bolzano province of Italy) has raised the profile of the disease and prompted efforts to harmonise vaccines, antibody assays and movement controls within the EU (Anon 2000).

This paper describes the seroconversion to IBR virus of a large closed dairy herd of high health status with high standards of biosecurity, that was known to have been serologically free of the infection since 1987. The herd showed virtually no clinical signs and there were no detectable effects on its performance or productivity. The virus was reactivated, isolated and identified.

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MATERIALS AND METHODS

Herd

The predominantly autumn-calving 230-cow Holstein-Friesian dairy herd was in East Anglia (a region of low cattle density), in an isolated rural position surrounded by arable farms, with no cattle in other herds within about 2 km. The milking parlour and main farm buildings used by the cows were about 20 m from the road, and their grazing pastures extended alongside several minor roads. The boundaries were well fenced or hedged with no apparent possibility of direct or indirect contact with other cattle, and live animals or carcases were loaded at one end of the farm, away from the main herd. The herd was managed on a conventional cubicle and straw yard system, and was fed out of parlour with predominantly home-grown maize or grass silage, and a protein blend. Male calves were sold when they were about a week old, and heifer calves retained for breeding were reared on a separate similarly isolated premises about 1 km away.

The herd had maintained high standards of disease security since 1987, when it was one of the first in Britain to achieve IBR-monitored negative status in the former Cattle Health Scheme (CHS) operated by the State Veterinary Service (Agricultural Development and Advisory Service 1987). During the five years before the IBR virus infection was detected, the cattle had not attended agricultural shows or similar events, no live animals had been purchased and only essential visitors had been admitted to the farm. The genetic improvement of the herd relied on artificial insemination (AI) and embryo transfer.

History of IBR virus infection

The entire herd was initially tested for IBR virus by ELISA (Edwards and others 1986) in 1987 when it applied for CHS membership, and it was retested annually until the scheme was taken over by Cattle Health 2000 (Bloxham Laboratories) in 1996. It remained in Cattle Health 2000 until 1998 and the last complete herd test was in July 1997. The IBR virus status of the milking herd was then monitored at intervals of approximately three months by using bulk milk antibody ELISA provided by the Veterinary Laboratories Agency (VLA) (Pritchard 2001). Because it was managed separately, the heifer herd was monitored independently; 92 yearling heifers were blood sampled and tested for IBR virus antibodies in July 1999. There was no evidence that the herd had been exposed to IBR virus until a low-positive bulk milk result (OD ratio 0·42) was recorded in March 2000.

Another bulk milk sample was immediately tested and confirmed the previous result, and the entire milking herd was examined for clinical evidence of IBR or IPV. The farm's records were examined for unexplained changes in milk yield and quality, somatic cell count, and reproductive performance, and extensive enquiries were made into possible sources of infection. The herd's movement records were examined and details of likely risk factors, including outbreaks of IBR in the vicinity, movements of personnel and vehicles and wildlife activity, were obtained from the owner, farm workers and other sources. The numbers and sources of embryo transfers and AIs were also recorded.

At the beginning of May 2000, the entire milking herd of 231 cows and 20 of 100 heifers, aged between five months and two years, were blood sampled and tested for IBR virus infec-

tion by ELISA; five of the heifers were retested in December 2000. In addition, bulk milk samples were tested for bovine virus diarrhoea (BVD) virus and *Leptospira borgpetersenii* serovar Hardjo antibodies by ELISA (Pritchard 2001). The herd was known to have been free from these infections and there was concern that they may have entered the herd at the same time as IBR virus. Any cows that aborted were examined for serological evidence of IBR virus, BVD virus, *Neospora caninum* and *L* Hardjo infections, in addition to statutory testing for *Brucella abortus*. The owner was also asked to send aborted fetuses to VLA – Bury St Edmunds, where they were examined for evidence of IBR virus infection by virus isolation, histopathology and immunocytochemistry. Tests for other abortifacient agents were also made, for purposes of differential diagnosis, by the standard methods used in VLA regional laboratories.

Virus detection, reactivation and isolation

Fluorescent antibody tests (FAT) for IBR virus (Edwards and others 1983), and attempts to isolate the virus from nasopharyngeal and conjunctival swabs, were initially carried out on 20 cows randomly selected from the milking herd in May 2000. Further efforts to isolate and identify the virus were made by corticosteroid reactivation (Sheffey and Rodman 1973). Two cull cows (A and B) identified as seropositive for IBR virus in the recent herd test, were isolated and given five consecutive daily doses of dexamethasone sulphate (Dexadreson; Intervet UK) intramuscularly at 0·1 mg/kg. Guarded wire nasopharyngeal swabs were taken from both nostrils from days 3 to 9 (taking the first day of reactivation treatment as day 1). The swabs were immediately put into virus transport medium for attempts to isolate the virus.

One-day-old Madin-Darby bovine kidney (MDBK) cell monolayers in a 25 cm^2 flask were inoculated with 0·2 ml of swab eluate or 1:10 tissue homogenate and incubated at 37°C for one hour. The inoculum was removed and the cultures were washed and incubated at 37°C. The cultures were inspected daily for a cytopathic effect and passaged if they were negative seven days after inoculation.

Characterisation of the reactivated virus

The DNA profile of the virus isolated was examined by restriction fragment length polymorphism (Banks 1993) and compared with the profiles of the viruses in the commonly used vaccines Tracherine (Pfizer) and Bovilis (Intervet), to determine whether they were vaccinal or field strains; the recently introduced gE-deleted marker vaccines (Bayer/Intervet) were not available in Britain at the time. The DNA profiles of the strains were compared by digesting them with *Hind*III and *Hpa*I restriction endonucleases. On day 9, the two dexamethasone-treated cows were blood sampled and their antibody concentrations for IBR virus were compared with those measured before the reactivation.

Experimental inoculation

As a guide to the virulence of the reactivated virus (designated strain 00/262), a 13-week-old, IBR virus-seronegative calf was inoculated intranasally with $2{\cdot}28 \times 10^5$ plaque-forming units (pfu) of a second passage of the virus. An age-matched seronegative control calf was sham inoculated and kept isolated from the virus-infected calf, and both calves were monitored daily for clinical signs of IBR for 14 days, by taking their rectal temperatures and nasal swabs. The nasal swabs were weighed before and after collection to estimate the quantity of mucus present, and frozen at –80°C before they were thawed and eluted with 1 ml 0·01M phosphate-buffered saline pH 7·2, plus antibiotics. After elution, the virus was titrated in a 10-fold series on MDBK cells in microplates. The DNA profile of the virus isolated from the infected calf was checked against the profile of strain 00/262 to confirm its identity.

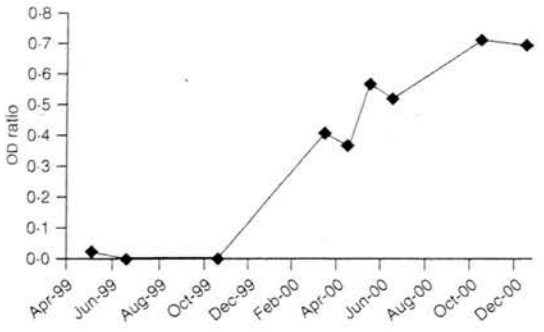

FIG 1: Results of bulk milk antibody tests between April 1999 and December 2000. OD Optical density

Further milk antibody testing

Further samples of bulk milk were collected in 2001 and 2002 and examined for IBR virus antibodies, and the IBR virus antibody status of each of the first and second lactation cows that had first calved and entered the herd in the autumn of 2001 and 2000 respectively, was assessed in May 2002, to provide a guide to the extent of the circulation of virus within the herd (Pritchard and others 2002). Some older cows that had initially been sampled in May 2000 were also retested.

RESULTS

Bulk milk antibody tests

The results of the bulk milk antibody tests made before, during and after the main period investigated are shown in Fig 1; optical density (OD) ratios below 0·10 are negative (<5 per cent seroreactors). The OD ratios increased from 0·00 in June and October 1999 (0·02 in April 1999) to 0·42 (low positive) in March 2000 and 0·72 (high positive) by October 2000. Tests for *L* Hardjo and BVD virus were consistently negative.

Herd serology

The herd blood test in May 2000, showed that 162 of the 231 cows tested (70·1 per cent) were seropositive for IBR virus (OD >0·25) and 29 (12·6 per cent) were in the inconclusive category (OD 0·15 to 0·25). The OD values of the seropositive sera were generally low, with only 14 having ODs greater than 0·6. The 20 heifers tested in May 2000, and the five retested in December, were seronegative.

Virus reactivation and isolation

The FAT and attempts to isolate the virus from the 20 cows sampled initially were unsuccessful. However, after their reactivation treatment, IBR virus was isolated from cows A and B on day 7, and from cow A only on day 8. Both cows showed significant increases in serum antibody concentrations for IBR virus after reactivation: in cow A the OD increased from 0·50 to 0·88, and in cow B from 0·64 to 0·95. Neither cow showed any clinical signs during the reactivation period, apart from a decrease in daily milk yield from about 20 to 22 litres to virtually none by day 9.

Characterisation of reactivated virus

The DNA of the isolate was clearly identified by *Hind*III digestion as a BHV-1 (IBR-like) strain, according to the scheme of Metzler and others (1985), and although it was very similar to the profile of Tracherine (Fig 2, tracks 2 and 5) there was a clear difference between the *Hpa*I profiles (tracks 3 and 4); this difference also applied to Bovilis.

The clinical signs in the experimentally infected calf were limited to mild anorexia and depression on days 5, 6 and 7 after inoculation. Its rectal temperature was greater than 39·5°C from day 5 to day 9, peaking at 41°C on day 8. The

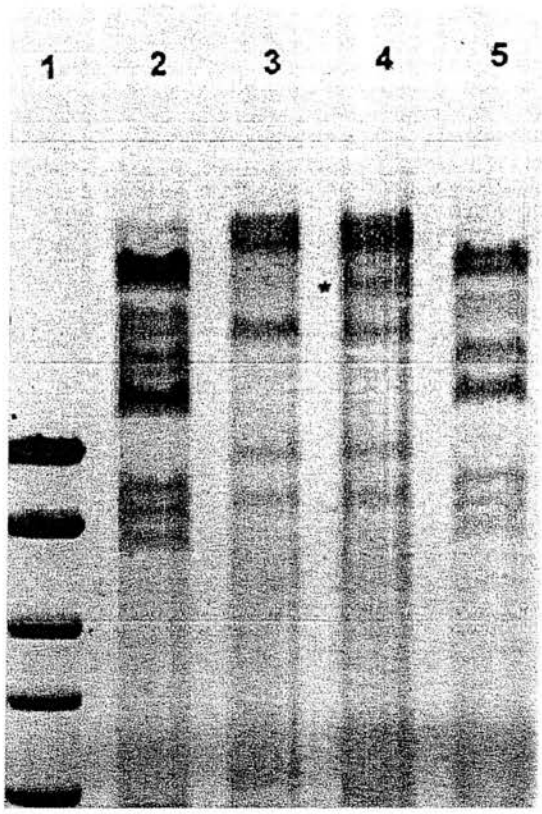

FIG 2: Inverted image of gel electrophoresis of DNA profiles of bovine herpesvirus type 1 strains. Track 1, Molecular weight marker; Track 2, 00/262 *Hind*III; Track 3, 00/262 *Hpa*I; Track 4, Tracherine *Hpa*I; Track 5, Tracherine *Hind*III. * Point of difference between the two strains after digestion with *Hpa*I

uninfected calf showed no clinical signs. Virus was isolated from the nasal swabs from the infected calf only on days 4 and 5, at titres of 1·68 and 1·12 x 10^2 pfu/100 mg nasal mucus respectively. When euthanased, the serum from the infected calf had a low positive OD for IBR virus (0·29). The DNA profile of the virus isolated from the infected calf was identical to that of the 00/262 strain reactivated from cows A and B.

Clinical signs and herd performance

The herdsman recalled, with hindsight, that a few milking cows (less than 5 per cent) had had a slight bilateral watery ocular discharge during March and April 2000. However, no other clinical signs had been seen before or were seen after the increase in bulk milk OD ratio that indicated the introduction of IBR virus infection into the herd. A comparison of production data showed that the herd's average annual milk yield increased from 9039 litres in 1999 to 10,152 litres in 2000. Other comparative data for 1999 and 2000 respectively were: milk protein (3·32 and 3·24 per cent), butterfat (3·65 and 3·70 per cent), bulk tank somatic cell count (27,000 and 45,000/ml), calving index (379 and 374 days) and services per conception (2·04 and 1·96). There were four abortions in 2000 compared with two in 1999, mainly at about seven months gestation, and four cows were found not to be in calf when they reached full term. The cows that aborted were all seropositive for IBR virus. No IBR virus infection was detected in the one fetus tested.

Investigations into the source of infection

There was no evidence that the infection had entered by any breaches in farm security or changes in management practices. There had been no outbreaks of IBR in the few other cattle herds in the locality and they had not used live IBR vaccines. The heifers (which had been reared apart from the cows since birth) were clearly not the source of infection because they were consistently seronegative for IBR virus.

The semen used during the previous five years had all been imported. Sixty-six straws purchased in 1998 from a stud in Canada were used in 33 cows between April 1998 and March 1999. Anecdotal information revealed that the stud had experienced an IBR virus breakdown in 1999 but no further details were available. However, if the stud had been the source of infection, the inseminated cows would have been expected to seroconvert and antibody would have been expected to be detected in the bulk milk tests carried out in June and October 1999 unless the seroprevalence was below the detection threshold (<5 per cent) when the samples were collected. All 14 heifers born to the cows inseminated with the imported semen were seronegative when tested in July 1999. No embryos were implanted in 1999 but two homebred cows were implanted with embryos from the USA in January 1998. Both calved in September 1998 and were included in the subsequent negative bulk milk antibody tests.

The proximity of the pastures and buildings to minor roads made it possible that the virus could have been transmitted from infected cattle on hauliers' vehicles. However, the herd was housed at least 20 m from the road when the infection was thought to have entered between October 1999 and March 2000. Anecdotal evidence emerged that in late summer 1999 four heifers of unknown IBR virus status had escaped from a small suckler herd about 2 km away. One of these heifers was known to have been roaming loose overnight but there were no reported sightings of any contact with the dairy herd. The bulk milk test carried out in October 1999, two months after the reported escape, was negative for IBR virus.

Milk antibody testing in 2001 and 2002

No bulk milk antibody tests were carried out during the main period of the foot-and-mouth disease epidemic in 2001. In October 2001, the bulk milk OD ratio for IBR virus was 0·73, and in 2002 it was 0·48 in April, 0·62 in May and 0·74 in June. Individual milk samples collected in May 2002 showed that two (2·5 per cent) of the 80 first calvers and seven (13·2 per cent) of the 53 second calvers had seroconverted to IBR virus. Fifteen of 18 randomly tested older cows were seropositive, all with significantly increased antibody concentrations (OD>0·2) compared with May 2000.

DISCUSSION

The importance of farm security in preventing the introduction of endemic diseases into naive cattle herds has been increasingly emphasised during recent years (Duncan 1994). The role of introduced animals as a source of infection has also been emphasised (Pritchard 1996a). The overall term 'biosecurity' came to the fore during and after the 2001 foot-and-mouth disease epidemic in Britain. It is, therefore, of concern that this herd should have inexplicably become infected with IBR virus. The herd had no clinical or previous serological history of exposure to IBR virus, was managed to high standards of biosecurity in an arable area of very low cattle density and relied exclusively on embryo transfer and AI for genetic improvement. There are a few other reports of subclinical IBR breakdowns in herds of high health status and AI studs where no source of infection was identified (Van Oirschot and others 1993; J. C. Bosch, personal communication). Paisley and others (2001) recorded that one of the few infected herds in Norway, in which 27 of 56 cattle were seropositive, was identified during routine bulk milk testing of a dairy herd that had never experienced clinical disease.

The value of regular tests of bulk milk antibody concentrations as an inexpensive and convenient method of monitoring the disease status of a herd was clearly demonstrated

by the detection of IBR virus infection in this previously naive herd. Unfortunately, after the negative result recorded in October 1999, the herd was not retested until the low positive result was recorded in March 2000, leaving a period of about five months during the winter when the infection was probably introduced. However, it was just possible that the herd had acquired the infection earlier and that the seroprevalence initially remained below the 5 per cent threshold for detection by the bulk milk ELISA. The generally low antibody titres in individual cows in May 2000 further suggested that the infection had been acquired recently, with the titres not yet boosted by the reactivation of latent virus. This also appeared to explain why the initial bulk milk OD ratios were much lower than expected (Pritchard 2001) from the serological results from the individual cows.

As a typical herpesvirus, BHV-1 exhibits the phenomenon of latency, that is, the virus may persist, mainly in the trigeminal (IBR virus) or sacral ganglia (IPV/IPB), without causing disease, but can be activated and excreted in response to various stress factors, including transportation, parturition and other diseases (Wyler and others 1989). As a result, after they recover from an acute infection, cattle remain potential carriers of the virus for life, and provide the main means by which the infection is transmitted from herd to herd and is imported into disease-free countries. The virus is most often spread by aerosols from infected animals. After an experimental infection, cattle can shed the virus for up to 16 days (Straub 1991). However, no stock had entered this herd for five years and the possibility of an escapee from a suckler herd as the source was pure conjecture. Although the cows were housed 20 m from the road, aerosol contact with virus shed from cattle on passing vehicles could not be ruled out completely. It has been shown that the virus can be transmitted by aerosols over distances of up to about 4 m (Mars and others 2000), but not over the long distances that foot-and-mouth disease virus (Gloster and others 1982), or Aujeszky's disease virus (like IBR virus also an alpha herpesvirus) (Gloster and others 1984), have been reported to be spread.

It has long been recognised that semen from bulls, actively or latently infected with IBR virus, could be an important potential source of infection, either through natural service or through AI (Huck and others 1971, Drew and others 1987); IBR virus was introduced into a herd in Switzerland by imported semen, despite the strict control measures applied on AI studs (Kupferschmied and others 1986). In this herd, the timing of the introduction of the infection and the results of the bulk milk antibody tests made semen a very unlikely source. Imported semen from the same bull was used by another IBR virus-free herd in East Anglia without any evidence of subsequent seroconversion to IBR virus (C. J. Tomlinson, personal communication). Provided that embryos are washed and treated with trypsin correctly to remove any virus adhering to the zona pellucida, Straub (1990) considered that there was no risk of IBR virus infection being introduced by embryo transfer; there was certainly no evidence that it was the source of infection here.

Feral deer were known to be present in the vicinity and they might conceivably have come into transient contact with the cattle. Red deer (*Cervus elaphus*) are known to excrete the virus after being infected experimentally with IBR virus, but the titre excreted is low and they are not usually considered to be a source of infection for cattle. Seronegative carrier animals have been implicated in the introduction of IBR virus into herds (Lemaire and others 1995, 2000). It is possible that a strain of virus of low virulence may have been present in a seronegative carrier when the closed herd policy was introduced, but it is extremely unlikely that the seronegative status would have persisted during the intervening years.

In the near future, it should be possible to characterise the BHV-1 biotype of the virus more definitively on the basis of DNA sequencing. However, by the currently available methods, the isolate was classified as a type 1 (IBR-like) virus distinct from the vaccinal strains. Type 1 strains are thought to have been introduced into Britain from North America in the late 1970s and they have been shown to cause clinically more severe disease than type 2a and 2b viruses (Edwards and others 1991). However, the isolate appeared to be remarkably non-pathogenic, even in this naive herd. The herd's milk yield (which usually decreases markedly during outbreaks of IBR) was greater in 2000 than in 1999, although the increase could have been attributable to other factors, particularly dietary changes. The herd's reproductive performance remained very good throughout. There were slightly more abortions in the period following the infection, but the total number of abortions during 2000 was still within the range (3 to 5 per cent) associated with the inevitable sporadic losses expected in most herds (Pritchard 1990). By 2001, the herd's average milk yield had increased to 10,320 litres per cow and only two abortions were recorded. The experimentally infected calf showed only mild clinical signs and excreted low levels of virus, although the trial failed to demonstrate that the virus was completely non-pathogenic. Owing to the movement restrictions imposed during the foot-and-mouth disease epidemic, and other constraints, it was not possible to carry out a larger animal inoculation study.

Pritchard (1992, 1996b) recorded that endemic IBR virus infections in dairy herds in East Anglia, and possibly elsewhere, generally had either a low or a high within-herd prevalence. When the seroprevalence in the milking herd was below about 20 per cent there was usually very little evidence of active infection among naive animals, including incoming heifers, despite the potential for the reactivation and excretion of latent virus by seropositive animals. Conversely, in herds with a high prevalence (>60 per cent seropositive), most incoming naive animals seroconverted within two or three years. Once this herd had become infected, the consistently high bulk milk antibody results, together with the results of the milk antibody tests on individual cows, indicated that it belonged to the latter type. The slight decrease in bulk milk OD ratio in April and May 2002 was probably attributable to the dilution effect of incoming seronegative heifers after calving. However, the seroconversion rate by second lactation cows (13·2 per cent within 18 months) was lower than usually encountered in herds with a high prevalence.

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1.4b

Papers and Articles

Prevalence of antibodies to bovine virus diarrhoea virus and other viruses in bulk tank milk in England and Wales

D. J. Paton, K. H. Christiansen, S. Alenius, M. P. Cranwell, G. C. Pritchard, T. W. Drew

Veterinary Record (1998) **142,** 385-391

Bulk tank milk samples from 1070 dairy herds in England and Wales were tested by ELISA for antibodies to bovine virus diarrhoea virus (BVDV). A subset of 341 herds was tested by ELISA for antibodies to bovine herpesvirus 1 (BHV-1), bovine respiratory syncytial virus (BRSV) and bovine coronavirus (BCV). None of the herds had less than 40 dairy cows and none had been vaccinated against BVDV. The prevalence of BVDV antibody-positive herds in the national population was estimated at 95 per cent and approximately 65 per cent of the herds had a high level of bulk tank antibody suggestive of recent infection with BVDV. Dairy herds in East Anglia and the south-east of England had a significantly lower risk of being BVDV antibody-positive than herds in the rest of England and Wales. However, these regional differences tended to diminish with increasing herd size. Around 69 per cent of the herds were BHV-1 antibody-positive and all the herds were antibody positive to BRSV and BCV. Comparison with earlier serological surveys revealed that there had been little change in the prevalence and distribution of BVDV antibody-positive herds in England and Wales over the last 20 years, but that there had been an increase in the prevalence of BHV-1 antibody-positive herds.

BOVINE virus diarrhoea virus (BVDV) is a common pestivirus infection of cattle and other ruminants that occurs almost worldwide (Houe 1995). The virus causes a diverse range of subclinical and clinical disease and is responsible for considerable economic loss (Bitsch and Ronsholt 1995, Houe 1995). Cattle infected in utero may become persistently infected carriers of BVDV. Persistently infected animals shed large amounts of virus during their lifetime and generally remain antibody-negative. Postnatal exposure of susceptible cattle to BVDV results in a short period of virus replication and shedding followed by the development of antibody which is often long lasting (Duffell and Harkness 1985).

There have been no studies of the seroprevalence of BVDV in England and Wales since those carried out approximately 10 and 20 years ago. However, in Great Britain, interest in control measures for BVDV infection has been renewed by the introduction of a new inactivated BVDV vaccine (Brownlie and others 1995) and by the initiation of national BVDV control schemes in Scandinavia (Alenius and others 1997). Until September 1996, no BVDV vaccines were licensed for use in Great Britain.

The recent development of tests for BVDV antibodies in bulk tank milk has considerably facilitated herd-based surveillance, and a good correlation has been demonstrated between the level of BVDV antibody in bulk tank milk and the proportion of seropositive cows in a dairy herd (Niskanen and others 1991, Niskanen 1993). It was decided to take advantage of this method to investigate the current prevalence of BVDV infection in the national dairy herd before the method becomes compromised by the use of vaccination.

The opportunity was also taken to test a subset of the samples for antibodies to bovine herpesvirus 1 (BHV-1, synonymous with infectious bovine rhinotracheitis virus), bovine respiratory syncytial virus (BRSV) and bovine coronavirus (BCV). These three viruses are common pathogens of cattle that can be spread by acutely infected animals. Cattle may also be latently infected with BHV-1. An estimate of the seroprevalence of BHV-1 in British cattle herds was last made 10 years ago (Edwards 1988). Little published information is available on the prevalence of BRSV and BCV in Britain, although the infections are known to be widespread.

Materials and methods

Collection of samples and herd data

Bulk tank milk samples were collected by private veterinary surgeons at the request of their local Veterinary Investigation Centre (VIC). Each of 12 VICs in England and Wales (Fig 1) was supplied with 135 sampling kits which were distributed among veterinary practices selected randomly from those involved in local cattle work. Each practice was issued with approximately 10 to 15 sampling kits and was asked to obtain milk samples from dairy herds in the course of routine visits. Samples were collected between July 22 and December 31, 1996. Only herds with 40 or more dairy cows which had not been vaccinated against BVDV were included in the study.

The sampling kits consisted of a 25 ml sterile plastic universal tube containing a milk preservative (final concentration when mixed with milk: Bronopol 6 ppm, Kathon 17·5 ppm, copper nitrate 0·07 ppm, Brilliant blue 0·09 ppm), a disposable sampling ladle and a form requesting the following information: number of milking cows in the herd, number of cows contributing milk to the bulk tank on the day the sample was collected, and whether or not the farmer reared any of his own replacement heifers. A prepaid envelope and a tube to protect the sample were provided to post the sample to the Central Veterinary Laboratory (CVL).

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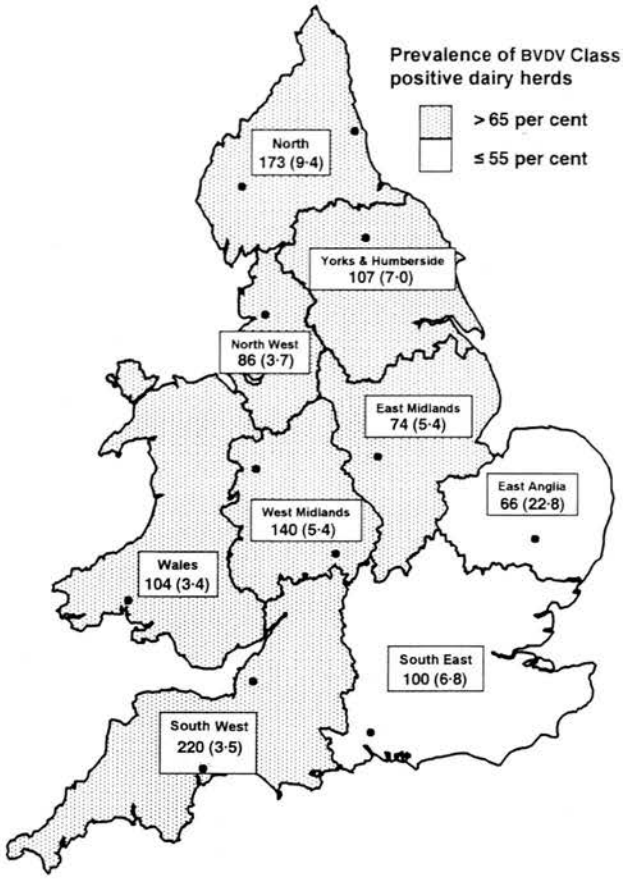

FIG 1: Location of Veterinary Investigation Centres (•), number of dairy herds sampled in each region (with per cent of the dairy herd population sampled in each region in brackets) and regional prevalence of BVDV class 3-positive herds

Antibody tests

After collection, the samples were stored at 4°C until tested at the CVL for antibody to BVDV strain Oregon C24V, using an indirect ELISA and a single batch of cell culture-derived positive and negative antigen (Edwards 1990). After centrifugation to remove the cream, the skimmed milk samples were tested at a dilution of 1:2 and a net optical density (OD) was calculated from the difference in absorbance between wells containing positive and negative antigens.

In order to use the Swedish system of classification and interpretation of the results of the BVDV antibody ELISA, 30 randomly selected samples from each VIC were also tested with a commercially available BVDV antibody ELISA (Svanovir; Svanova Biotech) at the National Veterinary Institute in Uppsala, Sweden. This test forms the basis for assigning herds to four different classes within the Swedish BVDV control scheme (Lindberg 1995). Herds in classes 0 or 1 are likely to have a very low or low prevalence of seropositive milking cows, and are unlikely to contain actively infected animals, unless they have been recently introduced. Herds in classes 2 or 3 are likely to have a moderate or high prevalence of seropositive cows, suggesting that infection has been present in the herd in the relatively recent past. Class 3 herds have the highest probability of active infection within the herd (Alenius and others 1997). The CVL ELISA results for the 360 samples were compared with the Svanova test results to derive CVL ELISA class limits for the four classes of interpretation of the test. The class limits were set to maximise the number of samples correctly classified by the CVL ELISA (Table 1). For comparison with previous serological surveys, herds placed in classes 1, 2 or 3 (CVL ELISA OD ≥0·135) were categorised as BVDV antibody-positive and are referred to as CLASS123-positive herds. Herds placed in class 3 (CVL ELISA OD ≥0·9) are referred to as CLASS3-positive herds. They are the herds most likely to have active infection and to contain persistently infected animals.

TABLE 1: Classification of 360 bulk tank milk samples from dairy herds in England and Wales by BVDV class (OD level) according to CVL antibody ELISA and Svanova antibody ELISA

	Svanova ELISA					
CVL ELISA	Class 0 (<0·05)	Class 1 (0·05-0·249)	Class 2 (0·25-0·549)	Class 3 (≥0·55)	Total herds	Per cent agreement
Class 0 (<0·135)	**14**	1	0	0	16	88
Class 1 (0·135-0·399)	2	**25**	2	0	32	78
Class 2 (0·4-0·899)	0	6	**45**	13	78	61
Class 3 (≥0·9)	0	0	27	**225**	234	95
Total herds	16	32	74	238	**360**	86

The same subset of 360 samples was tested at the National Veterinary Institute in Uppsala, Sweden, for antibodies to BHV-1, BRSV and BCV. All testing was by indirect ELISA and in each case herds with an OD of ≥0·05 were considered antibody-positive and herds with an OD ≥0·8 were considered strongly positive (Alenius and others 1991, Elvander and others 1995, Elvander 1996).

Scope and methods of statistical analysis

The study herds were categorised by region and the number of milking cows in the herd on the basis of the system used by the Ministry of Agriculture, Fisheries and Food (MAFF) agricultural and horticultural census. Their distribution was then compared with that of the national herd in 1995 (MAFF 1996).

National and regional estimates of mean OD levels and of the prevalence of virus antibodies in the dairy herd population were based on methods for simple random sampling (Levy and Lemeshow 1980). The prevalence of BVDV-seropositive dairy cows within the national herd was estimated from mid-point values for the likely percentage of seropositive cows responsible for each class of bulk tank antibody result (class 0 = 2·5 per cent, class 1 = 15 per cent, class 2 = 45 per cent, and class 3 = 82·5 per cent, Lindberg 1995).

Herd characteristics investigated in the study were region, number of milking cows in the herd (herd size), number of cows contributing milk to the bulk tank and whether the farmer reared any of his own dairy heifer replacements. The relationship between herd characteristics and the mean OD level and prevalence of each virus was examined using the computer software Epi Info Version 6.01 (Dean and others 1990). Using the Egret statistical computer software (Statistics and Epidemiology Research Corporation 1993), the risk of a herd being BVDV antibody-positive or BHV-1 antibody-positive was analysed by multiple logistic regression for the following herd characteristics: region, herd size and number of cows contributing milk to the bulk tank. In all the analyses, variables with $P<0.05$ were considered statistically significant.

To investigate the effect of cattle density on the geographical distribution of BVDV, the point estimates for mean BVDV OD for each region were regressed against the cattle density in the region. A similar procedure was used for BHV-1, BRSV and BCV. The total number of cattle and the numbers of dairy cattle per square kilometre were used as measures of cattle density for each region; the numbers were extracted from the 1995 MAFF agricultural and horticultural census (MAFF 1996).

Results

Description of herds

The distribution of 1620 sampling kits to veterinary practices yielded bulk tank milk samples from 1148 (71 per cent) dairy herds in England and Wales. Of the herds sampled 1070 (93 per cent) met the criteria for entry into the study and provided data about the size of the herd and the location of the farm. The 1070

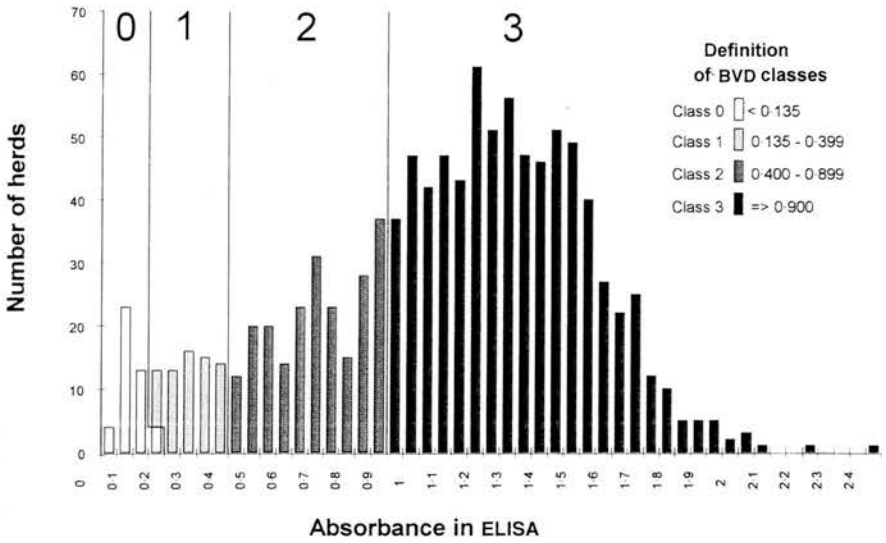

FIG 2: Frequency distribution of BVDV antibody levels in bulk tank milk samples from 1070 dairy herds in England and Wales and their categorisation by BVDV class

milk samples were submitted by 137 veterinary practices, with between 59 and 108 samples coming from the catchment areas of each of the 12 participating VICs. The location of the VICs and the number and percentage of herds sampled within each region are shown in Fig 1. The study herds constituted 5·2 per cent of all dairy herds with 40 or more cows in England and Wales. Between 3 and 9 per cent of the dairy herds were sampled from each region except East Anglia where 23 per cent of the dairy herds were sampled.

The average number of milking cows in the herds was 117 (median 102, range 40 to 400). The mean number of milking cows in the herds in East Anglia and the south east was significantly larger than the sample average, while the mean number of milking cows in the herds in Wales, the East Midlands and the north was significantly smaller than the sample average. In relation to the distribution of herd sizes in the dairy cattle population, small herds were under represented and large herds were over represented.

The mean number of cows contributing milk to the bulk tank on the day that the milk sample was collected was 90 (median 80, range 16 to 310). The average percentage of the milking cows in each herd contributing milk to the tank was 77 per cent (median 80 per cent, range 20 to 100 per cent). On average, the herds in East Anglia, the south east and the south west had a significantly lower percentage of cows contributing milk to the tank (68 per cent, 73 per cent and 76 per cent, respectively) than the herds in the rest of England and Wales combined (80 per cent). This difference may be due to differences in calving patterns. The differences between East Anglia, the south east and the south west were also statistically significant but the differences among the remaining regions of England and Wales were not. Only 4 per cent of 1062 farmers reported that they did not rear any of their own replacement heifers and regional differences were not statistically significant.

Herd size and farm location data were available for 341 of the 360 dairy herds whose bulk tank milk was tested for antibodies to BHV-1, BRSV and BCV. The mean number of milking cows in this subsample of 341 herds was 114 (median 100, range 40 to 370). The lowest number of cows contributing milk to the tank was 20 and the highest was 300 (mean 86, median 74). Regional differences in herd size and number and percentage of cows contributing milk to the tank were similar to those in the sample of 1070 herds. Overall, 3·2 per cent of 339 farmers reported that they did not rear any of their own replacement heifers.

Prevalence of antibodies to BVDV

The distribution of BVDV ELISA antibody levels in bulk tank milk among the 1070 study herds is shown in Fig 2. The mean OD for the 1070 herds was 1·01 (median 1·09, range 0 to 2·4). The prevalence of BVDV antibody-positive herds (CLASS123) in the study was 95·1 per cent and the prevalence of herds with a high level of antibody (CLASS3) was 65·5 per cent (Table 2). The percentages of dairy herds in England and Wales in each BVDV antibody class (with the 95 per cent confidence interval in brackets) were 4·6 per cent (3·4 to 5·8 per cent) in class 0, 6·9 per cent (5·4 to 8·4 per cent) in class 1, 23·0 per cent (20·5 to 25·5 per cent) in class 2, and 65·5 per cent (62·7 to 68·3 per cent) in class 3. The prevalence of BVDV antibodies in the dairy cow population of England and Wales was calculated to be 66 per cent.

Regional mean OD levels and prevalences are given in Table 2. Dairy herds in East Anglia and the south east had a significantly lower mean OD and prevalence of BVDV antibody-positive herds (CLASS123 and CLASS3) than herds in the rest of England and Wales combined (Fig 1). The prevalence of BVDV CLASS123-positive herds was significantly lower in East Anglia than in the south east, but there was no significant difference in the prevalence of BVDV CLASS3-positive herds or the mean OD level between the two regions. There was also no significant difference between the

TABLE 2: Mean level of BVDV antibodies in bulk tank milk samples and prevalence of BVDV-positive dairy herds in England and Wales and by region

Region	Number of herds	BVDV OD Mean (95% CI)	Per cent of herds in CLASS123 (95% CI)	Per cent of herds in CLASS3 (95% CI)
North	173	1·047 (0·993-1·101)	98·8 (97·2-100·0)	67·6 (60·8-74·4)
Yorks and Humberside	107	1·043 (0·971-1·115)	97·2 (94·1-100·0)	70·1 (61·6-78·6)
East Midlands	74	1·042 (0·942-1·142)	95·9 (91·5-100·0)	67·6 (57·1-78·1)
East Anglia	66	0·719 (0·586-0·852)	75·8 (65·7-85·9)	40·9 (29·3-52·5)
South east	100	0·877 (0·781-0·973)	91·0 (85·5-96·5)	52·0 (42·4-61·6)
South west	220	1·031 (0·974-1·088)	95·9 (93·3-98·5)	67·3 (61·2-73·4)
West Midlands	140	1·031 (0·964-1·098)	97·1 (94·4-99·8)	67·1 (59·5-74·7)
North west	86	1·076 (0·992-1·160)	97·7 (94·6-100·0)	70·9 (61·5-80·3)
Wales	104	1·108 (1·033-1·183)	99·0 (97·1-100·0)	74·0 (65·8-82·2)
England and Wales	1070	1·013 (0·987-1·039)	95·4 (94·2-96·6)	65·5 (62·7-68·3)

CI Confidence interval

TABLE 3: Multivariable models of herd characteristics associated with (a) BVDV antibody level (OD), (b) BVDV CLASS123 positive status and (c) BVDV CLASS3 positive status for 1070 dairy herds in England and Wales

Variable	Regression coefficient (β)	95% confidence interval (β)	Odds ratio
(a) BVDV OD			
Model 1			
East Anglia	−0·3498	−0·4583 to −0·2414	NA
South east	−0·1961	−0·2864 to −0·1058	NA
Herd size	0·0007	0·0002-0·0011	NA
Intercept	0·9723		
Model 2			
East Anglia and South east (region)	−0·4649	−0·6438 to −0·2860	NA
Herd size	0·0004	0·0001-0·0009	NA
Region by herd size interaction	0·0015	0·0003-0·0027	NA
Intercept	1·0018		
(b) BVDV CLASS123			
East Anglia	−2·6550	−3·3820 to −1·9276	0·0703
South east	−1·4965	−2·3213 to −0·6722	0·2239
Herd size	0·0062	0·0-0·0129	1·0063
Intercept	2·9530		
(c) BVDV CLASS3			
East Anglia	−1·2355	−1·7533 to −0·7178	0·2907
South east	−0·7981	−1·2242 to −0·3716	0·4502
Herd size	0·0025	0·0-0·0050	1·0025
Intercept	0·5168		

NA Not applicable

TABLE 4: Mean level of BHV-1 antibodies in bulk tank milk samples and prevalence of BHV-1-positive dairy herds in England and Wales and by region

Region	Number of herds	BHV-1 OD Mean (95% CI)	Per cent of herds with BHV-1-OD ≥0·05 (95% CI)	Per cent of herds with BHV-1 OD ≥0·8 (95% CI)
North	56	0·89 (0·69-1·10)	78·6 (67·9-89·3)	50·0 (36·9-63·1)
Yorks and Humberside	32	0·69 (0·41-0·97)	65·6 (49·0-82·2)	37·5 (20·6-54·4)
East Midlands	31	0·70 (0·45-0·94)	71·0 (54·9-87·1)	45·2 (27·5-62·9)
East Anglia	22	0·72 (0·33-1·11)	50·0 (28·8-71·2)	31·8 (12·0-51·6)
South east	41	0·76 (0·54-0·98)	70·7 (56·7-84·7)	46·3 (31·0-61·6)
South west	64	0·68 (0·52-0·85)	65·6 (54·0-77·2)	40·6 (28·6-52·6)
West Midlands	33	0·78 (0·53-1·02)	69·7 (53·9-85·5)	45·5 (28·4-62·6)
North west	27	1·43 (1·11-1·74)	80·8 (65·5-96·1)	76·9 (60·5-93·3)
Wales	35	0·37 (0·20-0·54)	63·9 (48·1-79·7)	16·7 (4·4-29·0)
England and Wales	341	0·77 (0·68-0·85)	69·2 (64·3-74·1)	43·1 (37·9-48·3)

CI Confidence interval

prevalence of BVDV or OD levels among the regions excluding East Anglia and the south east.

Nationally, there was no statistically significant association between the number of dairy cows in a herd and its BVDV antibody level or status (BVDV OD, CLASS123, CLASS3). However, after adjusting for the effect of the regions of East Anglia and the south east on BVDV OD levels and prevalence, there was a significant positive relationship between the number of dairy cows in a herd and its BVDV antibody level or status (Table 3). On average, each additional dairy cow in a herd was associated with an increase of 0·0007 in the OD level. Similarly, the odds of a herd being CLASS123-positive increased by a factor of 1·006 for each additional cow in the dairy herd, and the odds of it being a CLASS3-positive herd increased by a factor of 1·003.

Stratification of the data by herd size suggested that the regional differences in BVDV antibody levels and status might diminish with increasing herd size. However, a statistically significant interaction between region and herd size was found only when BVDV OD level was used as the outcome and East Anglia and the south east were combined as one region (Table 3). In this model, each additional dairy cow in a herd in East Anglia or the south east was associated with an increase of 0·0019 in the OD level. In the rest of England and Wales, the OD level increased by 0·0004 for each additional diary cow in the herd. Based on this model, the expected BVDV OD level in a 100-cow dairy herd was 0·73 in East Anglia and the south east and 1·044 in the rest of England and Wales. In a 200 cow dairy herd, it was 0 922 in East Anglia and the south east and 1·087 in the rest of England and Wales, and in a 310-cow dairy herd, the expected OD value in East Anglia and the south east converged with that in the rest of England and Wales at 1·134.

The results of the various multivariate analyses did not change significantly when the number of cows contributing milk to the bulk tank was used in the models instead of the number of dairy cows in the herd. The exclusion from the analyses of herds in which a small proportion of the dairy cows contributed milk to the bulk tank also did not affect the results. The association between a farmer's dairy heifer replacement policy and the BVDV antibody level or status of the herd was not statistically significant.

On a regional basis, there was a significant relationship between the mean BVDV OD of the sample herds and the total cattle density. East Anglia with 11·8 cattle per square kilometre and the south east with 26·5 cattle per square kilometre had significantly lower mean BVDV OD levels than the rest of England and Wales where there were from 39·9 to 88·5 cattle per square kilometre. The natural log of the cattle density in each region explained 81 per cent (95 per cent confidence interval, 59 to 98 per cent) of the variation in the mean BVDV OD among the regions.

Prevalence of antibodies to BHV-1

The distribution of BHV-1 ELISA antibody levels among the subsample of 341 dairy herds is shown in Fig 3a. Most of the herds (74 per cent) had either a very low level of antibody (OD <0·05) or were strongly positive (OD ≥0·8). The mean OD for the 341 herds was 0·77 (median 0·49, range 0 to 2·57). The prevalence of BHV-1 antibody-positive herds (OD ≥0·05) was 69·2 per cent and of strongly positive herds (OD ≥0 8), 43·1 per cent.

The regional mean BHV-1 OD levels and prevalences are given in Table 4. Herds in Wales had a significantly lower mean OD than herds in England and herds in the north west had a significantly higher mean OD than herds in the rest of England and Wales combined. The prevalence of BHV-1 antibody-positive herds varied from 50·0 per cent in East Anglia to 80·8 per cent in the north west, but the regional differences were not statistically significant. However, there were significant regional differences in the prevalence of herds with strongly positive BHV-1 antibody levels, which mirrored the BHV-1 OD results.

Nationally, there was a statistically significant linear relationship between the herd BHV-1 OD value and herd size. For each additional dairy cow in a herd, the OD value increased on average by 0·0029. When adjustments were made for this effect by using multivariable analyses, changes occurred in the regional associations detected by univariate analysis. Herds in the north and the north west of England had significantly higher mean OD levels whereas herds in Wales had significantly lower mean OD levels than herds in the remaining six regions of England. Adjusted for region, the BHV-1 OD level increased by 0·0026 for each additional dairy cow in a herd. Herds in East Anglia had a significantly lower probability of being BHV-1 antibody positive than herds in the rest of England and Wales. Regional differences in the prevalence of herds with a strongly positive BHV-1 antibody reaction were not altered by the inclusion of herd size in the logistic regression model. A similar result was obtained if the number of cows contributing milk to the bulk tank was used instead of herd size.

The 11 herds in which the farmer did not rear his own dairy replacement heifers were all BHV-1 antibody-positive compared with 223 (68 per cent) of the 328 herds in which the farmer did rear some or all of his own replacement heifers. This difference was statistically significant.

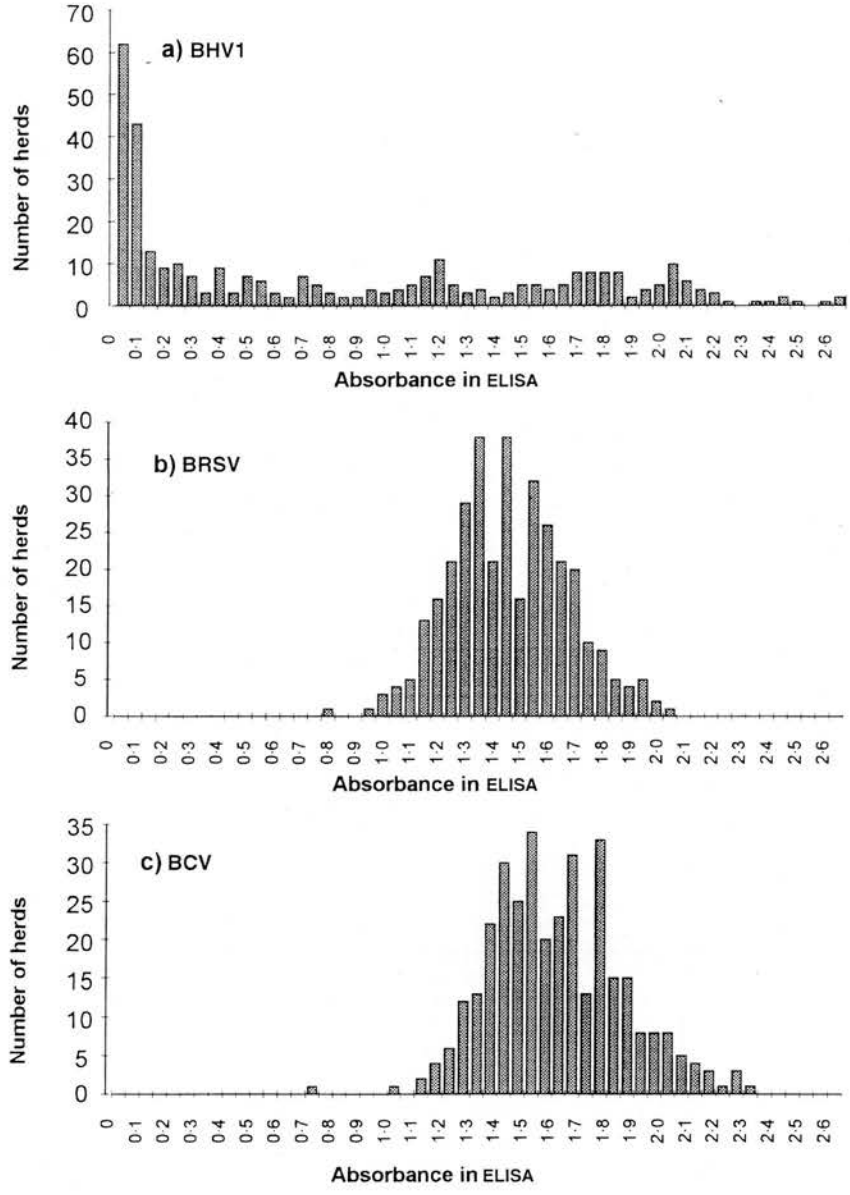

FIG 3: Frequency distribution of antibody levels in bulk tank milk samples from 341 dairy herds in England and Wales (a) BHV-1, (b) BRSV, (c) BCV

On a regional basis, there was a significant positive linear relationship between the median BHV-1 OD of the sample herds and the total dairy cattle density. However, when the north west region with its exceptionally high median OD level (1·77) and high dairy cattle density (30 per square kilometre) was removed from the analysis, there was no significant relationship between the median BHV-1 OD level and dairy cattle density.

Prevalence of antibodies to BRSV and BCV

All 341 dairy herds were antibody-positive to both BRSV and BCV with only one herd having an OD <0·8 to both viruses (Figs 3b and 3c). The mean BRSV OD was significantly lower in herds in the north west than in the rest of England and Wales while the mean BCV OD was significantly lower in Yorkshire and Humberside and in the south east (Table 5). Herds in East Anglia had significantly higher mean BRSV and BCV OD levels than herds in the rest of England and Wales (Table 5). The mean BRSV OD was also higher in herds in the south west than in the rest of England and Wales. There was no significant association between herd size or number of cows contributing milk to the bulk tank and the mean BRSV or BCV OD level. The regional mean BRSV and BCV OD levels were not related to the density of cattle in the regions.

Relationships between prevalence of antibodies to different viruses

The distribution of ELISA antibody levels by region for all four viruses is depicted in Fig 4. Herds that were BVDV CLASS123 positive were 10 times more likely to be BHV-1 positive and five times more likely to be BHV-1 strongly positive. The odds ratios for herds that were BVDV CLASS3 positive were 2·2 and 1·7, respectively. There were significant positive linear relationships between BVDV, BHV-1 and BCV OD levels and between BRSV and BCV OD levels.

TABLE 5: Regional distribution of BRSV and BCV antibody levels in bulk tank milk samples from dairy herds in England and Wales

Region	Number of herds	BRSV OD Mean (95% CI)	BCV OD Mean (95% CI)
North	56	1·34 (1·29-1·39)	1·52 (1·47-1·57)
Yorks and Humberside	32	1·35 (1·27-1·42)	1·48 (1·39-1·56)
East Midlands	31	1·41 (1·36-1·46)	1·54 (1·46-1·62)
East Anglia	22	1·60 (1·52-1·68)	1·81 (1·72-1·90)
South east	41	1·37 (1·31-1·43)	1·50 (1·42-1·57)
South west	64	1·44 (1·39-1·50)	1·62 (1·56-1·68)
West Midlands	33	1·34 (1·26-1·42)	1·61 (1·51-1·71)
North west	27	1·29 (1·21-1·36)	1·52 (1·45-1·59)
Wales	35	1·41 (1·34-1·47)	1·59 (1·52-1·65)
England and Wales	341	1·39 (1·37-1·41)	1·57 (1·54-1·60)

CI Confidence interval

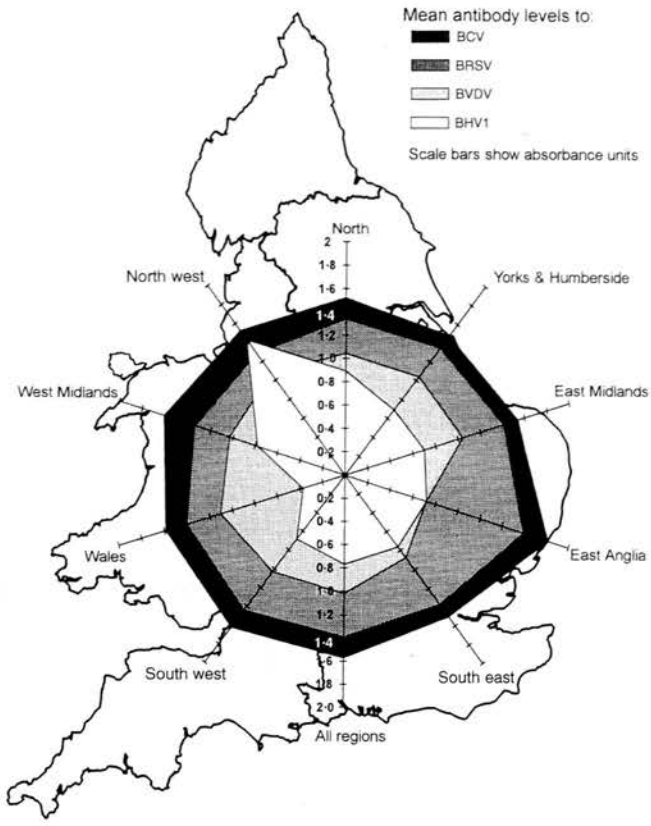

FIG 4: Comparison of mean BVDV, BHV-1, BRSV and BCV antibody levels by region

Discussion

The use of a bulk tank milk test enabled over 1000 samples, or 5 per cent of the dairy herd population in England and Wales, to be screened for antibodies to BVDV. The results demonstrated a very high level of exposure to BVDV in the national dairy herd. On the basis of Swedish research correlating antibody levels in bulk tank milk with within-herd seroprevalence (Niskanen and others 1991, Niskanen 1993), between 94 and 97 per cent of herds were estimated to have a BVDV antibody level indicative of seropositive cows in the milking herd (classes 1, 2 and 3). Between 87 and 90 per cent of herds had moderate or high BVDV antibody levels (classes 2 and 3), and approximately 65 per cent of herds had a high level of antibody (class 3). Considering that many of the class 0 herds would also have contained small numbers of seropositive animals, the proportion of herds without any seropositive cows is likely to be extremely small. The survey has provided the first estimate of the proportion of dairy herds in England and Wales that are likely to have had recent active BVDV infection or to have persistently infected animals in the herd (class 3).

The observed level of exposure of the national dairy herd to BVDV is remarkably similar to the levels reported by Edwards and others (1987) and Harkness and others (1978) despite marked differences in the methods used. Using sero-surveillance of 10 to 12 individual animals per herd, both sets of authors estimated that approximately 93 to 95 per cent of herds would contain at least one BVDV antibody-positive animal. They also found that about 62 to 65 per cent of the cattle had antibodies to BVDV, close to the seropositive rate of 66 per cent calculated in this study.

The regional variation in the prevalence of BVDV infection was similar to that recorded by Harkness and others (1978). To a large extent it reflected the density of the cattle population in England and Wales. East Anglia and the south east with their low cattle population densities had relatively high proportions of BVDV antibody-naive herds. These regions also have large blocks of arable land and isolated farms that reduce direct contact between herds. However, in very large herds, the difference in risk of BVDV infection between herds in East Anglia and the south east and herds in the rest of England and Wales was less marked, possibly as a result of the more frequent purchasing of cattle in larger herds.

The lack of change in the BVDV infection status of dairy herds in England and Wales over the last 20 years suggests that the advances made in the understanding of the pathogenesis and epidemiology of the disease and in the development of improved diagnostic methods have not been translated into better control. The small proportion of BVDV-naive herds suggests that current farm management practices do not provide adequate disease security and that improvements in biosecurity, with or without the removal of persistently infected animals and systematic vaccination, will be needed to reduce the frequency of BVDV infection significantly.

National schemes for the control and ultimate eradication of BVDV are under way in the Scandinavian countries. These rely on strict herd biosecurity measures in conjunction with the identification and removal of persistently infected animals (Waage and others 1994, Bitsch and Ronsholt 1995, Alenius and others 1997). Tests of bulk tank milk for antibody are used to screen dairy herds for BVDV infection. If there is a suspicion of active infection with BVDV, serology on a sample of eight- to 18-month-old homebred cattle is used to establish whether there have been recent seroconversions.

At this stage, it is not known what impact these BVDV control schemes will have on European community trade in cattle and on control of the disease in Great Britain. If the schemes are successful, other countries may be induced to attempt eradication in their national cattle herds. Milk surveys conducted in Finland and Sweden before their control schemes began (Niskanen 1993) showed a more favourable situation than in England and Wales, since 97 per cent and 16 per cent of herds, respectively, were free of BVDV antibodies (class 0). However, Denmark with a BVDV prevalence closer to that of England and Wales (Houe and Meyling 1991) has embarked on a national control scheme with some success. They have used a regional approach, starting with the areas of lowest prevalence. A similar strategy could be used in Britain, exploiting the lower prevalence of BVDV infection in East Anglia and the south east of England. In areas where adequate biosecurity cannot be established, vaccination may be a useful tool to reduce losses and to protect naive herds.

In an earlier study of the prevalence of BHV-1 infection in England and Wales, Edwards (1988) found that the prevalence of herds containing serological reactors to BHV-1 had increased from around 18 per cent in 1970 to 1973 to 48 per cent in 1984 to 1986. He predicted that the prevalence of BHV-1-infected herds in Great Britain would continue to rise owing to the spread of a newly introduced, more virulent subtype of BHV-1 around 1977 and the increased use of live attenuated BHV-1 vaccines. This prediction made nearly 10 years ago is borne out by the current survey that estimated that between 64 per cent and 74 per cent of the dairy herds in England and Wales now contain BHV-1 antibody-positive cattle. Over the same period, diagnoses of infectious bovine rhinotracheitis made at the VICs suggest that the incidence of clinical disease due to BHV-1 is declining in Great Britain (Veterinary Laboratories Agency 1996). If this is the case, then BHV-1 infection may have reached a steady state in the dairy cattle population of England and Wales. A similar estimate (75 per cent) has been made for the prevalence of BHV-1-positive herds in the Netherlands where the infection is considered endemic (van Wuijckhuise and others 1993).

Because the BHV-1 vaccination status of the 341 herds sampled was not known, it is possible that some of the positive results may have been due to vaccination rather than natural infection. However, as the use of BHV-1 vaccines in England and Wales appears to be low, the survey results are probably not greatly distorted by vaccination. A recent survey of 159 dairy herds by the VICs involved in the study revealed that only 9 per cent of the farmers vaccinated their herds against BHV-1 (D. J. Paton and others, unpublished observations). Four per cent of the farmers vaccinated their herds against BRSV.

In a study of 23 dairy herds in East Anglia between 1987 and 1991, only 22 per cent were completely free of BHV-1 seroreactors (Pritchard 1992) and in most antibody-positive herds less than 20 per cent of adult cattle were seropositive. The present study's estimate of a 50 per cent prevalence of antibody-negative herds in East Anglia probably indicates that bulk milk testing is unable to identify herds containing very small numbers of seroreactors (Frankena and others 1997, Hartman and others 1997). It also indicates that the national prevalence of dairy herds with BHV-1-seropositive animals is probably higher than the 69 per cent found in this survey. The polarisation of herds in the study into either strongly positive or virtually negative BHV-1 categories has been observed by other researchers (Espuna and others 1988, Pritchard 1992).

The pattern of association between BHV-1 antibody status and the location of a herd resembled that for BVDV. Edwards (1988) found a similarly high prevalence of BHV-1 infection in the north west and low prevalences in Wales and East Anglia. The dairy cattle densities in the regions may explain some of the difference in the prevalence of BHV-1 in herds in the north west and East Anglia. The north west has the highest and East Anglia has the lowest density of dairy cattle in England and Wales. However, it is more difficult to explain why Wales with its moderately high density of dairy cattle had a very low prevalence of BHV-1 antibody-positive herds, even after adjustments for the lower average size of Welsh dairy herds.

Nationally, there was a strong association between a herd being BVDV antibody-positive and its being BHV-1 antibody-positive. It is therefore likely that similar factors affect the risk of a herd being infected by either virus. Common risk factors would explain the similar geographical distribution of the two viruses and the effect of herd size on disease prevalence. In this limited study of risk factors for BVDV and BHV-1, herds in which all replacement dairy heifers were bought in were all BHV-1 and BVDV antibody-positive. In the case of BHV-1, the difference in antibody status between these herds and the others was slightly more marked, making the association between buying-in and a positive BHV-1 antibody status statistically significant. Hence, one common factor that may affect the risk of a herd being infected with either BVDV or BHV-1 is the introduction of purchased cattle into the herd.

This study was the first national survey of the prevalence of BRSV and BCV in dairy herds in England and Wales. As with BHV-1, the BRSV vaccination status of the herds was not known. However, as the use of BRSV vaccines appeared to be low, its effect on BRSV antibody levels was considered minimal. The results confirmed the widely held view that most adult cattle in England and Wales have evidence of exposure to BRSV, as is the case in other countries (Verhoeff and Van Nieuwstadt 1984, McCullough and others 1987). Sweden is the only other country that has published information on a nationwide survey for BRSV antibodies in bulk milk samples. In Sweden, the proportion of BRSV antibody-positive herds ranged from 51 per cent to 89 per cent and the highest prevalence was found in areas with the highest population of cattle (Elvander 1996). In the current survey, all the herds were BRSV antibody-positive and there was no significant association between the mean BRSV OD level of dairy herds in a particular region and its cattle density. East Anglia, with the lowest cattle density in England and Wales, had the highest mean OD for both BRSV and BSV, in sharp contrast with its position as the region with the lowest mean BVDV OD. The BRSV findings support the results of a survey by Hogg (1992) of 503 farms affected by respiratory disease; 70 per cent of the calves tested had antibodies to BRSV, and the highest proportion (77 per cent) of BRSV-seropositive herds was in the south east and East Anglia.

The reasons for the differences in the prevalence of the four viruses in the national dairy herd are uncertain. Both BVDV and BHV-1 are able to establish long-lasting infections. In the absence of effective controls for either virus, a higher prevalence of BVDV might suggest that the continuous shedding of virus from persistently infected animals may be more effective at spreading BVDV than the periodic shedding from animals latently infected with BHV-1, even though latency occurs more readily than persistent infection. However, BRSV and BCV are both more prevalent than either BVDV or BHV-1, even though neither is known to induce a carrier state.

Bulk tank milk testing to detect antibodies provides a useful, low cost method for determining the disease status of the national herd. It is also widely used in disease control schemes, for example, for brucellosis, enzootic bovine leucosis, BVDV, BHV-1 and *Leptospira hardjo* infections. Periodic bulk tank milk testing may be a useful means of providing regular herd surveillance, and in the case of BVDV, may be combined with targeted serology to establish the presence of persistently infected animals (Houe 1995). Bulk milk tests provide an inexpensive and convenient method for assessing the disease status of herds and thereby allow appropriate precautions, such as the vaccination or disease-screening of individual animals, to be undertaken before they are added to a herd.

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Making the Best Use of Bulk Milk Antibody Tests

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ABSTRACT

Measuring antibodies for BVDV, IBRV and *L.hardjo* infections by ELISA using milk samples taken from the bulk tank provides a simple low cost method of assessing the disease status of dairy herds. This allows appropriate control measures to be adopted. It can also be used for disease monitoring, as a supportive diagnostic tool when investigating herd problems such as milk drop and reproductive failures, and for national surveillance. This paper discusses the use of bulk milk ELISA tests as recently introduced by the Veterinary Laboratories Agency and outlines their potential role in dairy practice.

KEYWORDS: Cattle, Antibodies, Bulk milk, BVD, IBR, *L.hardjo.*

INTRODUCTION

Bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR) and leptospirosis represent three of the most important endemic infectious diseases affecting cattle in Britain. In addition to the economic and welfare aspects of clinical and sub-clinical disease, unless controlled, these infections all tend to persist thereby presenting important implications for the movement of stock between herds of different status.

Since the mid 1980s, antibody testing of bulk milk samples has been an integral part of voluntary or compulsory eradication schemes for IBR virus, BVD virus and *Leptospira hardjo* infections in several countries including Germany, France, the Netherlands and in Scandinavia. It has primarily been used to identify infected dairy herds with a view to implementing control measures, including vaccination. Combined with individual cow blood (or milk) tests, it is also used to detect seroreactors or individual persistently infected animals in the case of BVDV infection. In these countries, bulk milk antibody tests have provided a simple and convenient means of surveying the disease status of their national herd (Niskanen 1993, Wuijckhuise and others 1998). It was also apparent from the World Buiatrics Congress in Edinburgh 1996 and from the pre-congress workshop session on bovine abortion, that bulk milk antibody testing is used in some countries, in conjunction with individual animal blood or milk antibody testing, as a diagnostic tool for the investigation of disease outbreaks, including abortions. The recent introduction by the Veterinary Laboratories Agency (VLA) of bulk milk antibody testing by enzyme-linked immunosorbent assay (ELISA) for BVD, IBR and *L.hardjo* now offers similar opportunities to veterinary surgeons in Britain. Bulk milk ELISA is also used in the statutory control programmes for brucellosis and enzootic bovine leucosis.

GENERAL POINTS

By definition, bulk tank samples only reflect the status of cows whose milk is currently entering the tank and therefore do not include male animals, young stock, dry cows and those cows whose milk is being withheld for whatever reason. Samples will inevitably be affected by dilution and the unequal contribution of individual cows to the tank. The test measures past exposure and, like tests on sera, will not detect very recent infection or distinguish between field and vaccinal challenge.

Bulk milk ELISA can be used to determine whether a herd is "positive" or "negative" for exposure to a particular infection. However, unlike its use with notifiable diseases, this qualitative approach using a fixed cut-off point, is of limited value for common endemic infections. It may also fail to identify herds with a very small number of reactors - a limitation which is of particular concern when the test is used to support national eradication schemes such as those used for IBR in mainland Europe (Hartman and others 1997).

The tests offer greater potential by exploiting the basic principle that the optical density (OD) from a bulk milk ELISA gives an approximate indication of the proportion of seropositive cows, particularly with IBR and BVD since detectable titres generally persist for life. This information provides a good guide to whether infection is, or has recently been, active in the herd or whether the herd is naive. It must be appreciated that the seroreactors may all be amongst older or purchased cows and a high OD does not necessarily indicate active or recent infection. In general, to comprehensively establish herd status, at least two tests several weeks apart are required, together with an evaluation of statistically representative or specifically targeted, individual blood or bulked milk samples . Nevertheless, as a preliminary screening test, even a single bulk tank sample

provides a reasonable guide to the likely situation in the herd.

ADVANTAGES

The main advantages of bulk milk antibody testing are cheapness, ease of collection and the ability to provide a general idea of seroprevalence without the need to blood sample large numbers of animals (particularly useful when screening for *L.hardjo*). The tests provide a good starting point for differential diagnosis, in addition to their value for serological monitoring. It is envisaged that veterinary practices could record the information from bulk milk tests and incorporate it into preventive medicine programmes, particularly in relation to purchased animals (Pritchard 1996a). Regular testing of bulk milk samples every few months provides a simple low cost method of confirming continuing freedom from infection in known disease-free herds; including those at potential risk of introducing new infection, and thereby allows opportunity for prompt action if indicated. For example, a positive result in a previously negative herd could herald an upsurge in infertility, abortions or milk drop.

LIMITATIONS

Inevitably, the bulk milk ELISA will give a biased result if the status of cows contributing to the bulk tank is significantly different to the rest of the herd. Furthermore, any estimate of within-herd sero-prevalence is inevitably somewhat crude and can only provide an approximate guide. It is important not to expect too much of the test - ODs could show variations of up to 10 or 20 percent with samples collected only a few weeks apart depending on calving pattern, the number of cows contributing to the sample and their relative milk yields and antibody titres. The proportions of fat and colostrum can also have an effect. Paired (acute and convalescent) bulk milk samples taken several weeks apart are more difficult to interpret than paired individual cow blood samples because of the large number of variables involved, but this approach may still be of value in some circumstances.

SAMPLE COLLECTION

The sample collected should be representative of the milking herd. Wait until milking has finished and ensure that the contents of the tank are thoroughly agitated before collection. Collect sample from more than one tank if appropriate and either combine proportionately or test separately if this is likely to provide any useful information (eg. heifers and younger cows' milk collected in one tank). Samples can also be collected from different age cohorts, for example, when it is desirable to assess the status of first or second lactation homebred cows, or unvaccinated naive animals, as a guide to current/recent infection. For this, take an equal volume of milk from 6-10 cows in this category, mix well and submit an aliquot of the composite sample for testing.

Avoid collecting milk from the top of the tank to minimise the amount of cream present since this can significantly interfere with the result (although it will be removed at the testing laboratory anyway). Use a clean ladle or similar receptacle for sample collection and **do not allow preservative to get into the bulk tank**. Preliminary findings suggest that it is not essential that the bronopol preservative ("MMB preservative solution") used in the tubes is added immediately. Depending on environmental temperature, it could be added at the laboratory provided the sample is received within about 48 hours of collection. Once preservative has been added, the sample is stable for several months provided it is kept refrigerated. For longer term storage the samples can be frozen. The amount of preservative used (currently 150 µl/25 ml of milk) has no effect on the OD value.

THE BULK MILK ELISA TESTS

The bulk milk antibody tests used by the VLA for BVD and IBR are based on the indirect ELISA tests developed by the Central Veterinary Laboratory (CVL) for use with serum samples. The BVD test was initially calibrated against the Swedish Svanova BVDV antibody ELISA which has been used extensively in Sweden (Niskanen 1993). The CVL IBR ELISA was initially trialled alongside a commercially available kit that had been validated for use on bulk milk samples. The test currently used by the VLA for *L.hardjo* is based on the Dutch "Ceditest" (id-dlo) ELISA. In the Netherlands, this has been used since 1989 as part of a voluntary national control scheme under which a herd is considered to be *L.hardjo*-free if it passes five consecutive tests over an 18 month period at a specified cut-off point. This is a relatively crude ELISA and does not distinguish between infection with *L.hardjobovis* (the main pathogenic species) and *L.hardjoprajitno* or *L.saxkoebing*. Since it is essentially used as a screening test this shortcoming is not particularly significant since a high bulk milk OD is unlikely to be associated with the latter infections. Within the VLA, we have modified the test to make it more robust and to identify various disease categories. With all three tests, to improve robustness and eliminate any problems with antigen standardisation, results are expressed as a **signal to positive ratio** (OD ratio) using reference field control positive samples unique to the VLA and are therefore not comparable with results from other laboratories.

With BVD, calibration against the Swedish test allowed OD ratios to be subdivided into four main categories based on the Swedish experience. With IBR and *L.hardjo*, the ELISA tests were trialled within the VLA by examining milks from nearly 300 herds of known status, including completely

seronegative herds and some where all the cows were individually tested. The categories developed (negative, low positive, mid positive and high positive) are designed to reflect epidemiological status. Results falling in the mid positive ranges represent the inevitable "grey area" which is a feature of most serological tests and further monitoring or more comprehensive sampling is needed to clarify the precise situation in these herds.

THE TESTS IN PRACTICE

1. BVD

In Britain there has been renewed interest in control measures for BVD following the recent launch of a killed vaccine and developments in Scandinavia where several schemes exist for the control and potential eradication of the infection. The recent survey by the VLA using bulk milk samples from over a thousand dairy herds in England and Wales (Paton and others 1998) highlighted the considerable regional differences in infection levels and the potential risks associated with the movement of cattle.

The bulk milk ELISA provides a good starting point for BVD investigations. The categories adopted, based on OD ratios, are shown on Table 1 below which summarises interpretation, with a suggested course of action. In positive herds, particularly those in the mid to high positive ranges, individual animal or cohort blood or milk sampling of young homebred cows and uncalved heifers, will help to determine whether infection is currently active (Table 2).

Table 1: Bulk milk antibody categories for BVD with brief guide to interpretation and action

Category	OD ratio	Approx % seropos.	Interpretation/Action
Negative	<0.10	<5	**Naive.** Maintain herd security/quarantine measures. Test purchases for BVD virus (including calves born to seropositive dams). Monitor bulk milks every 3 months.
Low positive	0.10 - 0.35	<25	**Basically naive.** Proceed as above or if recent infection is suspected re-test in 3 - 5 weeks or use paired serology.
Mid positive	>0.35-0.70	25-65	Difficult to interpret. Re-test/monitor bulk milks or take individual blood or cohort bulk milk samples (see Table 2).
High positive	>0.70	>65	**Likelihood of PI animals increases with OD ratio** but may represent historical infection. To check for **active** infection blood sample young **homebred** cattle (Table 2). Results provide a basis for control strategy, including vaccination, if appropriate.

Table 2: Main outcomes from cohort serology in BVD-positive herds, with possible control options

9-18m Heifers	1st lactation cows	2nd lactation cows	Comments and main control options
All negative	All negative	Some or all positive	Active infection unlikely unless 1st lactation cows tested have only been calved for a few weeks. Persistently infected (PI) animals probably died or been culled recently. Monitor 1st lactation cohort (bloods or bulked milk) and maintain good herd security as above.
All negative	Some or all positive	Most or all positive	Active infection (acute infection/PI animals) in milking herd but no PIs amongst heifers. Consider vaccinating incoming heifers and/or test milking herd for PI animals.
Some or all positive	Most or all positive	Most (or usually all) positive	Active infection in milking herd as above plus PI animals probably present amongst heifers. Screen heifers (and younger female and male calves if retained) for virus and consider testing milking herd for PI animals. (PCR or individual bloods).

Note: sample homebred animals only, 6-10 per cohort (uncalved heifers ≥9 months, first and second lactation cows).

Seronegative cows/cohorts identified from these tests can be re-sampled several months later to see if seroconversion subsequently occurs, to provide a further guide to whether infection is active in the herd and whether persistently infected animals are likely to be present. The bulk milk polymerase chain reaction (PCR) test for BVD virus recently introduced by the VLA will provide additional information for use when deciding on a BVD control programme and whether, for example, it is likely to be worthwhile blood sampling the whole milking herd to identify individual persistently infected animals.

2. IBR

As outlined by Pritchard (1996b), excluding completely seronegative herds, IBR status tends to fall into two fairly distinct categories. In infected herds where less than 20 percent of adult cows are seropositive (low positive herds), the seroreactors are frequently older or purchased animals and there is usually very little, if any, virus circulation or active infection. Conversely, in those dairy herds with at least 60 percent seroreactors (high positive herds) reactivation of the latent herpesvirus infection and circulation of virus (either field or vaccinal) is common. Most incoming seronegative animals (usually heifers) seroconvert to IBR within two years, sometimes accompanied by mild clinical signs such as upper respiratory infection, pyrexia or milk drop. In these herds vaccination of heifers if seronegative, is worth considering. Table 3 gives details of the bulk milk categories for IBR with a brief guide to interpretation and a suggested course of action.

3. *L.hardjo*

The lack of *L.hardjobovis* specificity and the failure of leptospiral antibodies to persist in the same way as those for BVDV and IBRV, complicates interpretation of bulk milk results.

Herds in the negative and low positive categories (Table 4) are unlikely to have active infection with *L.hardjobovis* unless very recent - note that it may

Table 3: Bulk milk antibody categories for IBR with brief guide to interpretation and action

Category	OD ratio	Approx % seropos.	Interpretation/Action
Negative	<0.10	<5	**Naive/unvaccinated.** Maintain herd security/quarantine measures. Consider blood testing purchases if IBR-free status is desirable. Monitor bulk milks every 3 months.
Low positive	0.10-0.40	<20	Some seropositive cows but **extensive active infection unlikely.** Retest in 2 weeks/monitor bulk milks as above for major changes. Maintain herd security.
Mid positive	>0.40-0.70	20-60	Difficult to interpret. Re-test/monitor bulk milks or take individual blood or cohort bulk milk samples, particularly from young homebred unvaccinated cows as with BVD (Table 2).
High positive	>0.70	>60	**Heavily infected/vaccinated.** Significant virus circulation likely due to reactivation and re-excretion of latent infection. Clinical recrudescence may occur sporadically in unvaccinated herds - consider vaccination, especially heifers if seronegative.

Table 4: Bulk milk antibody categories for *L.hardjo* with brief guide to interpretation and action

Category	OD ratio	Approx % seropos.	Interpretation/Action
Negative	<0.07	<2	**Naive/unvaccinated.** Maintain herd security/quarantine measures. Review risk factors. Monitor bulk milks every 3 months. Test/ antibiotic treat purchased replacements.
Low positive	0.07 - 0.40	<10	A few seropositive cows but **active infection unlikely**. May be waning historical infection/vaccination in old cows or cross reaction with *L.saxkoebing/hardjoprajitno*. Retest in 2 weeks to check for recent infection, otherwise proceed as above.
Mid positive	>0.40-0.70	10-30	Difficult to interpret. Re-test/monitor bulk milks or take individual blood or cohort bulk milk samples, or blood sample a statistical cross section of the herd (BCVA 1992).
High positive	>0.70	>30	**Heavily infected/vaccinated**. If unvaccinated and no obvious risk factors, re-test immediately to confirm. If vaccination has lapsed, sample (blood or pooled milk) young unvaccinated cows for evidence of active infection. Vaccinate if appropriate.

take three or four weeks to develop a positive ELISA reading (mainly IgG) compared with 10 days for the microscopic agglutination test (MAT) which mainly measures IgM. The presence of a small number of seropositive cows in an otherwise uninfected herd, with no obvious risk factors (shared bulls, frequent purchases, water courses, co-grazing with sheep) is not likely to be due to infection with *L.hardjobovis* unless the titre is historical or herd infection is very recent. A similar situation was not infrequently encountered when testing was carried out for membership of the Cattle Health Scheme formerly operated by MAFF and proved to be a problem when allocating so called "Elite" status. In these herds, as with negative herds, regular monitoring of bulk milks provides the reassurance that disease status has not changed and it also helps to fulfil the risk assessment required by the COSHH regulations.

EFFECT OF VACCINATION

The present tests do not distinguish between vaccinal and field exposure. There is little point in testing herds which are already carrying out a full vaccination programme because they will inevitably give a positive result. Where vaccination has lapsed (particularly relevant with *L.hardjo*), there may still be a high OD ratio reflecting historical exposure or waning vaccinal titres. In such cases, in order to check whether infection is still active, collect a pooled bulk milk sample from a cohort of 6-10 unvaccinated (usually younger) cows in the herd or collect individual blood samples from the same group. If these tests are all negative, infection is not likely to be currently active although regular on-going monitoring of unvaccinated cows is still advisable. Often individual blood samples collected from unvaccinated young cows entering a herd previously infected with *L.hardjo* in which vaccination has ceased, will show very high MAT titres (≥1/400) confirming active recent infection in these naive animals.

THE FUTURE

Losses from BVD, IBR and *L.*hardjo are often subclinical and many veterinary surgeons and farmers are unaware of the disease status of their herds. Bulk milk tests offer an ideal opportunity to improve this situation and stimulate an interest in monitoring infections, adopting appropriate control measures including vaccination, and screening new stock (Pritchard 1996a). Opportunities for the future include bulk milk antibody tests for *Salmonella typhimurium* and *Neospora caninum*. Knowledge of a herd's disease status is particularly relevant at present when many previously closed herds are now purchasing replacements as a consequence of the cull for bovine spongiform encephalopathy.

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Bulk Milk Antibody Testing for *Leptospira Hardjo* Infection

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ABSTRACT

Measuring antibodies for *L.hardjo* infection by ELISA using milk samples taken from the bulk tank provides a simple low cost method of assessing the disease status of dairy herds. It encourages the adoption of appropriate control measures and helps to fulfil the risk assessment required by the COSHH regulations. It is also of value for disease monitoring, particularly in naïve herds and as a supportive diagnostic tool when investigating herd problems such as milk drop and reproductive failure.

KEYWORDS: Cattle, antibodies, bulk milk, *Leptospira hardjo*.

INTRODUCTION

Bulk milk antibody tests have been widely used in Europe since the mid 1980s as an integral part of voluntary or compulsory disease eradication schemes. In Britain, the Veterinary Laboratories Agency (VLA) introduced bulk milk enzyme-linked immunosorbent assay (ELISA) for *Leptospira hardjo*, bovine virus diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) infections in 1997 to allow private veterinary surgeons to assess and monitor the disease status of their clients' herds and for use as a diagnostic tool. Bulk milk ELISA is also used in Britain in the statutory control programmes for brucellosis and enzootic bovine leucosis. Further comments on the use of bulk milk antibody tests and disease security aspects are included in previous papers by the author (Pritchard 1996, Pritchard 1998).

GENERAL POINTS

By definition, bulk milk samples only reflect the status of cows whose milk is currently entering the tank and do not include male animals, young stock, dry cows and those cows whose milk is being withheld for whatever reason. The test measures past exposure to field or vaccinal challenge and, like tests on sera, will not detect very recent infection *see The L.hardjo ELISA*.

Bulk milk ELISA can be used to determine whether a herd is "positive" or "negative" for exposure to a particular infection. However this qualitative approach, using a fixed cut-off point, is of limited value for common endemic infections. It may also fail to identify herds with a very small number of reactors. The test offers greater potential by exploiting the basic principle that the optical density (OD) from a bulk milk ELISA gives some indication of the likely proportion of seropositive cows contributing to the sample. This information provides a guide to whether the infection is, or has recently been, active in the herd. However, it must be appreciated that the seroreactors may all be amongst older or purchased cows and, depending on how long detectable antibodies persist (often for life in the case of BVD and IBR), a high OD does not necessarily indicate active or recent infection. In general, to comprehensively establish herd status, at least two tests several weeks apart are required, together with an evaluation of statistically representative or specifically targeted, individual blood or bulked milk samples - *see Sample collection*. Nevertheless, as a preliminary screening test, even a single bulk tank sample provides a reasonable guide to the likely situation in the herd.

The main advantages of bulk milk testing are cheapness, ease of collection and the ability to provide a general idea of seroprevalence without the need to blood sample large numbers of animals (particularly useful when screening for *L.hardjo*). The tests provide a good starting point for differential diagnosis in addition to their value for serological monitoring and preventative medicine programmes. Regular testing of bulk milk samples every few months provides a simple low-cost method of confirming continuing freedom from infection in known disease-free herds, thereby allowing opportunity for prompt action if indicated. It is important to accept that any estimate of within-herd seroprevalence is inevitably somewhat crude because of the dilution effect and unequal contribution of individual cows to the tank. However, it is usually accurate enough to broadly categorise the herd status.

SAMPLE COLLECTION

Wait until milking has finished and ensure that the contents of the tank are thoroughly agitated before collection. Avoid collecting milk from the top of the tank to minimise the amount of cream present since this can interfere with the test result. Use a clean ladle or similar receptacle for sample collection and do not allow the preservative in the 20ml sample tubes (available from VLA on request) to get into the bulk tank. It is not essential to use the tubes with preservative if the samples are kept cool and

submitted promptly to the testing laboratory.
Take the sample from more than one tank if appropriate and either combine proportionately or test separately if this is likely to provide any useful information. Bulked samples can also be collected from different cohorts - particularly young homebred cows or unvaccinated naïve animals - as a guide to current or recent infection in the herd. For these, take an approximately equal volume of milk (a single squirt will suffice) from at least 10 cows in the appropriate cohort, mix well and submit an aliquot of the composite sample for testing.

THE *L.HARDJO* ELISA

The test currently used by the VLA is based on the Dutch "Ceditest" id-dlo ELISA (Bercovich and others, 1990). In the Netherlands, this has been used since 1989 as part of a voluntary national control scheme under which a herd is considered to be *L.hardjo*-free if it passes five consecutive tests at a specified cut-off point over an 18-month period. We have modified the test to make it more robust and to identify various disease categories. Results are expressed as an OD ratio (OD/OD control positive) which has been standardised within the testing laboratory. Milks were originally examined by the VLA from over 200 herds of known status, including some where all the cows were individually tested with the microscopic agglutination test (MAT) for *L.hardjo bovis* at a titre of 1/50. On the basis of the findings the OD ratios were subdivided into four categories as shown in Table 1.

When used on sera in a recent VLA trial (Woodward & others 1997) the sensitivity and specificity of the id-dlo ELISA compared well with the MAT, which despite limitations is still the reference test. Moreover, ELISA offers the significant advantage over the MAT in not requiring live antigen. However, it is important to realise that the ELISA mainly measures IgG antibodies, (which take 3-4 weeks to appear) whereas the MAT predominantly measures IgM for which titres usually peak at about 10-20 days post infection. More significantly, IgG antibodies for *L.hardjo bovis* persist on average for about two years compared with only 6-12 months for IgM antibodies. Hence, the ELISA is better at detecting long-standing infection whereas the MAT mainly measures fairly recent infection.

Herds in the negative and low positive categories are unlikely to have active infection with *L.hardjo bovis* (the main pathogenic species) unless very recent. The presence of a small number of seropositive cows in an otherwise uninfected herd, with no obvious risk factors, was occasionally encountered when testing was carried out for membership of the Cattle Health Scheme formerly operated by MAFF. Possible explanations include historical exposure or vaccination of older cows (including purchased animals) or cross reactions with the closely related *L. hardjo prajitno* or *L. saxkoebing*. In these herds, as with negative herds, regular monitoring of bulk milk provides the reassurance that disease status has not changed. It also helps to fulfil the risk assessment required by the Control of Substances Hazardous to Health (COSHH) regulations.

Herds in the high positive category are likely to be heavily infected with *L.hardjo bovis* and, or vaccinated. High OD ratios are unlikely to be associated with *L.hardjo prajitno* or *L.saxkoebing* which have a different epidemiology and usually cause only sporadic infection. There is little point in

Table 1: Bulk milk antibody categories for *L. hardjo* with brief guide to interpretation and action.

Category	OD ratio	Approx % seropos	Interpretation/Action
Negative	<0.07	<2	Naïve/unvaccinated. Maintain herd security/quarantine measures. Review risk factors. Monitor bulk milks every 3 months. Test/antibiotic treat purchased replacements.
Low positive	0.07-0.04	<10	A few seropositive cows but active infection unlikely. May be waning historical infection/vaccination in old cows or cross reaction with *L.saxkoebing/hardjo prajitno*. Retest in 2 weeks to check for recent infection, otherwise proceed as above.
Mid positive	>0.40-0.70	10-30	Difficult to interpret. Re-test/monitor bulk milks or take individual blood or cohort bulk milk samples, or blood sample a statistical cross section of the herd (BCVA 1992).
High positive	>0.70	>30	Heavily infected/vaccinated. If unvaccinated and no obvious risk factors, re-test immediately to confirm. If vaccination has lapsed, sample (blood or pooled milk) young unvaccinated cows for evidence of active infection. Vaccinate if appropriate.

testing herds which are already adopting a full vaccination programme because they will usually give a strong positive result. Even when vaccination has lapsed, the bulk tank may still give a high OD ratio reflecting historical exposure and/or waning vaccinal titres. In these cases, in order to check whether infection is still active, collect a pooled bulk milk sample from unvaccinated cows as detailed earlier. If negative, the findings suggest that infection is not currently active, although regular on-going monitoring of the 'sentinel' unvaccinated cohort is still advisable.

Results falling in the mid-positive 'grey area' need to be interpreted cautiously, preferably with further monitoring or blood sampling to clarify the precise situation. All results should be evaluated in relation to known risk factors (shared bulls, frequent purchases, water courses, co-grazing with sheep) and the precise population of cows contributing to the bulk sample.

RESULTS TO DATE

Feedback from general practitioners who have followed up bulk milk results with individual cow blood sampling using the statistical approach outlined by BCVA (1992) has been encouraging, with an apparently good correlation in most cases between the proportion of seropositive animals detected and predictions from the bulk milk test. Inevitably, however, there have been reports of high bulk milk OD values unsupported by an appropriate number of seropositive cows. The main explanation relates to the differences between IgG and IgM antibodies as described above - when sera are re-tested by ELISA instead of MAT results become much more comparable.

The results of testing 3264 bulk milks received at Sutton Bonington Veterinary Investigation Centre during the 12 month period between 12/97 and 11/98 on behalf of VLA in England and Wales have shown 801 (24.5%) negative, 646 (19.8%) low positive, 720 (22.1%) mid positive and 1097 (33.6%) in the high positive category. These samples were submitted for various purposes, including predominantly: assessing herd status, disease monitoring and for differential diagnosis of reproductive failure and milk drop. They include some repeat samples from the same herds. The results are therefore difficult to interpret, being heavily biased and dependent on geographical distribution and local initiatives. They are also complicated by the more variable persistence of detectable antibodies compared with the equivalent data for IBR and BVD bulk milk tests. However, despite these factors, throughout the testing period the proportion of samples from herds with no evidence of active infection with *L.hardjo bovis* (negative/low positive) has remained constant at around 40-50% compared with 50-60% in the mid/high positive range. With the latter it is unfortunately not possible to distinguish between vaccinal and field exposure but it is thought unlikely that many samples came from fully vaccinated herds.

CONCLUSION

The introduction of bulk milk antibody testing for *L. hardjo* and other infections has greatly facilitated the assessment of disease status, monitoring and different diagnosis of endemic disease problems in dairy herds. Further bulk milk tests are likely to become available in the future.

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Milk offers an attractive alternative to serum for examining the antibody status of lactating animals. Samples are readily available, simple to collect, and perform robustly under laboratory testing conditions

Milk antibody testing in cattle

GEOFF PRITCHARD

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MILK antibody testing has played a significant role in cattle disease control and eradication programmes in many countries, particularly in mainland Europe and Scandinavia, since the mid-1980s. In Britain, bulk milk antibody testing is an integral part of the statutory control programmes for brucellosis and enzootic bovine leukosis. It is also used by veterinary diagnostic laboratories, and within formal cattle health schemes, for measuring bovine viral diarrhoea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV)/bovine herpesvirus 1 and *Leptospira hardjo* infections. A bulk tank antibody test for *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) was used for a recent prevalence study in Denmark, but proved unreliable. Milk antibody testing has, however, been successfully developed for several other important cattle infections including salmonella, respiratory syncytial virus and coronavirus. In 1997, the VLA introduced bulk milk antibody testing for BVDV, IBRV and *L hardjo* in its Regional Laboratories. This article discusses the use of these tests, with particular emphasis on practical and cost-effective approaches.

ANTIBODIES IN MILK

In ruminants, the main immunoglobulin in milk and colostrum is IgG (mainly IgG1). The majority of IgG in milk is produced locally by the udder, whereas in colostrum it is all derived from serum via a selective transport mechanism from the maternal circulation prior to parturition (Tizard 2000). As lactation progresses, the immunoglobulin concentration falls, before increasing again after mid-lactation.

Despite the dynamic mechanisms involved, most published work has demonstrated a good correlation between serum and milk antibody concentrations in individual animals for a variety of infections using enzyme-linked immunosorbent assay (ELISA). A recent study by the author and his colleagues at the VLA, using ELISAs for BVDV and IBRV on individual cows, demonstrated very good agreement between readings on milk samples and the serum 'gold standard' results (Pritchard and others 2001). This study also found that the stage of lactation had no significant effect on test performance. Since antibody concentrations tend to be lower in milk than sera, different cut-off values or dilution rates are usually needed to obtain equivalent test sensitivity with milk. Samples are generally tested as either skimmed milk or whey.

Why use milk antibody tests?

- To provide a simple and inexpensive alternative to blood sampling
- To promote interest in herd biosecurity
- To initiate disease investigations at minimal cost
- To determine the antibody status of a herd and monitor changes
- To identify which infections are currently active
- To provide guidance on vaccination policy

LABORATORY METHODS

Ideally, milk samples for antibody testing should be submitted to the laboratory in 25 ml screw-top plastic Universal tubes or similar receptacles containing a preservative. This enables the milk to be stored for several days (or even months, if necessary) at 4°C, or deep-frozen, without detectable loss of antibody titre. It is also possible to add preservative on arrival at the laboratory if milk samples are received in good condition, within two or three days of collection (eg, postal samples). The preservative used by the VLA is Milk Marketing Board (MMB) solution (150 mmol/25 ml milk), which contains bronopol, kathon, copper nitrate and brilliant blue. The presence of fat may affect the optical density (OD) measurement used with ELISA.

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and so milk samples are first centrifuged in the laboratory before a sample suitable for testing is taken from below the cream layer.

The BVDV and IBRV milk antibody tests performed within the VLA utilise in-house indirect (IgG) ELISAs. Initially developed for sera, these were validated for use with bulk milk in the field and against commercially available milk ELISAs, and for individual cows by comparing antibody concentrations with those in sera. The *L hardjo* ELISA was adapted from the Ceditest (ID-DLO) indirect (IgG) ELISA, which has been used successfully on bulk tank and individual cow milk and serum samples in the Netherlands since 1989 (Bercovich and others 1990).

Bulk and individual cow milk antibody concentrations are expressed as OD ratios (OD of test sample/OD of known field control positive), rather than straight ODs. This standardises results and minimises fluctuations due to reagent variation. Bulk milk OD ratios are allocated to one of four categories (negative, low-, mid- or high-positive). These provide a broad assessment of the status of the herd with respect to a particular infection (see later). The BVDV categories are based on those widely used in Sweden; those for IBRV and *L hardjo* were developed within the VLA based on results from herds of known antibody status.

As with tests on serum, milk antibody ELISA does not distinguish between antibodies stimulated by vaccines currently available in Britain and those associated with field infection. However, this may be possible in the future with the development of marker vaccines such as the glycoprotein E deleted IBRV vaccine available in mainland Europe.

GENERAL APPROACHES TO MILK ANTIBODY TESTING

INDIVIDUAL COWS

The examination of milk, rather than serum, for determining the antibody status of individual cows, provides a way of reducing initial costs to the farmer and is particularly useful for herd screening purposes. Although primarily of value in dairy herds, it can also be used successfully with quiet suckler cows. The use of foremilk has not been fully evaluated; however, it is probably acceptable if samples are grossly normal. Recorder jar samples are likely to be unreliable because of potential carry-over between cows.

BULK MILK

Bulk milk antibody testing may be used either qualitatively to detect the presence or absence of a particular infection, such as enzootic bovine leukosis, or quantitatively to estimate the proportion of seropositive cows in a herd. The latter is of particular value for common endemic infections and assumes that the antibody titre, or OD ratio, equates approximately to the proportion of seroreactors. It should be borne in mind that a high-positive bulk milk result does not necessarily indicate current or recent infection, because with viral infections, such as BVDV or IBRV, detectable antibodies may persist for life. OD values may also reflect recent or historical, part or whole herd vaccination.

Viewed on a herd basis, individual cow variations in antibody concentration and milk yield tend to even themselves out and the bulk milk results provide an often remarkably repeatable 'snapshot' of herd antibody status. This is evident from the almost identical readings obtained when herds are resampled within a few days. Despite this, a single bulk milk result can only ever provide an approximate guide. It is always prudent to submit a further sample or samples to confirm the findings before making major decisions, particularly when results are not consistent with herd disease history and/or known risk factors. For monitoring purposes, especially in herds known to be free of a particular infection, bulk milk should be retested at least every three months, depending on individual circumstances.

COHORT SAMPLES

It is often useful to examine the antibody status of a specific group or cohort of animals rather than, or in addition to, the whole herd. Testing a composite milk sample from part of a herd provides a cheap and convenient, albeit less precise, alternative to individual cow blood or milk samples. The sample should comprise an approximately equal volume of milk from as many cows as possible (ideally at least 10 to 15). Alternatively, the cohort can be put through the milking parlour first and the bulk tank sampled at an appropriate point during milking. A whole herd bulk tank sample can be collected at the end of milking for comparison, if necessary.

Bulk milk sample collection

- Bulk tank samples should represent the whole herd, as far as possible
- Wait until milking is complete and agitate the tank to mix milk before collection
- Use a jug or ladle to collect samples. Do not immerse tubes containing preservative
- If appropriate, combine samples in approximate proportions from different tanks
- Use preservative and store samples as described for individual milks

NB Results may be biased by the exclusion of some cows or groups (eg, bulls, dry cows, heifers and cows with milk withheld from tank)

Milk sample tube containing preservative

Individual cow sample collection

- Sample milk from any quarter
- Avoid colostrum and grossly abnormal specimens
- Collect into Universal containers containing preservative
- Preserved samples keep for several months at 4°C
- Freeze at –20°C for long-term storage

Cohort milk sample collection

- Combine milk samples to provide a crude indication of antibody status of a group
- Take approximately equal volumes of milk (a squirt) from at least 10 to 15 cows
- Combine into a single clean container
- Mix thoroughly and pour off milk into a 25 ml sample bottle for testing
- Use preservative and store as described for individual milks
- Useful cohorts include unvaccinated, young homebred, and known naive cows

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(above) Bulk milk antibody testing assists in the investigation of complex disease problems and (above right) provides an inexpensive, 'farmer-friendly' approach to establishing herd antibody status

Knowing the antibody status of the first-lactation (homebred) cohort is helpful when interpreting the whole herd bulk tank result. It identifies probable recent or current infection, rather than just historical exposure. Similarly, testing a cohort of unvaccinated cows in a herd where vaccination has lapsed can help to determine if infection is still active, and whether vaccination should be resumed. Any such decision should, of course, also include an evaluation of biosecurity. Recently purchased animals, or groups of cows managed separately, constitute other potentially useful cohort samples. If the identity of individual cows making up a seronegative cohort is known, the same group (effectively sentinels) can be serially retested at regular intervals (every three to six months) to monitor for active infection.

Cohort results can be interpreted using similar guidelines to bulk milks, provided enough individual cow samples have been included, but the approach essentially seeks to distinguish between positive and negative.

BULK AND COHORT MILK ANTIBODY TESTING

INITIAL ASSESSMENT OF HERD STATUS

A bulk milk antibody test, preferably combined with a first-lactation homebred cohort sample, provides a good initial working assessment of herd disease status – a factor often unknown to many farmers and their veterinary surgeons. Without this information it is difficult to know what disease security precautions and pre-entry testing measures are appropriate for purchased animals and which endemic diseases might be contributing to herd health problems.

MONITORING

Herds in negative or low-positive categories are potentially susceptible to infection, depending on the relevant risk factors. In these naive herds, regular monitoring of bulk milks (usually at three-monthly intervals) allows identification of any newly introduced infection. For herds already in a high-positive category, monitoring bulk milks is of less value, but serial testing of specific cohorts, sentinel groups or individual animals may be appropriate. Herds in the inconclusive, mid-positive category should generally be closely monitored to establish status. It should be borne in mind that the repeatability of results of follow-up bulk tank testing will inevitably be influenced by changes in the composition of the milking herd, such as those associated with calving patterns and the introduction of heifers or purchased animals.

Veterinary practitioners may find it useful to record graphically the changes in bulk milk antibody results for their clients' herds over a period of time. This increases awareness of what infections are active and serves to highlight any newly introduced infections. The records also provide a valuable source of health assurance for potential purchasers of breeding replacements (as, for example, promoted by the recently introduced Cattle Health Certification Standards [CheCS] initiative).

DISEASE INVESTIGATION

Although not a substitute for comprehensive veterinary investigation, bulk tank and cohort samples provide a useful starting point for the examination of complex multifactorial disease problems, such as abortions, infertility and milk drop, which could be attributable to the common endemic infections discussed here. Bulk/cohort milks can also be helpful when investigating respiratory disease and diarrhoea outbreaks. In these cases, the submission of milk samples to the laboratory in addition to other specimens, can provide potentially relevant

Uses of bulk milk and cohort antibody testing

- Assessment of disease status and biosecurity priorities
- Monitoring of endemic infections
- Providing an early warning system for newly introduced infections
- Convenient starting point for disease investigations
- Health assurance when seeking herd replacements
- Cattle health schemes, including those run by veterinary practices
- Veterinary research projects, facilitated by non-invasive sample collection
- Inexpensive epidemiological tool for antibody surveys
- National and regional disease surveillance
- Potentially very wide applications with respect to different infections and animal species

NB Bulk/cohort milks can only provide a rough guide to antibody status

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BVDV BULK MILK ANTIBODY TESTS: GUIDE TO INTERPRETATION AND ACTION

Category	*OD ratio*	*Approximate % seropositive*	*Comments*
Negative	<0·10	<5	Naive herd, probably unvaccinated (see text). Review and upgrade biosecurity, where appropriate. Consider vaccination if in high risk location. Quarantine purchases and test for BVDV antigen (and antibody, if pregnant). Test calves born to seropositive purchased dams for antigen. Monitor bulk milks every three months
Low-positive	0·10-0·35	5-25	Basically naive. Proceed as above or retest in three to five weeks to confirm status
Mid-positive	>0·35-0·70	>25-65	Situation less clear. Closely monitor bulk/cohort/sentinel milks or proceed as below
High-positive	>0·70	>65	PI animals are more likely as OD ratio increases but it may represent historical infection. Hence, additionally test milk from young homebred cow cohort and sera from six uncalved homebred heifers to clarify. Use bulk tank PCR to check for PI animals, or serial sentinel testing of milk/sera from known naive individual cows/cohorts to detect active infection. Consider whole or part herd vaccination (including naive added stock) and/or screen incoming heifers for PI animals

background information on endemic disease status and facilitate the interpretation of individual animal serology results. Testing paired (acute and convalescent) bulk milk samples can be useful for demonstrating active infection in a herd, although this is much less precise than paired individual blood or milk samples. However, as mentioned earlier, resampling of cohorts of animals previously identified as being seronegative, can provide a very sensitive indicator of current disease status.

MILK ANTIBODY TESTING FOR SPECIFIC INFECTIONS

The following section provides a more detailed description of the specific applications of bulk and cohort milk antibody testing when investigating the potential role of BVDV, IBRV and *L hardjo* in herd disease problems and formulating control programmes.

BVDV

Bulk milk testing, particularly when combined with the assessment of cohort milk samples from first- and, perhaps, second-lactation homebred cows, and sera from uncalved homebred heifers (about six is usually adequate), provides a very useful starting point for herd BVDV investigations. The results can assist in the formulation of control strategies, including whether to vaccinate all or part of a herd.

A high bulk milk result, in the absence of seroconversion in young homebred cows or uncalved heifers, suggests infection is historical. Follow-up monitoring of negative cohorts or individual seronegative cows determines whether infection is currently active and whether it is likely that persistently infected (PI) animals are present in the milking herd. The latter can be investigated further using a bulk tank polymerase chain reaction (PCR) test to detect BVDV. This requires 150 ml of milk without preservative (further details are available from the VLA Regional Laboratories). In small herds, the presence of PI animals may reduce the bulk milk antibody concentration. This is thought to be due to competitive binding of antibodies to soluble antigen or virus particles in the milk instead of to antigen immobilised on the ELISA plate. It should be noted that in the first year following BVDV vaccination of naive animals, although still protected from infection, OD ratios in milk (and sera) may remain low but they subsequently increase after revaccination.

IBRV BULK MILK ANTIBODY TESTS: GUIDE TO INTERPRETATION AND ACTION

Category	*OD ratio*	*Approximate % seropositive*	*Comments*
Negative	<0·10	<5	Naive/unvaccinated herd. Review and upgrade biosecurity, where appropriate. Consider vaccination if in high risk location. Quarantine purchases (and test for IBRV antibody?). Monitor bulk milks every three months
Low-positive	0·10-0·40	5-20	Basically naive; extensive active infection unlikely. Proceed as above, or retest in two to three weeks to confirm status
Mid-positive	>0·40-0·70	>20-60	Situation less clear. Closely monitor bulk/cohort/sentinel milks, or proceed as below
High-positive	>0·70	>60	Active infection with extensive reactivation and re-excretion of latent virus probable. Clinical recrudescence/seroconversion may occur in incoming stock. Test young homebred cow cohort and uncalved homebred heifers to clarify or use paired samples. Consider vaccinating uncalved heifers if seronegative (likely) as well as naive added animals

IBRV

As a typical herpesvirus, IBRV has the potential for widespread within-herd transmission through reactivation and re-excretion by latently infected (seropositive) animals, usually in response to some form of stress. Despite this, the within-herd seroprevalence in the field situation tends to fall into two fairly distinct categories which can usually be determined by bulk and cohort milk sampling.

In herds where consistently less than 20 per cent of the cows are seropositive (low-positive bulk milk category), there is very little, if any, virus circulation or active infection. In this situation, seropositive animals are frequently older or purchased animals that have been in the herd for several years without transmitting infection. Where appropriate, it is usually a simple matter to eliminate IBRV infection completely from these herds by a test-and-cull approach.

In high-positive herds (where at least 60 per cent of the cows are seropositive), there is invariably extensive circulation of field and/or vaccinal virus. Most naive animals (including homebred heifers reared separately) which enter these herds seroconvert within about two years. Depending on the pathogenicity of the virus strain(s) involved, this can be accompanied by clinical signs (often fairly mild or unrecognised), including upper respiratory disease, pyrexia, milk drop or infertility. In such herds, vaccination of heifers, which are usually seronegative (blood test, if in doubt), is worth considering.

LEPTOSPIRA HARDJO

The microscopic agglutination test (MAT), rather than ELISA, is the most widely used test (on serum) for *L hardjo* and is adopted for international trade purposes. The MAT measures mainly IgM, which demonstrates recent infection; titres peak after 10 to 20 days but decline within six to 12 months. The Ceditest ELISA, meanwhile, measures IgG; this immunoglobulin does not appear until three or four weeks after infection and persists for about two years. ELISA is therefore of much less value for milk drop diagnosis, but is a better guide to

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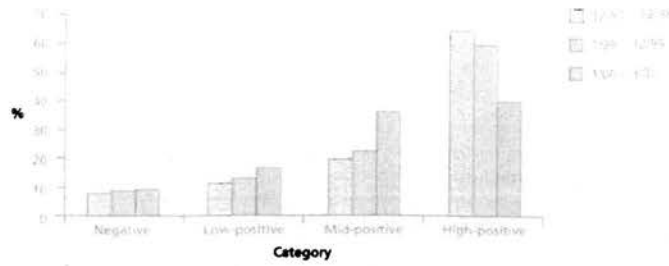
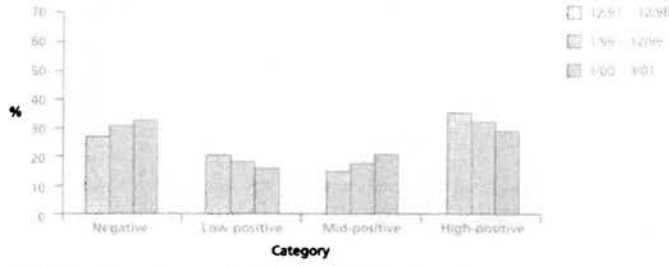


BVDV: bulk milk trends, December 1997 to March 2001 (n=19,436)



IBRV: bulk milk trends, December 1997 to March 2001 (n=5584)

Trends in bulk milk antibody test results

Approximately 38,000 bulk milk antibody tests for BVDV, IBRV or *L hardjo* were carried out by the VLA in England and Wales in the period between December 1997 and March 2001. The distribution of herds submitting samples appeared to broadly reflect the national cattle population. Samples were mainly submitted to Regional Laboratories for initial assessment of herd status; in many cases, this was prompted by promotional schemes offered by pharmaceutical companies. Other samples were submitted for follow-up monitoring; about 10 to 15 per cent were for strictly diagnostic purposes. The proportion of submissions received from vaccinated herds is not known; however, it is thought unlikely that many were included because it would not have provided any useful information.

Despite obvious selection bias, and the fact that the data did not constitute a random survey, quarterly analysis of the returns demonstrated a fairly consistent pattern during the three-year period under review.

L HARDJO BULK MILK ANTIBODY TESTS: GUIDE TO INTERPRETATION AND ACTION

Category	*OD ratio*	*Approximate % seropositive*	*Comments*
Negative	<0·07	<2	Naive/unvaccinated herd. Review risk factors, particularly shared bulls, purchased stock and watercourses. Upgrade biosecurity as appropriate. Consider vaccination if in high risk location. Quarantine purchases, blood test for *L hardjo* and/or give prophylactic antibiotic. Monitor bulk milks every three months
Low-positive	0·07-0·40	2-10	Basically naive, but may be historical infection/vaccination or cross-reaction with *L saxkoebing* or *L hardjoprajitno*. Proceed as above, or retest in two to three weeks to confirm status
Mid-positive	>0·40-0·70	>10-30	Situation less clear but infection with *L hardjobovis* probable. Proceed as below, or closely monitor bulk/cohort/sentinel milks, especially if history of discontinued or part herd vaccination (see text)
High-positive	>0·70	>30	Likely to be heavily infected and/or vaccinated. Retest immediately to confirm (NB zoonotic implications), especially if no obvious risk factors and unvaccinated. To investigate further, take individual cow/cohort blood or milk samples. Test unvaccinated cohort if vaccination has lapsed. Vaccinate and treat with antibiotic at drying-off, where appropriate

longer term status than MAT and more useful when purchasing replacements. These facts also explain some of the apparent discrepancies seen when comparing individual cow serum MAT results with milk or serum ELISA. Nevertheless, in a study in which 2144 serum samples were examined, Woodward and others (1997) reported 88·4 per cent sensitivity and 92·4 per cent specificity with the ELISA using the MAT as the reference standard. Although the *L hardjo* ELISA is a very sensitive test, in practice it may not distinguish between infection with *L hardjobovis* (the main pathogenic species) and the closely related serovars *L hardjoprajitno* and *L saxkoebing*. However, this is not usually significant when used as a bulk milk screening test (see below).

In unvaccinated herds, monitoring of bulk milk provides a convenient method of complying with the risk assessment required under the Control of Substances Hazardous to Health (COSHH) regulations. The bulk milk test also offers an alternative to the usual practice of testing a statistical number of individual animals to obtain a herd assessment. Low-positive bulk milk OD ratios are unlikely to represent active infection with *L hardjobovis*, since this would be expected to spread fairly quickly within a herd resulting in a mid- or high-positive OD ratio (retest, if in doubt). It is more likely that it represents cross-reaction with a related serovar. In herds fully vaccinated against *L hardjo*, the bulk milk antibody concentration is usually in the mid- or high-positive category. Even if *L hardjo* vaccination ceased two or three years previously, the bulk milk OD ratio may still be elevated, despite the absence of active infection and negative MAT titres in individual cows.

SUMMARY

Milk antibody testing provides an inexpensive, farmer-friendly approach to examining the infection status of cattle herds. It may also encourage some farmers to initiate disease investigations that would not otherwise be undertaken because of the cost of blood sampling. Importantly, bulk milk antibody testing offers a convenient means of providing potential purchasers with basic assurances on the endemic disease status of breeding replacements. It is likely to prove valuable, for example, when sourcing cattle for restocking after the recent devastating foot-and-mouth disease epidemic. There are also considerable potential opportunities for epidemiological surveillance and research projects, not only in cattle but also in lactating animals of other species.

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BVDV

The early results for BVDV were very similar to those obtained in a large epidemiological survey carried out by the VLA in 1996 (Paton and others 1998); this used bulk milks to provide a comprehensive picture of BVDV infection in dairy herds in England and Wales. There was an asymmetrical frequency distribution and progressive increase in number between the negative samples (7 per cent) and those in the high prevalence category (64 per cent). The marked fall (to 40 per cent) in the proportion of high-positive samples tested after 1999 probably reflected a lack of interest in monitoring known heavily infected herds, particularly those that had recently embarked on vaccination programmes.

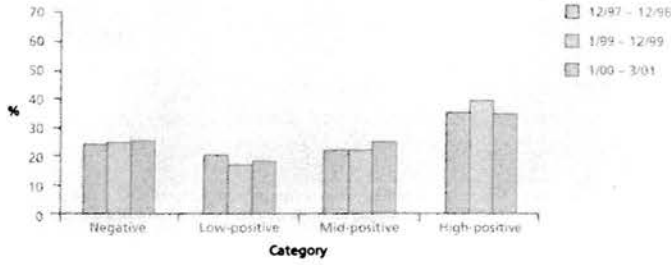
IBRV

The prevalence pattern for IBRV infection differed markedly from that for BVDV. As anticipated (see text), test results showed a bimodal frequency distribution, polarising into either high (30 to 36 per cent) or negative categories (28 to 34 per cent).

Leptospira hardjo

There was a fairly even spread between *L hardjo* seroprevalence categories, partly attributable to the lack of long-term persistence of detectable antibody. The proportion of samples submitted from negative herds (24 to 25 per cent) was virtually unchanged during the three-year period. Consistently, 54 to 59 per cent of samples were in the mid- to high-positive categories, suggesting either exposure to field infection, vaccination, or both.

***L hardjo*: bulk milk trends, December 1997 to March 2001 (n=12,504)**



Acknowledgements

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Detecting antibodies to infectious bovine rhinotracheitis and BVD virus infections using milk samples from individual cows

G. C. PRITCHARD, G. M. KIRKWOOD, A. R. SAYERS

MILK has become increasingly important since the 1980s for detecting the presence of antibodies to a wide range of bovine infections. Samples are simple and convenient to obtain and, unlike serum, testing does not incur the added cost of veterinary collection. Milk antibody testing therefore provides farmers and their veterinary surgeons with a potentially very useful and inexpensive method of initiating disease investigations and monitoring the presence of common endemic infections in the herd. The non-invasive method of sampling also readily lends itself to use in research projects and epidemiological surveys.

Several authors, including Niskanen and others (1989), Witte and others (1989) and Hoorfar and Wedderkopp (1995), have used an ELISA to examine the relationship between milk and serum immunoglobulins for a number of bovine infections. The in-house antibody ELISA methods employed by the Veterinary Laboratories Agency (VLA) for bovine viral diarrhoea (BVD) virus (Edwards 1990) and infectious bovine rhinotracheitis (IBR) virus (Edwards and others 1986) were originally developed for serum but have since been adapted for testing herd bulk-milk samples (Paton and others 1998, Pritchard 1998). This short communication describes the evaluation of the use of these tests on milk samples from individual cows, as an alternative to sera, for routine submissions to VLA regional laboratories. The effect of stage of lactation and other factors, including general robustness in working practice, were also considered. It was thought particularly opportune to develop the further use of milk as a farmer-friendly method of sample collection at a time when the cattle industry faced severe financial hardship and there were fewer requests for veterinary visits to investigate disease problems because of cost.

In ruminants, immunoglobulins (Igs) predominantly of the IgG_1 class, are selectively transported from the maternal circulation into the mammary secretions before parturition, ready for ingestion and absorption in colostrum by the sucking neonate (Husband 1998). Milk Ig concentrations fall as lactation progresses. Caffin and others (1983) noted that concentrations of IgG_1 in milk whey were similar at 30 and 150 days lactation but were significantly greater by 270 days. Unlike colostrum, most IgG in milk is produced locally from the udder rather than transported from serum (Tizard 2000). Milk IgG_1 is known to increase after experimental challenge with mastitis-causing bacterial pathogens, apparently due to selective transport rather than changes in mammary gland permeability from a generalised inflammatory response (Guildry and others 1980, Caffin and others 1983).

Despite the dynamic mechanisms involved, most authors have noted a significant correlation between antibody concentrations, as detected by ELISA, in milk and sera for a number of bovine infections. Although antibody concentrations are generally lower in milk than sera, equivalent test sensitivity can be achieved by using different cut-off values or different test dilutions (Niskanen and others 1988, Witte and others 1989). In the laboratory, milk samples are usually tested either as whey or skimmed milk. According to Niskanen and others (1988), BVD virus antibodies in milk reached a minimum 21 weeks after parturition before increasing again with the approach of the dry period; there was also a slight decline with increasing milk yield. These authors found little variation in antibody titres in skimmed milk samples between individual mammary quarters and no detectable effect from subclinical mastitis or raised somatic cell counts. However, Forschner (1988) commented that altered mammary secretions were unsuitable for IBR virus ELISA testing. False-positive results may occur more frequently with milk than serum (Witte and others 1989, Hoorfar and Wedderkopp 1995) but this probably reflects the methods of milk preparation and dilution before testing rather than any problem with milk as a sample medium. Milk may actually produce fewer false-positives since it acts as a very efficient blocker for non-specific binding (T. Drew, personal communication).

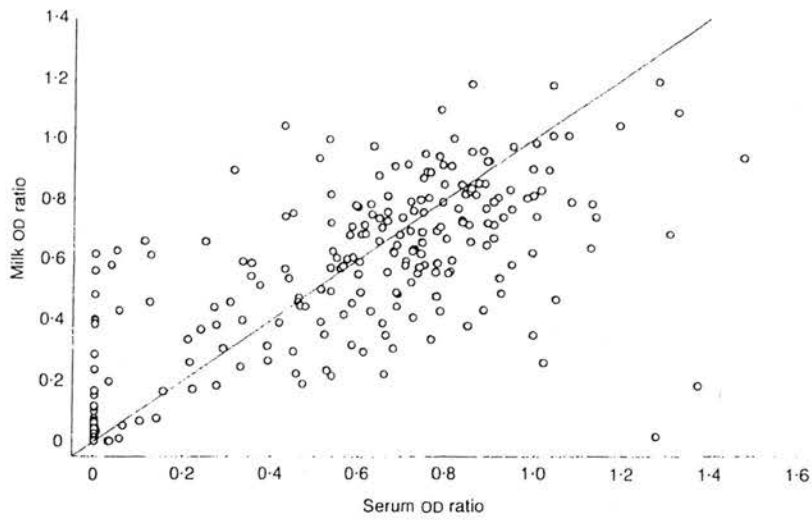

FIG 1: Scatterplot of bovine viral diarrhoea virus milk optical density (OD) ratio against serum OD ratio

Between May and October 2000, 394 matched sets of milk and sera submitted to the VLA – Bury St Edmunds for diagnostic or disease-screening purposes by local practising veterinary surgeons, were tested for BVD virus and IBR virus antibodies using the in-house indirect ELISAs referred to above. Details of age, breed and month of lactation were recorded. The samples were received as 61 separate submissions from 40 unrelated herds, mainly Holstein-Friesian, but also including 29 sets of samples from a Jersey herd and 19 from three unrelated beef suckler herds (South Devon, Lincoln Red and Jersey crosses). Two hundred and forty-three (62 per cent) samples came from nine herds (range 12 to 64 samples), and the remaining samples were received in batches of up to five per herd. These included 45 (11 per cent) from cows which had aborted and for which tests for BVD virus and IBR virus were requested in addition to statutory examinations for *Brucella abortus*.

Milk samples were submitted in labelled, sterile 25 ml plastic universal tubes containing 150 µl of Milk Marketing Board preservative solution (bronopol, kathon, copper nitrate and brilliant blue) (Wychem). With most of the abortion samples, preservative was not added until after arrival at the laboratory. Milks were stored at 4°C for up to three months, before being tested in batches with the corresponding sera. The sera were stored at –20°C for up to three months but were subsequently held at 4°C, for up to about two weeks, before testing. Since the presence of fat is known to affect optical density (OD) (Witte and others 1989), the milk samples were centrifuged before use and 2 ml milk samples were aliquoted from beneath the cream

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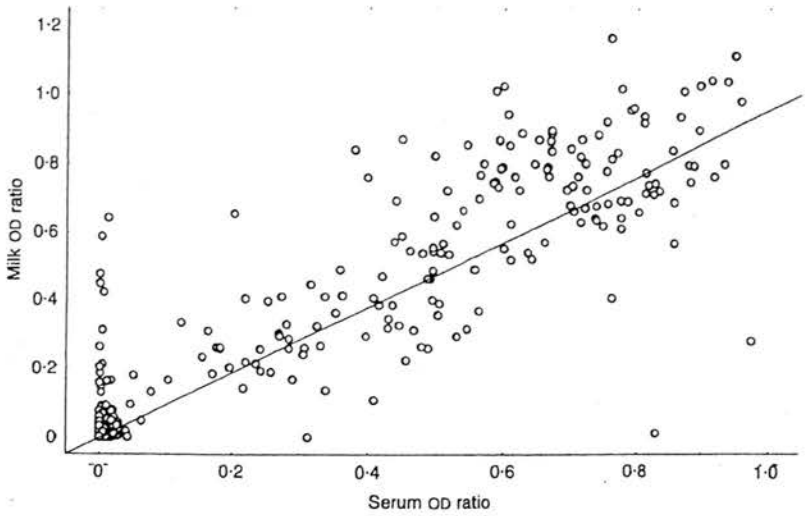

FIG 2: Scatterplot of infectious bovine rhinotracheitis virus milk optical density (OD) ratio against serum OD ratio

layer. Subsequently, 200 µl skimmed milk aliquots were diluted (1:1) with 200 µl phosphate buffer diluent, which contained polyvinylpyrrolidone blocking agent. Corresponding serum dilutions were 1:100 (IBR virus) and 1:50 (BVD virus).

The VLA ELISAs were used to detect IgG in the milk and sera using microtitre plates coated with sonicated and inactivated antigen. Antibody bound to solid-phase antigen was subsequently detected using an anti-bovine monoclonal antibody conjugated to horseradish peroxidase, visualised by addition of a substrate chromagen and expressed as OD. OD ratios (sample OD/control OD) for milk and sera were subsequently classified as seropositive or seronegative using the cut-off OD ratios of at least 0·25 for IBR virus and at least 0·20 for BVD virus.

As shown in Figs 1 and 2, a wide range of OD ratios were recorded for both infections. The diagonal lines represent equality. The milk and serum OD ratios were compared by paired *t* tests (Table 1). At the dilutions used, the milk IBR virus OD ratios were significantly higher than the serum values, but for BVD virus the difference was not significant (P=0·284). A logistic regression was fitted to the milk ELISA result (negative/positive) as a function of stage of lactation (up to one month, one to two months, two to three months, more than three months, and abortions), age class (less than four years, at least four years) and serum ELISA result (negative/positive) using the generalised estimating equations method in the statistical package STATA (StataCorp 1999). There was no detectable effect of stage of lactation on the performance of the test for IBR virus (P=0·137) or BVD virus (P=0·812). With BVD virus, for a given serum test result, older cows were more likely to be positive by the milk test than younger animals (P=0·022), but this effect was not detected with IBR virus (P=0·204). Several other authors, including Caffin and others (1983), have reported that IgG_1 concentration increases with age of cows.

Using serum ELISA as the definitive 'gold' standard, 153 (38·8 per cent) of the 394 sera were seropositive for IBR virus, and 188 (47·7 per cent) were seropositive for BVD virus. The sensitivity and specificity of the milk ELISA for IBR virus, with 95 per cent confidence limits, were estimated as 0·961 (0·917 to 0·986) and 0·925 (0·884 to 0·955), respectively. For BVD virus, the corresponding values were 0·973 (0·939 to 0·991) and 0·932 (0·889 to 0·962).

In summary, the VLA milk ELISAs performed well against the serum ELISAs. Importantly, there was no evidence that the stage of lactation had a significant effect. At the dilutions specified, and using the same cut-offs as those for serum, both milk tests showed slightly greater sensitivity than specificity. Increasing the cut-offs would have improved specificity at the expense of sensitivity but on balance this was considered inappropriate. The milk tests were robust, straightforward to perform, and suitable for routine veterinary diagnostic use as an alternative to sera in lactating animals. There was no evidence that they were affected by freezing and thawing, storage at 4°C for several months, the inevitable presence of colostrum in some of the abortion samples, or delaying the addition of preservative until receipt at the laboratory.

TABLE 1: Comparison of milk and serum optical density (OD) ratios for infectious bovine rhinotracheitis (IBR) virus and bovine viral diarrhoea (BVD) virus

Sample	Mean (sd) OD ratio*	Number of samples	Difference (sd) between means	*t* test	P
IBR virus					
Serum	0·246 (0·313)	394	−0·070 (0·145)	−9·621	<0·001
Milk	0·316 (0·381)				
BVD virus					
Serum	0·345 (0·393)	394	0·011 (0·200)	1·037	0·284
Milk	0·334 (0·353)				

*Sample OD/control OD

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Bulk Milk Antibody Testing: Where are we Now?

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ABSTRACT

During the last 20 years bulk milk antibody testing has been used in many countries to assess the disease status of dairy herds. Since 1997, the Veterinary laboratories Agency has performed over 60000 bulk milk antibody ELISA tests for BVD, IBR or *Leptospira Hardjo*. This paper details experiences gained during this period and includes practical applications, problem solving, frequently asked questions, newly introduced tests and future possibilities.

KEYWORDS: Cattle, bulk milk antibodies, ELISA, BVD, IBR, *L. Hardjo*

BACKGROUND

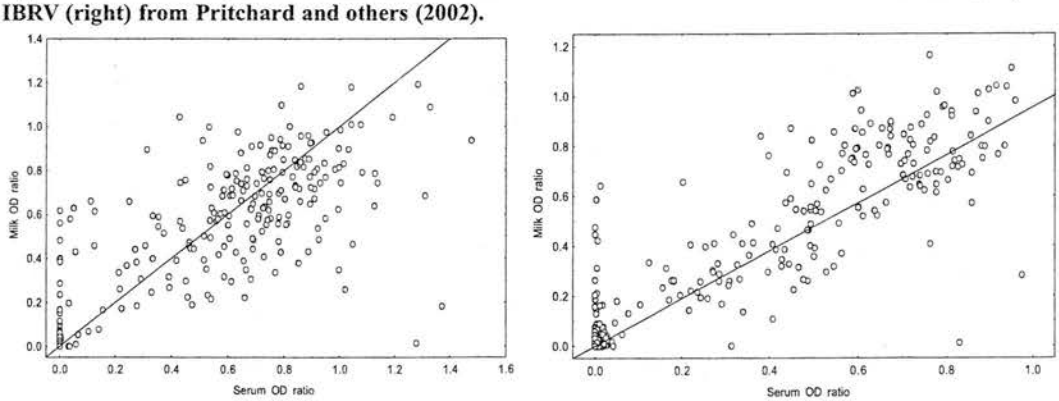
Since the mid 1980s, many countries, particularly in mainland Europe, have used milk as an alternative to serum to assess the antibody status of cattle for surveillance surveys and disease eradication schemes. As shown in Figure 1, milk from individual cows can also be used instead of serum for a number of tests, such as those to detect antibody to bovine viral diarrhoea virus (BVDV) and infectious bovine rhinotracheitis (IBR/bovine herpesvirus 1 (BHV1) infection. However, it is the possibility of determining the antibody status of a *whole* herd from a *single* milk sample conveniently taken from the bulk tank that is most appealing.

Milk is cheap, easy to obtain, and readily available. In ruminants the main immunoglobulin in milk is IgG (especially IgG1) produced locally by udder lymphoid tissue (whereas that in colostrum is derived from serum). Immunoglobulin concentrations fall as lactation progresses and increase as parturition approaches but overall there is a good correlation between concentrations in serum and milk. Moreover, on a "swings and roundabouts" principle it is generally accepted that the immunoglobulin concentration of a well mixed bulk tank sample correlates well with the overall antibody status of a herd.

Although Britain has not followed some other countries by operating large scale voluntary or compulsory eradication schemes for endemic diseases such as BVD or IBR, bulk milk antibody testing has nevertheless become firmly established as a useful low cost diagnostic and monitoring tool within dairy veterinary practice. Unfortunately the availability of the tests came slightly too late for inclusion in the former Cattle Health Scheme (CHS) introduced by the State Veterinary Service in 1987, although this approach was introduced for enzootic bovine leucosis (EBL) and *Brucella abortus*. Bulk milk antibody testing is now an integral part of cattle health schemes that followed the demise of the CHS in 1996, including the Premium Cattle Health Scheme (Scottish Agricultural College) and Herdcare (Biobest). The offer of free bulk milk antibody testing has also provided an attractive marketing tool for pharmaceutical companies wishing to promote awareness of the diseases concerned and the role of vaccination for their control.

The Veterinary Laboratories Agency (VLA) first introduced bulk milk antibody testing 10 years ago in a large epidemiological survey of the prevalence of BVD virus infection in dairy herds in England and Wales (Paton *et al.* 1998) using ELISA testing methodology developed for disease eradication in small dairy herds in Sweden (Lindberg and Alenius 1999). The survey also included antibody testing for three other common endemic infections namely IBRV, bovine respiratory syncytial virus (RSV) and bovine coronavirus. The VLA in-house indirect serum (IgG) ELISAs for BVDV and IBRV were later validated for use with milk and the tests were made available to veterinary practitioners in England and

Figure 1: Relationship between individual cow milk and serum ELISA OD ratios for BVDV (left) and IBRV (right) from Pritchard and others (2002).



Wales. A slightly modified version of the ID-DLO (Ceditest) ELISA (Bercovich *et al.* 1990), which has been used successfully in a national control programme in the Netherlands, was also introduced for *Leptospira Hardjo* since the microscopic agglutination test (MAT) used routinely by VLA for testing sera was not suitable for milk.

In some situations (such as the statutory monitoring of bulk milk samples for EBL or *B. abortus*), a simple qualitative positive or negative result is adequate provided the test offers an acceptable degree of sensitivity in relation to herd size. However, with common endemic infections for which many herds are likely to have at least some serological evidence of infection, just presence or absence of antibody is not enough to be of any practical value. Hence, when VLA first introduced bulk milk antibody testing for BVD, IBR and *L. Hardjo*, efforts were made to categorise the within-herd seroprevalence results (expressed as optical density (OD) ratios), into negative, low positive, mid positive and high positive. These categories were designed to offer a guide to subsequent course of action including vaccination and further testing (Pritchard 1998, Pritchard 2001).

The findings from bulk milk samples submitted to VLA Regional Laboratories (RLs) for surveillance and monitoring (rather than diagnostic) purposes, have changed very little during the period 1997 to 2005 apart from a reduction in samples from BVD high-positive herds. The frequency distribution of the various categories was much the same in 2005 (Figure 2) as that reported previously by Pritchard (2001). Although the data do not constitute a random survey and represent a biased (albeit very large) sample of herds in England and Wales, they nevertheless give some idea of the likely national picture. Vaccination is a major confounding factor since it is not possible to distinguish vaccinal antibodies from those derived from field exposure to infection. However, it is likely that when herds commence vaccination they will cease to monitor milk antibody status for the infection concerned. This is a probable explanation for the decline in samples from herds in the high positive BVD category compared with results from the national survey by Paton *et al.* (1998).

Full details of the use of the VLA bulk milk tests were presented at previous BCVA meetings (Pritchard 1998,1999) and also described extensively by Pritchard (2001). It is not proposed to repeat this information here but instead to present an overview based on experiences and frequently asked questions from over 60000 tests for BVD, IBR and *L. Hardjo* undertaken by VLA since 1997.

FREQUENTLY ASKED QUESTIONS (FAQs)

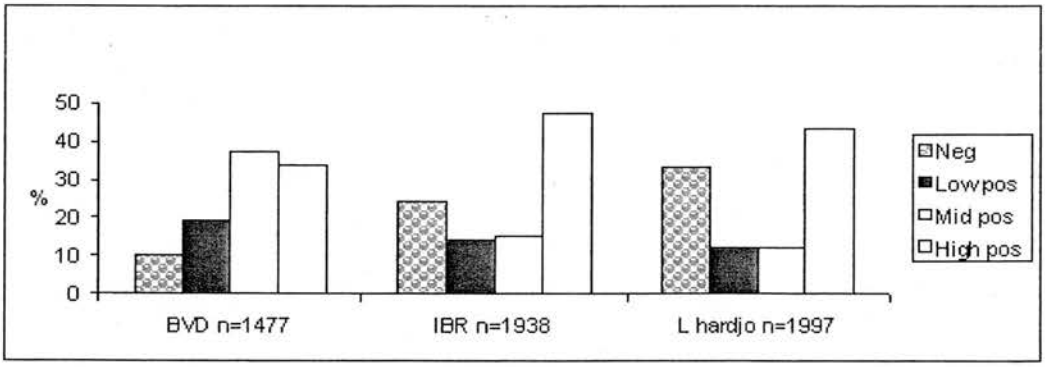
1. How accurate are the tests ?

The methodology used in bulk milk antibody testing inevitably lacks great precision and very minor changes in OD ratio should be ignored (correct the result to a the first decimal point). Do not lose sight of the fact that the tests use a very small sample of milk from a very large bulk tank which may comprise milk from 100 or more cows of varying stages of lactation and yield, so essentially it is just a "ball-park" figure. Moreover, the make-up of the bulk tank can vary from day to day depending on a number of factors including calving pattern, drying-off programme and milk being withheld because of antibiotic treatment for mastitis or other disease; individual cow milk yield and antibody titres also vary widely. However, despite these variables, experience has consistently shown that the bulk tank readings remain remarkably constant when a herd is in a stable disease situation. In practice the number of cows contributing to the tank does not appear to be an issue: with larger herds where samples are taken from both tanks the results are usually virtually identical.

2. The milk sample has been in my car for a couple of days, can it still be tested?

Milk ELISAs perform remarkably well in terms of robustness (better than serum) and repeatability is very good despite varying storage and transit times

Figure 2: Bulk Milk Antibody Test Results 2005.



and inevitable variation of test reagents. Provided the sample is not clotted and cheesy, the preservative can often be added several days later in the receiving laboratory without affecting test results but try to avoid this if possible.

3. We have just taken on a new dairy client and know nothing about the herd's disease status. Would a bulk milk antibody test be useful?

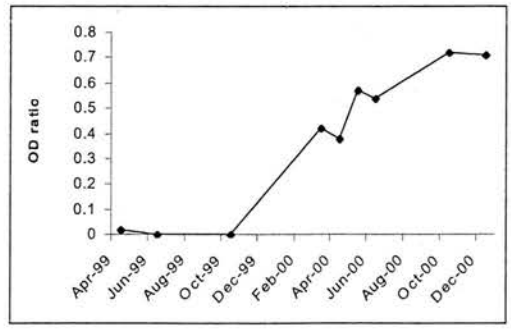
A bulk milk antibody result essentially provides a "snapshot" of a herd's antibody status and is much cheaper, simpler and more practical than statistical blood sampling. Initial herd screening to assess status is one of the main indications for using bulk milk antibody tests. Bulk milk tests have proved to be an invaluable tool when taking on the herd of a new client, source matching breeding herd replacements or setting up a herd health plan. They are also a useful first step or "foot in the door" when investigating chronic or complex disease entities such as reproductive failure or milk drop. The results should be assessed in the context of vaccination and disease history where available.

4. How frequently should bulk milk antibody testing be carried out?

Once the base line disease status has been established, the regular use of bulk milk testing at three-monthly intervals (or more frequently if there are ongoing risk factors such as purchased replacements or attendance at shows), provides a very useful and inexpensive method of monitoring for changes and early detection of incoming disease. It is also used in health schemes to ensure maintenance of disease-free status.

The value of regular monitoring was well illustrated (Figure 3) in an incident involving the introduction of IBRV infection into a high health status IBRV-free dairy herd. Similarly, the early detection of BVDV antibodies in a naïve herd in the absence of clinical signs, could permit prompt vaccination, which might in turn prevent or significantly reduce potentially disastrous sequelae. A certain amount of serendipity is always likely to be involved with the timing of sample collection in relation to prompt detection of disease but testing at strategic times such as at the end of the show season (if cattle are exhibited) or after cattle return from seasonal summer grazing is a useful approach.

Figure 3: Increase in IBRV OD ratio in a high health status dairy herd following introduction of infection (from Pritchard *et al.* 2003).



Follow-up monitoring is of much less value where the initial assessments indicate a result in the high positive category and such herds are often subsequently vaccinated. Be aware that antibody titres (ODs) for BVDV and IBRV essentially persist for the lifetime of the cow. Bulk tank OD ratios will only decline in heavily infected herds if there is no active infection and as seropositive older cows are gradually replaced by seronegative stock. Where a negative cohort has been identified for whatever reason, such as when checking whether BVDV infection is still present in a high positive herd (see 9) the same group of animals (record the ear numbers) can be retested after a few months (*i.e.* as sentinels) as a further check to establish whether infection is active.

5. Can I use paired bulk milk samples in the same way as paired sera?

Paired (acute and convalescent) bulk milk samples have been used successfully as a means of diagnosing recently introduced disease but it is generally preferable to use paired serology or paired individual milk samples for this purpose, particularly when morbidity is fairly low since it is much more targeted approach.

6. Do the bulk milk tests detect vaccinated animals?

Yes. There is little value in initiating bulk milk antibody testing in a fully vaccinated herd because it is likely to be fairly strongly positive. Vaccination is a major confounding factor with the interpretation of bulk milk tests and in general it is not possible to distinguish between field and vaccinal titres. However, where IBRV/BHV1 gE marker vaccine is used, ELISAs are available to distinguish vaccinates from animals that have seroconverted to field exposure, although test sensitivity may be an issue. Where vaccination has lapsed but there are concerns about whether infection is still present, making up a composite cohort sample bulked from 10-15 unvaccinated homebred cows (an approximately equal squirt of milk from each) and, or age cohorts as appropriate, offers a quick inexpensive farmer-friendly method of establishing status (Pritchard 2001). The lack of expected increase in bulk milk OD ratio often seen following primary vaccination with inactivated BVDV vaccines relates to an inherent limitation in the nature of the test linked to detection of structural protein (I Mawhinney, personal communication).

7. The bulk milk result is much higher than expected. What should I do next?

An unexpectedly high bulk milk result in a known previously naïve unvaccinated herd, or unexpectedly large increases in OD ratio, or changes in category compared with the previous sampling, inevitably cause concern. Unlike cross sectional blood sampling to determine herd status, with bulk milks we are using a single small volume sample to judge an entire herd. Moreover, as discussed at 1 above, the test offers only limited precision and minor changes should be ignored. It is ***essential to confirm the result*** before initiating precipitous action: ask the laboratory to check the result and also promptly submit a further sample for testing. Also consider possible explanations for increases in OD ratio such as recent introduction of purchased replacements.

8. The bulk milk result is much higher than expected and has been confirmed. Should I do anything else now, such as collecting cohort samples?

A confirmed result that is significantly higher than expected demands further investigation without delay. It is often worth testing parity (or other) cohort samples (separate pooled samples from first, second and third or older lactation groups as at 6) to check whether the infection involves a particular group of cows, such as those that have grazed separately or have come from a particular purchased source. When trying to decide if infection is currently active in a herd, the status of young unvaccinated homebred animals (first and second lactation cohorts) is particularly important since if they are positive it indicates that infection is likely to be fairly recent. Individual blood or milk samples could be taken for further investigations instead of bulk milk cohorts but the later approach is something the farmer can easily do to get the ball rolling and may be adequate for the purpose. When collecting cohort samples take them directly from the teats of individual cows (not from the jar) to avoid the possibility of carry over from the previous cow.

9. Which bulk milk antibody tests are the most reliable?

Regarding the individual tests, the IBR bulk milk ELISA has generally proved to be the most straightforward and trouble-free in terms of performance and interpretation. In common with other herpes viruses, seropositivity to IBRV is linked to potential infectivity. A high positive result invariably means extensive virus circulation within the herd due to reactivation and re-excretion of latent virus with incoming naïve animals likely to seroconvert within a year or two of entry, sometimes accompanied by clinical signs. Low positive herds have few seropositive animals (<20%) and virus circulation is usually minimal (Pritchard 1996).

L. Hardjo has caused most problems with interpretation, particularly when trying to compare the findings with individual cow serological results from the MAT, which is still the commonly used gold standard on sera and used for international trade purposes. The MAT mainly measures IgM antibodies, which are short lived and have variable persistence (averaging about six months) whereas the ELISA measures IgG which persists for two or three years. Hence, the latter is most useful for detecting chronic infection whereas MAT is best for recent infection. The ELISA does not appear to be completely Hardjo-specific and a low positive result may reflect exposure to other closely related leptospires, such as *L.* Saxkoebing; there is also the issue of the relative pathogenicities of hardjo Bovis and hardjo Prajitno, either of which may potentially be detected by the ELISA. High bulk milk ELISA OD ratios for *L. Hardjo* could be associated with historical infection and/or vaccination, in which case the MAT results (recent infection) might be negative. Seroconversion to the MAT occurs within about seven to 10 days, whereas to seroconvert to ELISA takes nearly four weeks. Despite these difficulties, the bulk milk ELISA is a reliable and sensitive indicator of either complete absence of infection or it's potential presence; significant increases into the higher categories are likely to be important. In problem situations, in addition to statistical individual cow MAT serology on 25-30 unvaccinated animals and age cohort analysis, the additional testing of individual cow or cohort milk samples by ELISA can help to clarify herd status.

With BVD, in general the higher the OD ratio the more likely it is that one or more persistently infected (PI) animals are present in the herd. However (unlike IBR), do not assume that active infection is present just because the bulk milk result is in the high positive category. Comparison of cohort milk samples from young and old homebred cows in many such herds, and examination of serological status of uncalved heifers, suggests that active infection is present in only about 20-50 per cent of high positive herds. This is probably because all PI animals have died or otherwise left the herd and seropositive cows are mainly confined to the older age groups or purchased animals. As a note of caution in very small herds, Sandvik *et al.* (2001) described a 14 cow dairy herd in Norway in which the presence of a single PI was associated with a much lower bulk tank OD value for BVDV than expected. This was attributed to competitive binding of antibodies to soluble antigen or virus particles in the milk rather than to antigens immobilized on the ELISA plates.

10. What about looking for antigen rather than antibody in the bulk tank?

In addition to antibody testing of bulk milk samples there is on-going interest in testing bulk tank samples for antigen or viral genome, as with the RT-PCR for BVD (which is available within VLA and can detect a single virus positive cow in a sample derived from up to 100 cows) or using bulk tank PCR for identifying herds infected with Johnes disease. The use of the bulk tank RT-PCR for BVDV could be adopted on herds in the high positive category to detect active infection as an alternative, or in addition, to cohort or sentinel antibody testing. It should also be considered in the small herd situation described at 9 above.

11. What other bulk milk tests are available for endemic diseases?

In addition to bulk milk antibody testing for BVD, IBR and *L. Hardjo*, the VLA has recently introduced bulk milk antibody testing for *Fasciola hepatica*. Results are categorised as negative (<25% seropositive), inconclusive (25% seropositive) and positive (>25%). This test was used successfully in a survey of dairy herds in East Anglia (Pritchard *et al.* 2005).

Bulk milk antibody testing using the Pourquier ELISA for *Mycobacterium avium* sub species *paratuberculosis* (Johne's disease) is also available from several private laboratories, including those under the umbrella of the self-regulatory body Cattle Health Certification standards (CheCS) but has not been taken up by VLA or SAC because of concerns about test sensitivity. However, this aspect will be evaluated in a national Johne's disease survey due to commence at the end of 2006.

Other potentially useful bulk milk antibody ELISA tests on the immediate horizon include those for *Neospora caninum*. Several commercially available ELISAs for *N. caninum* have been validated for use with milk; the serum ELISA (MAST) currently used by VLA, has also been validated for use in individual and bulk milk (S Felstead, personal communication). Bulk milk tests for bovine coronavirus and RSV were included in the survey by Paton and others (1998) and could potentially be introduced if there was sufficient demand, which seems unlikely.

12. How do you see milk antibody testing developing in the future?

There is clearly scope to extend bulk milk antibody testing further to include other infections such as salmonella or Q fever. However, there are other potentially more useful avenues to explore. For example, the VLA has recently developed a protein based microarray whereby antibodies specific for each of 176 different O serotypes of *Escherichia coli* were spotted onto a single array platform allowing serotyping to be performed in minutes in a single tube (M Woodward, personal communication). In principle, any protein can be spotted onto an array platform and in the case of bulk milk examination, multiple antigens can be spotted and attaching antibodies detected very much as in ELISA. Similar approaches are being developed for other situations such as bovine respiratory disease serology and identification of mastitis pathogens in milk samples (R Card, personal communication). The major advantage of microarray approaches is the potential for multiple analytes to be examined from the same samples in the same single test. In addition, the reading of array tests gives a numerical output that is ideal for automated data collection and analysis. It seems likely that the microarray approach will supersede conventional antibody testing during the next decade.

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Added Animals : The Challenge To Preventive Medicine

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ABSTRACT

Standards of health security in cattle herds continue to lag behind other livestock sectors. Serious and costly disease outbreaks occur because farmers fail to adopt even minimal precautions when introducing new stock. Many problems could be averted by strict adherence to a four-week quarantine period combined with thorough clinical examination, strategic laboratory tests and prophylactic treatments. This paper discusses preventive options for the more easily detectable conditions such as bovine virus diarrhoea virus infection, digital dermatitis and contagious mastitis. Suggestions for preventing the introduction of problematical subclinical infections including salmonella and *Mycobacterium paratuberculosis* are also presented. The profession is urged to take up the challenge and adopt a more pro-active role by advising farmers of the potential disease risks from incoming stock and persuading them to adopt appropriate preventive measures. Improved knowledge of the disease status of their own herds is an essential adjunct to this policy, particularly since added animals may themselves be at risk from infections already present.

INTRODUCTION

The general concepts of health security in cattle herds were discussed at an earlier meeting by Duncan (1994). Here, I have attempted to develop these further, concentrating on the potential disease risks associated with added animals. Selling preventive medicine to cattle farmers has never been easy, nor can there be any absolute guarantees but by adopting appropriate measures it should be possible to significantly reduce the probability of introducing many of the more common conditions.

In an ideal world all herds would be entirely self-contained, relying only on artificial insemination and embryo transfer (ET) to safely introduce new genetic material. In reality this is rarely possible and many owners who profess to having closed herds conveniently forget the occasional purchased animal, particularly a bull. In addition to providing a potential source of infection to the home herd, added animals may, themselves, be at risk if entering a herd of lower disease status - a lesson that has been well learnt by the pig industry. For example, introducing in-calf heifers from a herd known to be free of infectious bovine rhinotracheitis (IBR) virus or *Leptospira hardjo,* could result in them aborting if either of these infections is endemic in the new herd. Similarly, pregnant cattle seropositive for bovine virus diarrhoea (BVD) virus infection could be carrying persistently infected (PI) calves with potentially disastrous consequences for a naive herd.

Prior to the introduction, in 1987, of the now privatised Cattle Health Scheme, the cattle industry had generally given little thought to the concept of disease security. Standards of disease prevention in most cattle herds remain lamentably low and are often non-existent - young calves, in-calf heifers and adult breeding stock, including ET recipients, continue to be purchased from sources of completely unknown disease status, such as markets or dispersal sales, without taking any precautionary measures. Veterinary surgeons, particularly those working in Veterinary Investigation Centres, regularly encounter major disease outbreaks linked to added animals. Many of these incidents could almost certainly have been prevented by adopting the principles of quarantine, clinical examination, strategic laboratory tests and prophylactic treatment as discussed here.

THE QUARANTINE PERIOD

Maintaining strict isolation for a four week period after purchase is probably the most important single step in preventing the introduction of disease with added animals. The quarantine period should also apply to cattle returning from shows (show animals should be kept as a separate group throughout the season) or from communal grazing. It is essential for the following reasons:

1 It provides a "safe" opportunity for new stock to develop clinical signs of diseases which they may be incubating or those associated with latent infections, such as IBR, salmonellosis or Johnes', in which clinical signs may be precipitated by transportation or other stress factors.

2. It enables the farmer and his veterinary surgeon to observe the animals over a period of time, to carry out a full clinical examination and to initiate treatment if appropriate.

3. It permits prophylactic procedures such as antibiotic or anthelmintic treatment and vaccination to be undertaken.

4. It enables precautionary laboratory tests to be carried out, such as those for BVD virus or contagious mastitis pathogens and thereby provides an opportunity to either reject the animals or to initiate a rational control programme.

Providing four-weeks isolation is difficult for many farmers : it requires concerted effort and strong encouragement. Personnel responsible for isolated animals should observe strict hygiene precautions including thorough boot disinfection and the use of separate protective clothing and equipment. Isolation buildings should be clean and disinfected before use and different from those used for housing sick animals. They should be well lit to facilitate inspection and ideally be bird and vermin proof; faeces or urine from the isolated animals should not come in contact with the resident herd and manure should be stored separately.

Isolation premises should preferably be located completely apart from cattle in the main herd, or, as a minimum requirement, it should not be possible for them to come within at least three metres of the added stock - I well recall a serious BVD abortion outbreak associated with a newly purchased PI bull which was "isolated" in an open-fronted pen located where pregnant cows walked past to the parlour. During the summer months a separate field or paddock can be used for isolation but it should be three metre double-fenced. Milking presents obvious problems if the added animals are lactating. If separate facilities are not available, as is usually the case, then the added cattle should go through the parlour last after the rest of the herd has been milked, followed by a thorough disinfection of the parlour.

Although the purchase of pregnant cattle should be avoided because of the added risks posed both to them and by them (at calving), it must be accepted that in-calf heifers are a favoured choice of farmers when adding breeding stock to their herds. Pregnant added animals should calve in isolation, and if they are seropositive for BVD their calves should be tested for BVD virus as soon as possible after birth even though they will probably need to be retested three months later to be sure that they are not PI animals.

After isolation, it is useful to have a further few weeks acclimatisation period as is frequently practised in the pig industry. This is achieved by mixing the added animals with some less valuable, preferably non-pregnant, older cattle from the resident herd. Not only will they act as sentinels, thereby effectively prolonging the quarantine period, but they will also provide the added animals with limited and controllable exposure to diseases endemic in the main herd.

DISEASE STATUS OF ADDED ANIMALS

The main conditions likely to be introduced with added animals are shown in Tables 1 and 2, together with suggestions for preventive measures. These should provide a useful check list, to be modified according to the type of added animal and individual circumstances. Remember to include purchased ET recipients - the disease status of these is much neglected. Other potentially important diseases such as warble fly infestation, brucellosis, tuberculosis and enzootic bovine leucosis are subject to statutory control programmes but in some circumstances, it may be appropriate to carry out additional testing as a safeguard. With imported animals, the Ministry of Agriculture, Fisheries and Food may require isolation, for example, with some imports of Canadian cattle, or carry out specific testing on a risk assessment basis. Several other infections - mycoplasma, rotavirus, parainfluenza 3 virus, respiratory syncitial virus and *Haemophilus somnus* for example - could be introduced with added stock but there are no obvious cost-effective preventive options.

When possible, the more critical tests, such as those for persistent infection with BVD virus, should be undertaken on the farm of origin before agreement to purchase. With diseases like Johnes', for which there is no satisfactory laboratory test for detecting individual subclinical carriers, the intending purchaser should try to obtain written assurance from the vendor of freedom from clinical disease in the herd for at least three years. Any serological testing carried out during the quarantine period should be deferred until the animals have been held for two or three weeks to allow time for seroconversion to occur should the animals have become infected just before entry to the new herd. Similarly, the culture of pooled faeces samples aimed at detecting subclinical salmonella infection should be left until three weeks isolation has elapsed.

There will inevitably be occasions when clinical examination or laboratory tests reveal a condition that is completely unacceptable to the purchaser. This poses a dilemma and in such cases difficult decisions need to be made. If, for example, a group of purchased animals develop salmonellosis whilst in isolation, or *L.hardjo* infection is detected, and it

is not feasible to return them to the farm of origin, it may require vaccinating the main herd, in addition to treating the purchased animals. Despite such problems, the detection of a condition **before** added animals are introduced into a herd at least allows rational preventive medicine decisions to be made rather than reacting to a serious disease outbreak once it has occurred.

DISEASE STATUS OF THE RESIDENT HERD

The oft quoted advice is that added animals should come from limited sources of "known disease status" but how many farmers or their veterinary surgeons have precise information about the disease status of their own herds? With the more overt diseases the status of the home herd is usually known thereby allowing added animals to be protected by appropriate prophylactic procedures. However, with largely subclinical conditions such as IBR or BVD virus infections, the precise status of the home herd is frequently not known. Hence it is not possible to assess the implications of introducing, for example, an IBR seropositive bull, which could undergo reactivation and re-excrete latent virus following the stress of transportation.

Maintaining careful records of serological results from all disease investigations, such as those for abortions or respiratory disease outbreaks, including the ages of animals tested and whether they are home-bred or purchased, helps to build up the herd disease picture over a period of time including an approximate age - specific prevalence profile. In addition, it is useful to routinely check a herd's endemic disease status with serological examinations on bulk milk or blood or milk samples collected from specific cohorts or a cross section of the herd. Such information can establish whether infections are active or whether there are merely a few seropositive older stock which are not actually transmitting infection. If, for example young homebred cattle at least nine months old (after maternally derived antibody has completely declined) are seropositive, it indicates that they have been exposed to the infection in question, which may mean that it is endemic in the herd: with BVD infection it suggests the presence of PI animals in the group. First lactation homebred animals provide an ideal group for blood or milk sampling to detect active infection on farms where heifers are kept apart from the main herd until calving. Antibody titres for BVD and IBR virus infections persist for life. Hence if the oldest animals in a herd are seronegative it is extremely unlikely that the infections are active even though there could be one or more seropositive purchased animals present.

CONCLUSIONS

One of the probable consequences of the impending selective slaughter programme for bovine spongiform encephalopathy is an increased movement of heifers, including many imported animals, into depleted herds. Past experience has shown that introduction of cattle can provide an important source for outbreaks of potentially serious diseases such as IBR, BVD and salmonellosis. With this in mind, and armed with the lessons learnt from the recent epidemiological studies into *Salmonella typhimurium* DT104 infection, now is an opportune time for the veterinary profession to make a concerted effort to promote higher standards of disease prevention in cattle herds and reduce the unnecessary losses that can follow the introduction of new stock. This will require an active input to convince farmers of the value of this approach and to encourage them to consult their veterinary surgeons before purchase rather than leaving them to deal with the aftermath.

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Table 1 - Main bacterial and viral conditions likely to be introduced by added animals, with suggestions for preventive measures

Disease/ Infection	Preventive Measures			Vaccin option	Comments
	Clin exam	Prophy. Treat.	Lab. tests		
BVD	(+)	-	+	Yes	It is essential to test for BVD virus (up to 1.8% of cattle may be virus positive); serology also useful. Calve pregnant females in isolation and test calves at birth for virus if dam seropositive.
IBR	(+)	-	+	Yes	The significance of adding seropositive (potentially infectious) animals depends on virus strain , existing herd status and possible implications for sale of cattle, semen and embryos.
Leptospira hardjo	(+)	+	+	Yes	25 mg/kg dihydrostreptomycin given twice within 14 days reduces the risk of excretion. Could repeat if added animals are seropositive and entering a naive herd but may still need to vaccinate latter. Zoonotic.
Salmonella	(+)	(+)	+	Yes	Although not validated, culture of 50 g pooled faeces after three weeks isolation should help to detect carriers. Positive SAT titre for *S.dublin/S.typhimurium* on serum may indicate recent exposure. Zoonotic.
Johnes' disease	(+)	-	(+)	Yes	Individual animal screening to detect subclinical infection is hampered by lack of suitable tests, all of which can produce false positive and false negative results. Tests such as the adsorbed ELISA may be useful on a group basis. Request certification of clinical freedom. If scour develops in isolation check CFT titre on serum and examine faeces for acidfasts; culture if suspicious.
Streptococcus agalactiae *Staphylococcus aureus*	(+) (+)	(+) (+)	+ +	No No	)Should be readily preventable)by using CMT, culturing)positive quarters and treating)where appropriate.

Table 2 - Miscellaneous conditions likely to be introduced by added animals, with suggestions for preventive measures

Disease/Infection	Preventive Measures			Vacc option	Comments
	Clin exam	Prophy treat	Lab tests		
Digital dermatitis, Superfoul	+ +	(+) (+)	- -	No No	)Good clinical examination)essential. If in doubt use)prophylactic antibiotic)spray/footbath or footbath as)appropriate.
Ringworm	+	(+)	(+)	Yes	Can cause quite serious problems in naive herds, particularly amongst adult animals. Zoonotic.
Misc. udder/skin conditions (pseudo-cowpox, warts etc).	+	(+)	(+)	No	Troublesome and best avoided.
Ectoparasites	+	(+)	(+)	No	Use appropriate prophylactic or curative treatment
Liver fluke Lungworm	- -	+ +	(+) (+)	No No	)Use carefully selected,)appropriate anthelmintics
Campylobacteriosis	(+)	+	(+)	No	See Taylor (1994) re. hired bulls. Prophylactic treatment probably more cost-effective than laboratory testing.
Trichomoniasis	(+)	-	(+)	No	Not present in UK but check imported bulls.
Neospora caninum	-	?	(+)	No	Problematical, epidemiology unclear. Consider not retaining calves from seropositive cows because of risk of congenital infection.

Key to Tables 1 and 2

\+ = Indicated
(+) = Possible benefit
\- = Not indicated

1.53

Update

by
Geoff Pritchard

Geoff Pritchard qualified from the Royal (Dick) School of Veterinary Studies in 1973 having previously graduated in agricultural science from Wye College. After three years in large animal practice in the south of England he joined the Animal Health Division of MAFF and transferred to Norwich Veterinary Investigation Centre in 1980. He gained his FRCVS in 1984 for a thesis on transmissible gastroenteritis in pigs. His present interests include diagnosis, control and prevention of infectious diseases of cattle and he is actively involved in the cattle health scheme operated by the State Veterinary Service.

Diagnosing the cause of bovine abortion

For the purpose of this article 'abortion' is defined as the premature expulsion from the uterus of a live non-viable or dead fetus of a recognisable size at any stage of gestation and includes premature and stillborn calves.

DESPITE the success of the brucellosis eradication programme, abortions continue to cause considerable economic losses to the cattle industry. Sporadic abortions are inevitable in all herds. However, once the incidence exceeds 3 to 5 per cent, particularly when several cases occur close together, efforts should be made to determine the cause in order to instigate appropriate control measures. The precise number of abortions required to initiate a full investigation will depend on individual herd circumstances.

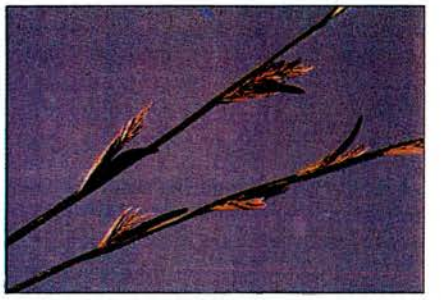
Abortions are usually caused by factors affecting the fetus or placental membranes but can also follow stress reactions in the dam. Apart from some specific infections, such as *Brucella abortus,* determining the cause can be difficult. Even if extensive laboratory examinations are undertaken, the success rate for diagnosing the cause of single abortions is low. It is more satisfactory for herd outbreaks particularly if a systematic approach is adopted.

In Britain, under the brucellosis legislation, cattle owners must report to the divisional veterinary officer all abortions that take place less than 271 days after insemination. Specimens (clotted blood, milk, vaginal swab) from the aborting cow are submitted to the local veterinary investigation centre by the farmer's veterinary surgeon. It is important to realise that laboratory examinations carried out on these routine statutory (BS7) submissions are specifically for brucellosis diagnosis.

Non-infectious causes

A good history and physical examination of the aborting cow are particularly important for conditions in this category. In most cases, diagnosis is unlikely to be achieved by laboratory examinations alone.

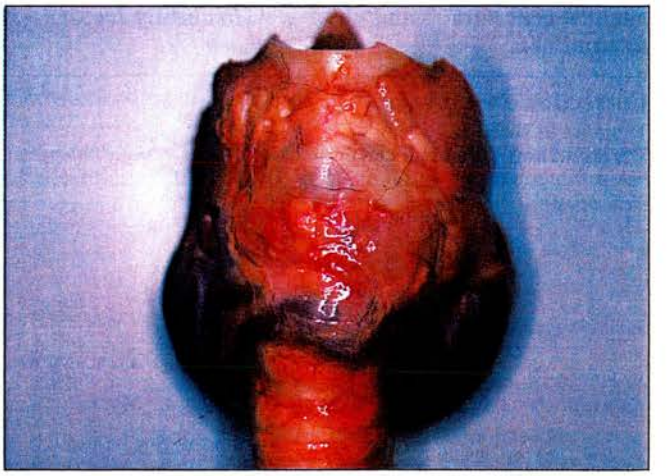
Causes include twinning, hereditary disorders, hormonal imbalances, trauma and poisonings (eg, nitrate and ergot). Possible nutritional deficiences include vitamin A, selenium, iodine and severe starvation.



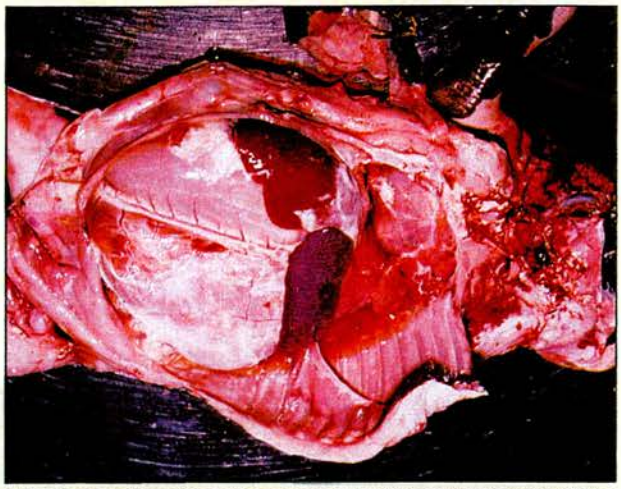
Ergot on perennial rye grass

Fetal goitre

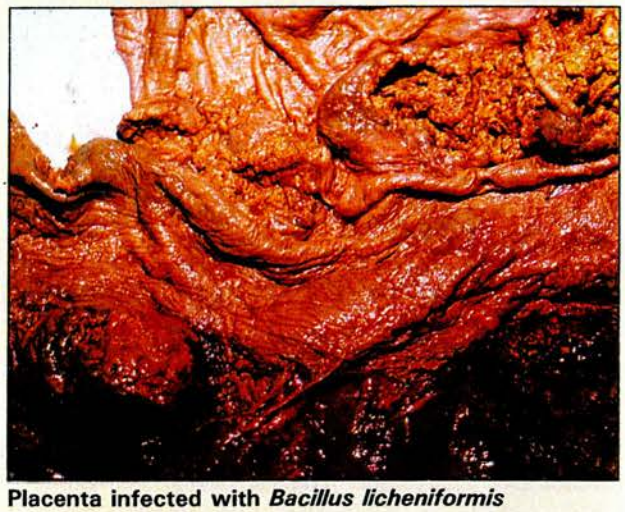
Stillborn and aborted calves with fetal goitre caused by congenital iodine deficiency are seen in parts of Britain, particularly the south west. Diagnosis is based on the gross and histological appearance of the thyroid gland, its weight (>0·03 per cent of fetal bodyweight) and total iodine concentration. Thyroxine estimation on serum or plasma from pregnant cows may be helpful. Check whether goitrogens (eg, kale and other brassicas) are being fed.



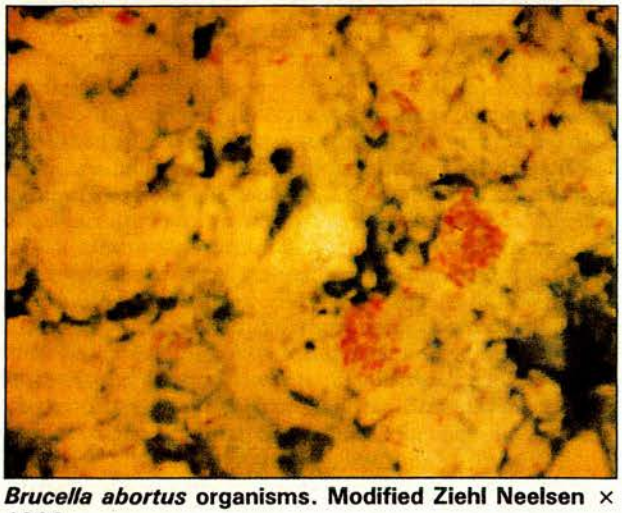
Enlarged fetal thyroids caused by congenital iodine deficiency



Pleurisy and peritonitis caused by *Campylobacter fetus fetus* infection



Placenta infected with *Bacillus licheniformis*



***Brucella abortus* organisms. Modified Ziehl Neelsen × 1000**

and stomach contents) and cotyledons. Veterinary investigation centres routinely culture on sheep blood, Farrells (for *B abortus*) and MacConkey agars in 10 per cent carbon dioxide and in selenite broth. It should be emphasised that isolation from fetal material does not necessarily mean the organism caused the abortion since its presence may be coincidental or it may be a contaminant.

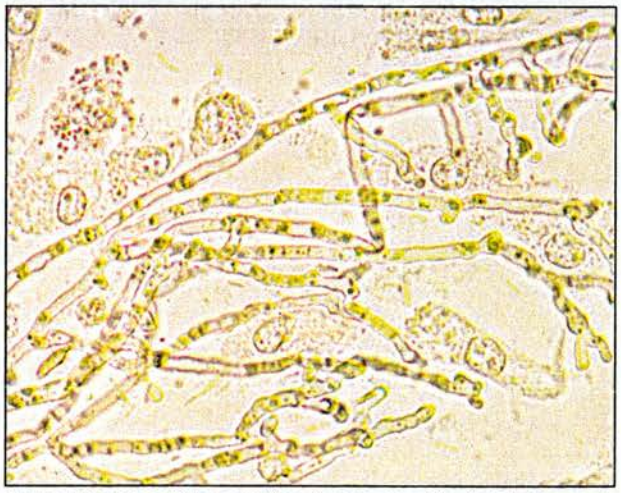
Serology, which has been invaluable for brucellosis eradication, is occasionally used to diagnose other bacterial infections such as *L monocytogenes*. Examination of paired sera is essential.

Fungal causes

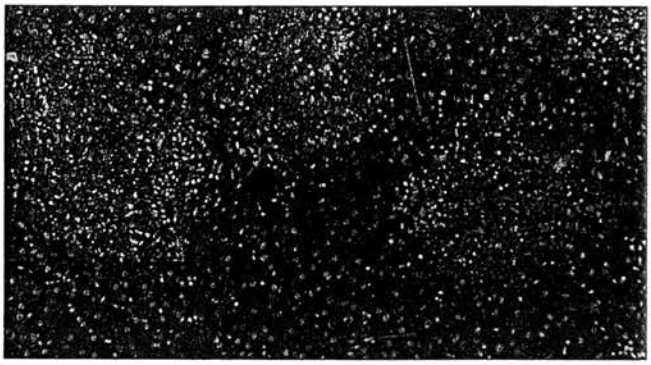
Mycotic abortion is quite common in some areas, depending on local factors such as rainfall and grass conservation practices. Fungal spores proliferate in wet spoilt hay, mouldy silage and brewers grains. The usual cause is *Aspergillus fumigatus* but fungal species belonging to *Absidia, Mucor* and *Rhizopus* genera are occasionally implicated. The incubation period varies from several weeks to months depending on the extent of exposure. Most abortions occur between the fifth and seventh months of gestation.

Placenta showing typical features of mycotic abortion

Mycotic skin lesions



Fungal hyphae *(Aspergillus fumigatus)* in fetal stomach contents. Potash Quink × 400



Multifocal hepatic necrosis due to IBR virus infection. H & E × 50

● **Fetal serology** IBR virus antibodies are not detected in fetal fluids because fetal death occurs very rapidly after infection. BVD virus antibodies may be detectable (using an indirect FAT) in fetuses infected after 90 to 125 days.

● **Dam serology** Serological diagnosis of IBR and BVD virus infections is currently based either on the serum neutralisation test (SNT) or the ELISA test. Seroconversion or significant increases in titre (ie, a fourfold rise in SNT titre or an increase in optical density of at least 0·2 with the ELISA test) from paired sera are considered diagnostic. Although paired serology is still worth undertaking in abortion outbreaks, titres are usually static because of the delay between infection and abortion. High titres can be maintained for several years after infection with either virus and they do not necessarily indicate recent exposure. The absence of a titre effectively eliminates the virus as a cause of abortion.

● **Herd serology** Serology is of most value when looked at on a herd basis. Unfortunately, veterinary surgeons are often unaware of the endemic infectious disease status of their clients' herds. This makes it difficult to interpret serology from individual aborting cows. Occasional serological screening of about 10 per cent of adult cattle (preferably 20 to 30 animals) to check BVD, IBR (and *L hardjo*) status is very worthwhile, not only for abortion investigations but also for diagnosis of milk drops and other vague illnesses. Single sample serology performed routinely on blood from aborting cows provides additional disease surveillance at low cost. Comprehensive monitoring for *L hardjo* and IBR infections is also available through the MAFF Cattle Health Schemes.

● **Screening sera for BVD virus** Sera from live weakly or congenitally abnormal calves born to the aborting group of cows should be examined for persistent infection with BVD virus. This is usually achieved by the immunoperoxidase (IPX) microculture test which is a cheaper, more rapid alternative to the conventional tissue culture methods. It should be noted that calves with congenital intracranial malformations are rarely viraemic but usually have precolostral antibody titres for BVD virus. The IPX test can also be used on sera from aborting cows to detect any (probably less than 0·5 per cent) that are persistently infected with BVD virus and therefore likely to produce infected fetuses or calves.

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Approach to sporadic bovine abortions

There is usually little point in attempting a full differential diagnosis for every sporadic abortion but—

- Carry out statutory (BS7) brucellosis investigation
- Request the VIC to test the serum for *L hardjo* antibodies (unless the herd is already fully vaccinated) and to culture the vaginal swab for salmonella
- Consider testing the aborting dam's serum for IBR and BVD antibodies (see viral abortion section)
- Physically examine the aborting cow
- Examine the placenta for evidence of infection with fungi or *B licheniformis*
- Check relevant history such as recent purchases, clinical disease, infertility, changes of diet and stock movements

Investigation of abortion outbreaks

When the critical threshold number of abortions in a herd has been reached, a full laboratory investigation should be initiated promptly

- Supply the laboratory with a brief history, including relevant findings from physical examinations of aborting cows and details of any weak or congenitally abnormal calves
- Submit entire fresh fetuses and placentae (very important) to the laboratory without delay
- If distance is a problem, send (in leak-proof containers) from each abortion—
  - Several complete cotyledons
  - Two ml of fetal stomach contents aseptically collected with Vacutainer or pasteur pipette
  - At least 2 ml aseptically collected fluid (preferably clear) from the thoracic or abdominal cavities
  - About 5 g each of fresh lung, liver, thymus and salivary gland
  - Air-dried acetone-fixed impression smears from fresh cotyledon, lung, liver and kidney
- Serology (*L hardjo*, BVD, IBR and possibly other infections)
  - Submit two full 7 ml Vacutainers of clotted blood from all cows that have aborted in the current season
  - Consider sending clotted blood from cows with recent unexplained infertility or illness (eg, milk drop) that could be associated with abortions
  - Consider taking a representative number of blood samples from unaffected cows for comparative purposes
  - Collect repeat (paired) samples two to three weeks later as appropriate
- Submit 2 ml clotted blood from live weakly or congenitally abnormal calves (preferably before they have sucked) to screen for BVD virus infection
- Submit clotted and heparinised blood samples for biochemical tests as appropriate (eg, thyroxine, selenium, nitrate)

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THE DIAGNOSIS OF INFECTIOUS CAUSES OF BOVINE ABORTION

GEOFF PRITCHARD, BURY ST EDMUNDS VETERINARY INVESTIGATION CENTRE

INTRODUCTION

An abortion represents one measurable end product of reproductive failure. It can be defined as the premature expulsion from the uterus of a live non-viable or dead fetus of a recognisable size at any stage of gestation and should include premature and stillborn calves.

In Britain, under the current brucellosis legislation, cattle owners must report to the Divisional Veterinary Officer all abortions that take place less than 271 days after insemination. Specimens (clotted blood, milk, vaginal swab) from the aborting cow are submitted to the local Veterinary Investigation Centre by the farmer's veterinary surgeon. It should be realised that the laboratory examinations carried out on statutory (BS7) submissions are specifically for brucellosis diagnosis. BS7 specimens are not suitable for a full differential diagnosis although they are often used for this purpose. This is one of the main reasons why, in the absence of brucellosis, the diagnostic success rate for bovine abortion appears to be so low.

Most bovine abortions are sporadic and probably have a non infectious aetiology. These are inevitable in most herds and their causes are unlikely to be diagnosed by a laboratory. However, abortion **outbreaks** are usually caused by infectious agents which can be identified if appropriate specimens are submitted to the laboratory. Infectious agents causing abortion in cattle in Britain include bacteria, viruses, fungi and protozoa. The most common causes currently identified by the diagnostic recording system (VIDA II) operated by the Veterinary Investigation Service are:

Leptospira hardjo
Salmonella dublin
Bovine virus diarrhoea (BVD) virus
Bacillus licheniformis
Mycoses (mainly *Aspergillus fumigatus*)

Abortions due to *B.licheniformis* and *Neosporum caninum* infections were described at the previous BCVA meeting. Further details of the main causes of bovine abortion and their diagnosis have been summarised by the author (Pritchard 1990). The purpose of this present paper is to provide a systematic approach to abortion diagnosis for practitioners without attempting to consider every possible cause in detail.

SPORADIC ABORTIONS

There is little point in initiating a full laboratory investigation for every sporadic abortion. Instead, the following procedure is suggested:-

1. Perform statutory (BS7) brucellosis investigation.

2. Check whether this is indeed a sporadic abortion or whether the farmer has failed to report previous cases. Include stillbirths and premature calvings in the assessment. If this appears to be part of an outbreak proceed to next section.

3. Clinically examine the aborting cow.

4. Check the placenta for gross evidence suggestive of infection with fungi or *B.licheniformis* and pursue diagnosis if suspicious.

5. Test the serum from the aborting cow for *L.hardjo* antibodies unless the herd is fully vaccinated. If appropriate proceed further according to recently issued BCVA guidelines on *L.hardjo* diagnosis and control.

6. Consider asking the laboratory to culture the vaginal swab for salmonella, particularly if *S.dublin* infection appears likely.

ABORTION OUTBREAKS

Decide on an appropriate critical threshold for the number of abortions that need to occur in a particular herd before a detailed laboratory investigation is merited. This will vary with the type of herd and value of cattle concerned. In general it should be when more than 3 to 5 per cent of the herd have aborted. However, rather than wait there may need to be a projection based on calving pattern and the rate at which abortions are occurring. Remember to include stillbirths and premature calvings when making the assessment. Note that twinning is a common cause of abortion in cattle and such cases should perhaps be excluded as should stillbirths obviously associated with dystocia. Once the critical action threshold has been reached the following procedures are suggested:-

1. Submit one or more entire fresh fetuses **and placentae** to the laboratory without delay. Provide a comprehensive history, including number and ages of aborting animals, use of a common bull, recent disease problems (milk drop, pyrexia, diarrhoea, nasal or vaginal discharge, mucosal disease cases, infertility, etc), herd replacement and vaccination policies, and recent changes in diet. Note whether calves have been born weak or with congenital nervous signs.

2. If distance from the laboratory poses transportation problems send (in clearly labelled leak-proof containers) sufficient samples from each abortion to screen for the main infectious causes:

   a. Several complete fresh cotyledons.

   b. 2 ml of fetal stomach contents aseptically collected with vacutainer or pasteur pipette.

c. At least 2 ml aseptically collected fluid (preferably clear) from the thoracic or abdominal cavities.

d. About 5 grams each of fresh lung, liver, thymus and salivary gland.

e. Air-dried acetone-fixed impression smears from fresh cotyledon, lung, liver and kidney.

f. A cotyledon/placentome and pieces of fetal liver, heart and lung in formal saline for histopathology (add thyroid if enlarged).

3. Submit two full 7 ml vacutainers of clotted blood from all cows that have aborted in the current season. Consider also sending clotted blood samples from cows with recent unexplained illness that might be associated with the abortions. A representative number of blood samples from unaffected cows could also be sent for comparative purposes (see also BCVA guidelines for *L.hardjo* diagnosis).

4. Repeat (paired) blood samples can be collected two or three weeks later but because of the usual delay between fetopathic infection and abortion, seroconversion has usually occurred before the **first** samples are collected which tends to make this an unrewarding exercise.

NB *The main value of serology for BVD and infectious bovine rhinotracheitis (IBR) is to eliminate these viral infections from the differential diagnosis ie if aborting cows are seronegative then these agents are almost certainly not involved. Unfortunately actual antibody titres do not provide a reliable guide to the timing of infection since high titres for BVD and IBR can be maintained for several years. Diagnosis of viral abortion in cattle remains problematical and usually demands a whole herd approach.*

5. If fetal and placental examinations and serology for BVD, IBR and *L.hardjo* fail to indicate a likely infectious agent then the range of serological tests can be extended to cover less common causes such as Q Fever or toxoplasmosis. Also, depending on history, biochemical tests such as thyroxine or nitrate estimations can also be considered.

CONCLUSION

In summary, efforts at reaching a diagnosis for the cause of bovine abortion should be targeted towards definite herd problems - attempting to reach a diagnosis with every sporadic abortion or with inadequate samples is pointless, expensive and inevitably unrewarding for all concerned. The one notable exception, however, is in the strategic screening of sera from aborting cows to routinely monitor herds for the main endemic infections such as *L.hardjo* or IBR.

ACKNOWLEDGEMENTS

This presentation is based largely on a paper by the author published in the In Practice journal and I am indebted to the editor for allowing me to repeat some of the text.

Reference

Pritchard, G. C. (1990) In Practice 12, 92-97.

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Bovine abortion?

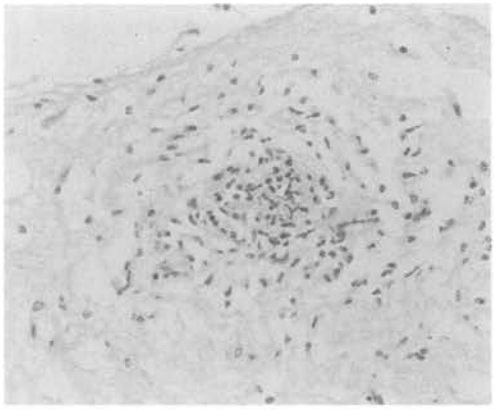
These questions refer to cattle abortion in Britain

1 Are most cattle abortions the result of infectious or non-infectious causes? Give examples of likely non-infectious causes.

2 What causes of abortion would you expect a veterinary investigation centre to be able to diagnose from samples sent in for statutory brucellosis testing? What other samples could you usefully submit?

3 How many abortions would you need to see in a herd before recommending a full laboratory investigation? Discuss.

4 List the three most common bacterial causes of abortion. What is the most common viral cause?



5 The histological lesion in the fetal brain pictured above relates to a condition that has recently been recognised as a potentially very important cause of bovine abortion. What is it and what samples are required for its diagnosis? What advice would you give for control?

6 The picture above shows a placenta from a cow that aborted at seven months' gestation. Gross examination reveals a marked placentitis with thickening and inflammation of the cotyledons and placental membranes. What are the most likely causes and how are these diagnosed? What advice would you give to the farmer in each instance?

7 Bovine viral diarrhoea virus is isolated from an aborted fetus. How would you interpret this finding and what action would you recommend?

8 A recently aborted cow in a herd that has not been vaccinated against either *Leptospira hardjo* or infectious bovine rhinotracheitis (IBR) has a microscopic agglutination test titre of 1/100 for *L hardjo* and is also seropositive for IBR with the ELISA with an optical density of 0·73. How would you interpret these findings? What advice would you give the farmer?

Answers on page 20

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Bovine abortion?

Ergot on perennial ryegrass; mycotoxins are one of the possible non-infectious causes of abortion in cattle

1

Unlike in sheep, non-infectious causes of abortion are almost certainly more common. Generally, an infectious cause can be found for up to about 15 to 20 per cent of abortions if adequate satisfactory material (ie, fresh fetus, placenta and clotted blood from the dam), often from several cases, is submitted to the laboratory. Histological examination of freshly removed placentomes, including placental membrane, can significantly increase the diagnostic rate for mycotic and bacterial abortions.

Twinning and chromosomal abnormalities are likely non-infectious causes. Other possibilities include nutritional deficiencies (eg, vitamin A, manganese and perhaps iodine), plant poisonings (eg, hemlock, juniper, rape), mycotoxins (eg, zearalenone, ergot), stress (eg, hyperthermia, dog worrying, low flying aircraft) and various hormonal, physical and toxic factors.

2

The current brucellosis legislation requires that samples of clotted blood and milk and a vaginal swab from any cow that aborts before 271 days after insemination or service (whether the calf is born alive or dead) be submitted to the local veterinary investigation centre specifically for *Brucella abortus* diagnosis. However, other examinations can be performed if requested. The vaginal swab may be cultured for bacterial infections, such as salmonella or *Actinomyces pyogenes,* but contamination is a major problem and examinations on a fetus or fetal specimens (stomach contents, thoracic cavity fluid and pieces of fresh lung, liver and kidney) and/or placenta or placentomes are likely to be much more rewarding. Examinations for antibodies using the dam's serum can provide evidence of past exposure to various infections (not necessarily the cause of the abortion). In the case of abortifacient agents such as *Leptospira hardjo* and *Neospora caninum* a high titre suggests recent exposure.

3

Difficult. Most abortions are sporadic and a full laboratory investigation is not merited after just one case, although in an unvaccinated herd it is worth checking whether the cow has a titre to *L hardjo*; this is also useful as a monitoring exercise, particularly in view of the COSHH implications. It is worthwhile grossly examining the placenta for obvious placentitis and pursuing as appropriate.

Depending on the calving pattern and herd history, a more comprehensive laboratory investigation should be undertaken after the second or third abortion in a short period of time (perhaps within two or three weeks) or certainly after 3 or 4 per cent of the herd have aborted. (Bear in mind that farmers frequently underestimate the cost of an abortion. At a recent workshop held in Edinburgh the true cost of a bovine abortion was estimated at between £650 and £750 depending on the type of herd [R. D. Murray, personal communication].) Serological screening of blood samples from all cows that have aborted during the season can help either to implicate or eliminate the cause but the findings only provide circumstantial evidence. Fresh fetuses, placentae and, if possible, placentomes are required if any meaningful effort is to be made to diagnose the problem. Many non-infectious causes require a comprehensive history before any conclusions can be reached.

4

The three most common bacterial causes of abortion are *L hardjo, Salmonella* species and *Bacillus licheniformis.* Various other bacterial infections, including *A pyogenes, Campylobacter fetus* species (*C fetus fetus* or *C fetus venerealis*), *Listeria* species, *Haemophilus somnus* and the rickettsial organism *Coxiella burnetii* (Q fever), are diagnosed less frequently. A wide range of further bacteria have been implicated in sporadic abortions.

Bovine viral diarrhoea (BVD) virus is the most commonly recorded viral cause of abortion. There is a very high seroprevalence in the national herd (about 65 per cent) but BVD abortion is difficult to diagnose and many cases go unrecognised. Some strains of infectious bovine rhinotracheitis (IBR) virus cross the placenta and cause abortion, but this is fairly uncommon.

5

This is a case of *Neospora caninum* infection. The characteristic histological lesions occur in the brain and comprise multifocal non-suppurative encephalitis, malacia, gliosis and meningitis. Non-suppurative myocarditis and placentitis may also be recognised. In cattle, infection results in abortion or the production of congenital or acquired progressive paraplegia in calves. A recent survey by the Veterinary Investigation Service revealed a 4·2 per cent incidence of *N caninum* infection in aborted fetuses based on histopathology and immunocytochemistry. This may be a conservative estimate. Infection with this protozoan parasite has also been reported in dogs, goats, horses and sheep.

Diagnosis requires the aborted fetus (or fixed brain, heart and placenta), while definitive histological confirmation necessitates specific immunocytochemistry. Serological examination of clotted blood samples from recently aborted cows using the indirect fluorescent antibody test (IFAT) provides evidence of recent exposure to infection. Titres of 1/640 and over are considered to be significant. Titres decline over a period of about 150 days.

Control and prevention remain problematical. Infection is presumed to follow the ingestion of neospora oocyst-contaminated herbage or other feed. Farmers are advised to cover or prevent access to feed by carnivorous animals and birds. At present, there is no evidence that the use of anti-protozoal drugs is of any value. Persistent

infection may follow abortion and if proven there would be a case for culling infected cows.

6

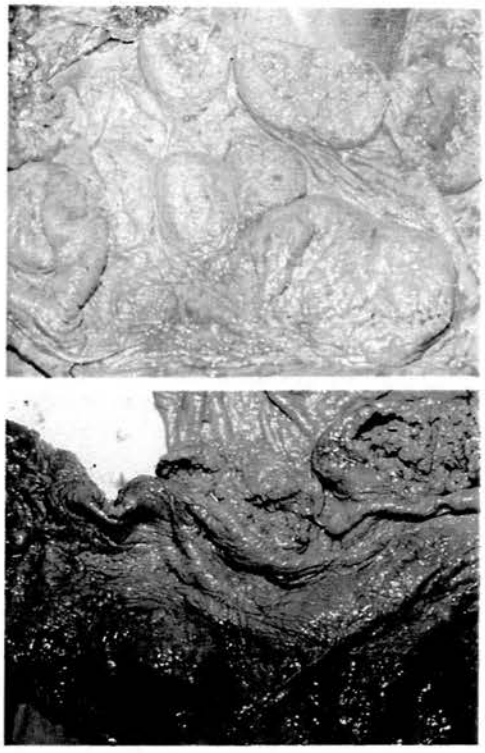
This is a case of *Chlamydia psittaci* abortion. This potentially zoonotic infection tends to produce a suppurative placentitis, as is occasionally seen with *Campylobacter fetus* infection. A drier, more leathery placentitis, often yellowish-brown in colour, is associated with mycotic abortion – notably *Aspergillus fumigatus* and, to a lesser extent, *Bacillus licheniformis* (see right). Note that *Brucella abortus* infection (see '2' above) can also produce a placentitis with necrotic foci and intercotyledonary thickening.

Bovine chlamydiosis may be diagnosed by modified Ziehl-Neelsen stained impression smears of any part of the bovine placenta. Serum samples can be examined for chlamydial antibody using ELISA. High diagnostic titres may be present at the time of abortion but paired sera should be examined whenever possible. Culture of chlamydia from placental tissue or the detection of chlamydial DNA by the polymerase chain reaction (PCR) test will reinforce the diagnosis. Chlamydial antibody is often, but not invariably, present in fetal fluids.

B licheniformis infection is readily confirmed by culturing fetal stomach contents and placental material, as is *C fetus* which may also be suspected by microscopic examination of stained impression smears. Fungal abortion is best diagnosed by demonstrating fungal hyphae in fetal stomach contents (see below) using wet smears stained with lactophenol cotton blue or potash 'Quink' (Parker). Stained 'squash' preparations from thickened cotyledons can also be helpful, as can histology (preferably of placentomes). The isolation of fungal organisms from the placenta is not conclusive on its own because of the possibility of environmental contamination.

Fungal hyphae in fetal stomach contents

Bovine chlamydiosis is as yet an uncommon cause of abortion in Britain (3·6 per cent of 2959 sera from aborted cows screened for chlamydial antibody within the VI Service during the past three years had optical densities high enough to be consistent with chlamydial abortion). It has also been tentatively linked with several syndromes including milk drop, mucopurulent nasal discharge and pyrexia. Contact with sheep (with enzootic abortion) constitutes a possible source of infection for some outbreaks. Treatment of at-risk pregnant cattle with long-acting oxytetracycline, as practised for sheep, may be helpful but this has not been confirmed. The farmer should be advised to prevent



(top) Mycotic abortion due to *Aspergillus fumigatus* infection. (bottom) Placenta infected with *Bacillus licheniformis*

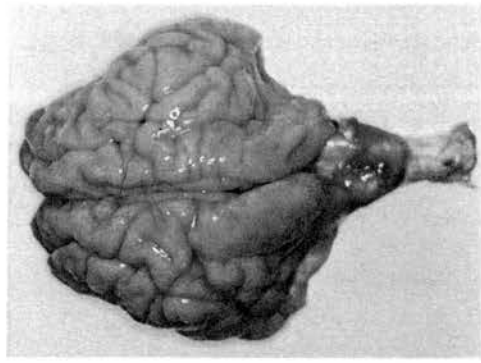
contact between cattle and sheep and vaccinate the latter (no chlamydial vaccine is presently licensed for use in cattle in Britain). Aborting cows should be isolated until discharges have ceased and their placentas should be carefully disposed of. Such cows may subsequently prove to be infertile.

Mycotic abortion is acquired from the ingestion or inhalation of fungal spores; the usual source of these is wet spoilt hay, mouldy silage and brewers' grains. Farmers should avoid giving mouldy feed to pregnant cattle or, if there is no alternative, to dilute it heavily . *B licheniformis* abortion has been associated with mouldy hay, feed, bedding and, in particular, silage effluent, especially when spread directly onto grazing land used by pregnant cattle. Farmers should be advised to adopt silage-making techniques that minimise the chance of abnormal fermentation and to dispose of effluent carefully.

C fetus is an uncommon cause of sporadic abortion – *C fetus venerealis* infection is usually associated with the recent purchase of an infected bull. Control can be achieved with the use of antibiotics and artificial insemination, as appropriate; cultural examinations on sheath washings to screen purchased replacements should be considered.

7

The isolation of BVD virus from a fetus is a rare finding because of the long time interval between infection and abortion. It indicates that infection occurred before the development of immunological competence (ie, before 90 to 125 days gestation) and provides very good evidence that this was the cause of the abortion. If abortion had not occurred, the calf would probably have subsequently died from a BVD-related illness such as mucosal disease.



(above) Calf born unable to stand due to mid-gestational BVD virus infection. (right) Brain from the calf, showing severe cerebellar hypoplasia and cerebral cavitation

The suggested course of action is as follows.

- Blood sample the dam to check whether she is also BVD virus-positive and cull if applicable (she will probably be BVD antibody-positive and virus-negative). If virus-positive, alternatively consider retaining her in the herd to 'immunise' maiden heifers well before they are served.
- Check whether the dam was recently purchased and whether she was pregnant at the time. If so, there should not be any further BVD abortions that season unless other pregnant cattle were purchased from the same source. There may, however, be consequences if she is viraemic and mixed with seronegative pregnant cattle or if a persistently infected fetus is born into an immunologically naive herd.
- If the dam is home-bred then, depending on the calving pattern, there are likely to be other cows carrying fetuses that have been exposed to BVD virus. This could result in further abortions, the birth of apparently normal but persistently infected calves or calves born with congenital defects (see above), depending on the developmental stage at the time of infection. Therefore, blood sample that season's calves – particularly potential heifer replacements — to check whether any are persistently infected and cull or retain for 'immunisation' purposes as discussed above. Ideally, defer screening calves until they are at least two or three months old to reduce the effect of maternally derived antibody. Re-sample virus-positive animals two or three weeks later in case they are undergoing acute infection rather than being persistently infected.
- Discuss longer term strategies including whole herd testing for BVD carriers and statistical serological screening. Advise blood sampling purchased replacements for BVD virus, including bulls, since about 1 per cent of cattle are persistently infected. At the time of writing, no BVD vaccine is licensed in Britain but should one become available this would clearly have a significant influence on control programmes.

Further reading

BCVA (1992) Guidelines for the Diagnosis and Control of *Leptospira hardjo* Infection in Cattle. Frampton-on-Severn, Gloucestershire, British Cattle Veterinary Association

PRITCHARD, G. C. (1990) Diagnosing the cause of bovine abortion. *In Practice* **12**, 92-97

8

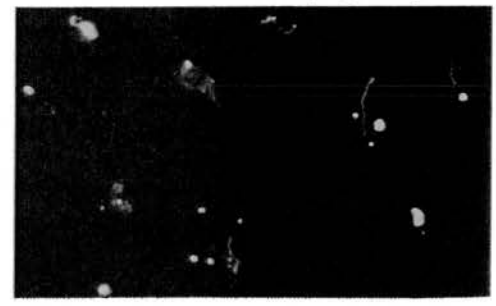
The optical density of 0·73 for IBR indicates that this cow has been infected with IBR during her lifetime or has been vaccinated. Although this is a moderately high optical density (the scale is 0 to 2) it does not necessarily indicate that IBR was the cause of the abortion because titres persist indefinitely and 30 to 35 per cent of adult cattle are seropositive anyway. Confirmation of IBR abortion is by demonstration of virus in fetal tissues or by fetal histopathology (revealing focal necrotic liver lesions). Rising antibody titres after abortion do not necessarily confirm the diagnosis because titres may rise as a result of reactivation of latent infection following the stress of abortion.

The *L hardjo* titre is consistent with past exposure to *L hardjo* although there may be cross reaction with the closely related *L saxkoebing* which can be acquired from wildlife sources. Typically, recent infection with *L hardjo* results in higher titres (ie, 1/400 to 1/6400), but by the time of abortion, which can be up to 12 weeks later, titres have generally fallen. Titres can also reflect vaccination which inevitably hampers diagnostic interpretation especially if this is not stated when the sample is submitted to the laboratory.

Follow-up action is essential if this is the first evidence of *L hardjo* infection in an unvaccinated herd because of the risk of further abortions and the COSHH implications. Ideally, a sufficient number of adult cattle (usually 20 to 30 animals depending on herd size) spread through the main age groups should be blood sampled to determine whether this is a recently introduced disease (a few high titres) or whether it is established and endemic. The main risk factors, such as the use of shared bulls, replacement policy and access to rivers and streams, should be considered when interpreting findings and deciding whether to vaccinate. If serology fails to demonstrate further evidence of infection, and vaccination is not undertaken, then the herd should be regularly monitored (including routine examination of blood samples from aborting cows or those with unexplained milk drop). In the event of a series of *L hardjo* abortions (cows with high titres), prompt vaccination without waiting for further blood sample results may be indicated, perhaps combined with antibiotic administration.

The definitive diagnosis of *L hardjo* abortion is problematical. It is best achieved by fluorescent microscopy on fresh (less than 24 hours since abortion) fetal tissue. The success rate remains low, however, mainly because many fetuses are decomposed by the time they reach the laboratory.

Fluorescent antibody test for *Leptospira hardjo*



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Analysis of 16S rDNA sequences from pathogenic *Leptospira* serovars and use of single nucleotide polymorphisms for rapid speciation by D-HPLC

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ABSTRACT

Leptospira have a worldwide distribution and include important zoonotic pathogens yet diagnosis and differentiation still tend to rely on traditional bacteriological and serological approaches. In this study a 1.3 kb fragment of the *rrs* gene (16S rDNA) was sequenced from a panel of 22 control strains, representing serovars within the pathogenic species *Leptospira interrogans*, *Leptospira borgpetersenii*, and *Leptospira kirschneri*, to identify single nucleotide polymorphisms (SNPs). These were identified in the 5′ variable region of the 16S sequence and a 181 bp PCR fragment encompassing this region was used for speciation by Denaturing High Performance Liquid Chromatography (D-HPLC). This method was applied to eleven additional species, representing pathogenic, non-pathogenic and intermediate species and was demonstrated to rapidly differentiate all but 2 of the non-pathogenic *Leptospira* species. The method was applied successfully to infected tissues from field samples proving its value for diagnosing leptospiral infections found in animals in the UK.

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1. Introduction

Leptospira are an important group of spiral-shaped bacteria which include major animal and zoonotic pathogens that are widely distributed in the environment and a wide range of wild and domestic animals. Human infections can result from contact with contaminated soil, water, or body fluids (especially urine) of infected carrier animals such as wild rodents (Bahaman and Ibrahim, 1988; Levett, 2001) and can cause potentially life threatening disease, particularly of the hepatonephritic type (Weil's disease) which has mortality rates approaching 15% (Faine et al., 1999). In the UK leptospirosis is an endemic disease in animals and an important cause of infertility, abortion, stillbirth and weak neonates in cattle and pigs (Dhaliwal et al., 1996a; Smyth et al., 1999) and milk drop in cattle (Dhaliwal et al., 1996b).

The *Leptospira* genus consists of a heterogeneous group of pathogenic and saprophytic species belonging to the order *Spirochaetales*. The genus *Leptospira* comprises 20 genomospecies, based on phylogenetic analysis of 16S rRNA (Morey et al., 2006; Matthias et al., 2008; Slack et al., 2008, 2009) and these have been broadly divided into pathogenic, saprophytic and intermediate types according to molecular typing and pathogenicity factors.

Serology, using microscopic agglutination tests (MAT), is currently the primary diagnostic tool for current and past infections. In reference laboratories cross-absorption agglutination test and monoclonal antibodies are used for serovar identification (Faine et al., 1999; Feresu et al., 1994). These methods use viable bacterial cultures, which can take between two to twelve weeks to grow, due to the fastidious nature of these organisms. However, interpretation of MAT results can be subjective.

Molecular approaches may be used when organisms are likely to be present. Several PCR methods have been developed (Stoddard et al., 2009; Fearnley et al., 2008; Fernandes et al., 2008; Slack et al., 2006a; Gravekamp et al., 1993) for detection whilst numerous molecular typing methods have been described including restriction fragment length polymorphism (Djadid et al., 2009; Woodward and Redstone, 1993), variable number tandem repeats (Slack et al., 2006b; Majed et al., 2005; Perolat et al., 1993), PFGE (Herrmann et al., 1992; Galloway and Levett, 2008), ribotyping (Perolat et al., 1994), arbitrarily primed-PCR (Perolat et al., 1994), multiplex PCR (Richtzenhain et al., 2002), 16S rRNA gene sequencing (Morey et al., 2006), *ompL1* species specific PCR (Reitstetter, 2006), multilocus sequence typing (Ahmed et al., 2006) and *wzx* gene sequence variation (Wangroongsarb et al., 2007).

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Table 1
Leptopira strains from the VLA panel and GenBank *Leptopira* strains included for comparison and DHPLC.

Serovar	Serogroup	Reference strain/subtype	Species	GenBank Accession no.
Australis[a]	Australis	Ballico	*L. interrogans*	AM050579
Australis	Australis	Ballico	*L. interrogans*	AY996794
Australis	Australis	Ballico	*L. interrogans*	FJ154556
Autumnalis[a]	Autumnalis	Akiyami A	*L. interrogans*	AM050580
Autumnalis	Autumnalis	Akiyami	*L. interrogans*	AF157069
Autumnalis	Autumnalis	Akiyami	L. interrogans	FJ154543
Autumnalis	Autumnalis	Akiyami A	*L. interrogans*	AY461864
Ballum[a]	Ballum	Musl27	*L. borgpetersenii*	AM050581
Ballum	Ballum	Musl27	*L. borgpetersenii*	AY631884
Ballum	Ballum	Musl27	*L. borgpetersenii*	AY461856
Bataviae[a]	Bataviae	Swart	*L. interrogans*	AM050582
Bataviae	Bataviae	Swart	*L. interrogans*	DQ991469
Bataviae	Bataviae	Van Tienen	*L. interrogans*	AY461865
Bim	Autumnalis	1051	*L. kirschneri*	AY996802
Bratislava[a]	Australis	Jez Bratisalva	*L. interrogans*	AM050583
Bratislava	Australis	Jez Bratisalva	*L. interrogans*	FJ154547
Bratislava[c]	Australis	Isolate HB	*L. interrogans*	AM050584
Bratislava[c]	Australis	Isolate BE	*L. interrogans*	AM050585
Bratislava[c]	Australis	Isolate K5A	*L. interrogans*	AM050586
Bratislava	Australis	Jez Bratisalva	*L. interrogans*	AY461863
Canicola[a]	Canicola	Utrecht V	*L. interrogans*	AM050566
Canicola	Canicola	Hond Utrecht IV	*L. interrogans*	AY461866
Canicola	Canicola	Moulton	*L. interrogans*	X17547
Celledoni[b]	Celledoni	Celledoni ATCC 43285	*L. weilii*	AY631877
Codice[b]	Codice	CDC ATCC 43284	*L. wolbachii*	AY631879
Copenhageni[a]	Icterohaemorrhagiae	Wijinberg	*L. interrogans*	AM050565
Copenhageni	Icterohaemorrhagiae	LI-130	*L. interrogans*	AY461869
Copenhageni	Icterohaemorrhagiae	Fiocruz LI-130 (*rrs*1)	*L. interrogans*	NC005823
Copenhageni	Icterohaemorrhagiae	Fiocruz LI-130 (rrs2)	*L. interrogans*	NC005823
Grippotyphosa[a]	Grippotyphosa	Moskva V	*L. kirschneri*	AM050567
Grippotyphosa	Grippotyphosa	Moskva V	*L. kirschneri*	AF157067
Grippotyphosa	Grippotyphosa	RM52	*L. kirschneri*	AY461877
Hardjo[a]	Sejroe	Hardjoprajitno	*L. interrogans*	AM050568
Hardjo	Sejroe	Hardjoprajitno	*L. interrogans*	AY461867
Hardjo	Sejroe	Hardjoprajitno	*L. interrogans*	FJ154553
Hardjo	Sejroe	Hardjoprajitno	*L. interrogans*	AY996796
Hardjo	Sejroe	Hardjo Lepto-0184	*L. interrogans*	AY99697
Hardjobovis[a]	Sejroe	Sponslee	*L. borgpetersenii*	AM050569
Hardjobovis	Sejroe	L550	*L. borgpetersenii*	CP000348
Hebdomadis[a]	Hebdomadis	Hebdomadis	*L. interrogans*	AM050570
Hebdomadis	Hebdomadis	Hebdomadis	*L. interrogans*	FJ154551
Hurstbridge[b]	Hurstbridge	BUT6ATCC BAA-1107	*L. fainei*	AY631885
Icterohaemorrhagiae[a]	Icterohaemorrhagiae	RGA	*L. interrogans*	AM050571
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	*L. interrogans*	AY631894
Icterohaemorrhagiae	Icterohaemorrhagiae	Par	*L. interrogans*	AF157084
Icterohaemorrhagiae	Icterohaemorrhagiae	Jac	*L. interrogans*	AF157073
Javanica[a]	Javanica	Veldrat Bataviae 46	*L. borgpetersenii*	AM050572
Javanica	Javanica	Veldrat Bataviae 46	*L. borgpetersenii*	Z21630
Javanica	Javanica	Veldrat Bataviae 46	*L. borgpetersenii*	FJ154600
Javanica	Javanica	Veldrat	*L. borgpetersenii*	AY461862
Korat[b]	Not designated	Korat-H2	*L. wolffii*	EF025496
Lai	Icterohaemorrhagiae	56601	*L. interrogans*	NC004342
Lyme[b]	Not designated	10 ATCC 43289	*L. inadai*	AY631896
Malaysia[b]	Tarassovi	Bejo-Iso9	*L. kmettyi*	AB279549
Manhao 3[b]	Manhao	L60ATCC 700520	*L. alexanderi*	AY631880
Mini[a]	Mini	Sari	*L. borgpetersenii*	AM050573
Mini	Mini	Sari	*L. borgpetersenii*	FJ154592
Mini	Mini	Sari	*L. borgpetersenii*	AY461859
Mozdoc[a]	Pomona	Pomona 5621	*L. kirschneri*	AM050574
Mozdoc	Mozdoc	5621	*L. kirschneri*	AY461879
Panama[b]	Panama	CZ214 ATCC 43288	*L. noguckii*	AY631886
Pomona[a]	Pomona	Pomona	*L. interrogans*	AM050575
Pomona	Pomona	Pomona	*L. interrogans*	AY996800
Pomona	Pomona	Pomona	*L. interrogans*	FJ154544
Pomona	Pomona	Pomona pomona	*L. interrogans*	AY461872
Ranarum[b]	Ranarum	Iowa City Frog ATCC 43287	*L. meyeri*	AY631878
Sejroe[a]	Sejroe	M84	*L. borgpetersenii*	AM050576
Sejroe	Sejroe	M84	*L. borgpetersenii*	AF157071
Sejroe	Sejroe	M84	*L. borgpetersenii*	FJ154593
Shermani[b]	Shermani	LT821 ATCC 43286	*L. santarosai*	AY631883
Tarassovi[a]	Tarassovi	Perepelicin	*L. borgpetersenii*	AM050577
Tarassovi	Tarassovi	Perepelicin	*L. borgpetersenii*	AY461861
Tarassovi	Tarassovi	Perepelicin	*L. borgpetersenii*	FJ154595
Zanoni[a]	Pyrogenes	Zanoni	*L. interrogans*	AM050578
Zanoni	Pyrogenes	Zanoni	*L. interrogans*	DQ991473
Not designated[b]	Not designated	5399 ATCC BAA-1107	*L. broomii*	AY796065

[a] Strains supplied by KIT (January 1998-October 2005), which were sequenced and used for DHPLC and comprise a panel of 19 antigens used for routine MAT based seordiagnosis.
[b] Strains supplied by KIT (November 2008) which were used for DHPLC (sequences obtained from GenBank).
[c] Strains from VLA culture collection, isolated from infected pig foetal tissue, which were sequenced and used for DHPLC.

In an attempt to find a rapid, sensitive and accurate method for speciating *Leptospira* we have chosen Denaturing High Performance Liquid Chromatography (D-HPLC). This method is commonly used for detection of single nucleotide polymorphism (SNP) based on separation of homo- and hetero-duplexes. DNA amplicons from test strains are mixed with a 'reference' or wild type strain, denatured and allowed to reanneal slowly. Hetero-duplexes, double stranded DNA containing a mismatch bubble, will be formed where there is a mismatch at the SNP site; whereas homoduplexes, double stranded DNA with no mismatches will be formed in the absence of SNPs. At a denaturing temperature heteroduplexes will denature more readily and will therefore be eluted from D-HPLC column earlier than homoduplexes. Once distinct chromatograms are established for each SNP, D-HPLC provides a useful platform for high-throughput SNP (or mutation) detection. In recent years, D-HPLC has been used for SNP discovery in the human genome project (Kwok and Chen, 2003), for gene scanning (Yu et al., 2006), for interleukin-10 promoter scanning (Guzowski et al., 2005), for mutation detection in antibiotic resistant bacteria (Randall et al., 2005), and to identify bacterial species by analysis of the 16S–23S intergenic spacer region (Hurtle et al., 2002).

The aim of this study was to investigate the potential of D-HPLC to rapidly identify *Leptospira* at the species level and to apply this technique to clinical diagnostic samples. The 16S rDNA gene was chosen and, based on sequences already in the database, the front end was used. Primers were designed and products were sequenced in order to confirm there was sufficient variation. Sequencing results provided evidence that D-HPLC would be a suitable method of differentiating strains. We would predict that clusters of strains with the same differences would have the same D-HPLC profile. Sequences of the 20 known genomospecies were compared and 14 genomospecies including pathogenic ($n = 9$), non-pathogenic ($n = 2$) and intermediate ($n = 3$) strains were selected for D-HPLC. Additionally 3 recent isolates and 21 infected tissues samples which had given positive Leptospira PCR results were included to demonstrate the applicability to field cases.

2. Materials and methods

2.1. Strains

Thirty reference strains acquired from Koninklijk Instituut voor de Tropen (KIT) (WHO Laboratory, Royal Tropical Institute, N.H Swellengrebel Lab1105 AZ, Amsterdam) were used for this study (Table 1). Of these, 19 comprise a panel of antigens used routinely for MAT based serodiagnosis at the Veterinary Laboratory Agency, (VLA, Weybridge) and these were used to sequence 16S rDNA prior to D-HPLC work. Sequences for the remaining 11 reference strains were already available in Genbank and hence D-HPLC work alone was performed. Three additional isolates from the VLA culture collection, (HB, BE and K5A), that had been isolated from infected pig foetal tissue (Williamson et al., 2004) and identified with monoclonal antibodies as *L. interrogans* serovar Bratislava were also included for 16S rDNA sequence analysis and D-HPLC. Prior to DNA extraction, strains were checked for other bacterial contamination by streaking onto 5% sheep blood agar plates and strains from the panel of 19 and the 3 from the VLA culture collection were tested with reference antisera in an MAT. Strains had undergone 4–48 passages, since being supplied by KIT between January 1998 and February 2005 whist the remaining 11 strains had undergone 2–3 passages, since receipt in November 2008.

2.2. DNA extraction from tissues

DNA was extracted from 1 cm^3 of kidney tissue taken from diagnostic material submitted to VLA regional laboratories from private veterinary surgeons for disease diagnosis, in which pathogenic *Leptospira* had been detected by PCR (Fearnley et al., 2008). These samples derived from 3 rats, 1 dog, 1 fox and 1 badger and 13 abortion submissions (10 bovine, 1 porcine, 1 equine and 1 alpaca) in which leptospiral fetopathy was included in the differential diagnosis. An automated robotic MagNA Pure LC instrument (Roche Applied Science, Switzerland) was used to extract DNA with the commercially available DNA isolation kit II for tissue (Roche, cat. No. 3186229) according to manufacturers' instructions.

2.3. DNA extraction from bacteria

Seven to ten day old *Leptospira* strain cultures (20 ml), grown in EMJH medium at 30 °C, were boiled for 10 min prior to cells being harvested and washed in TE buffer (10 mM Tris HCl, 1 mM EDTA pH 8.0) by centrifugation. The bacterial cell pellet was resuspended in 250 µl lysis buffer (100 mM tris HCl, 100 mM EDTA, 50 mM sodium chloride pH 7.4) with 30 µl 10% SDS and 5 µl 20 mg/ml Proteinsase K added, and incubated at 65 °C for 16 h. Following incubation, DNA was extracted using a Phaselock gelTM Light tube (CP Laboratories), using manufacturer's instructions. DNA concentrations were determined at optical density 260 nm using a spectrophotometer (Helios λ, SLS).

2.4. PCR and sequencing

PCR primers (Table 2) were designed on the basis of the published *Leptospira* 16S rDNA sequences (GenBank – http://www.ncbi.nlm.nih.gov/). Primers Lepto 16S F1 and R1 were designed to amplify a 1.318 kb fragment of the *rrs* gene. All other primers were internal primers designed to enable sequencing of the entire amplicon.

PCR amplification was performed using Expand High Fidelity Taq Polymerase (Roche) with proof reading ability, according to manufacturer's instruction. This was used with 4 mM $MgCl_2$, 0.2 mM dNTP (Amersham Pharmacia), 100 pmol of primers and 300 ng of genomic DNA. PCRs were performed using the following amplification protocol: 1 cycle at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 63 °C for 45 s, and 72 °C for 1.5 min; followed by a final extension at 72 °C for 10 min.

The amplified 1.318 kb PCR products were run on 0.8% agarose gels and visualised by ethidium bromide staining. The bands were excised using Qiagen QIAquick gel extraction kit (Cat. 28704) and sequenced with primers listed above. The DNA sequences were analysed using SeqMan software (DNASTAR, Lasergene 6). The GenBank accession number for all 16S rDNA fragments sequenced in this study is given in Table 1.

2.5. Sequence comparisons

Sequences from the VLA panel and the 3 isolates were aligned using ClustalW and a consensus 16S rDNA sequence was constructed using Megalign software (DNASTAR, Lasergene 6). Published *Leptospira* 16S rDNA sequences obtained from GenBank were then aligned

Table 2
Primers used for 16S rDNA sequencing and DHPLC.

Primer name	Sequence
Lepto 16S F1	5′-GGC GGY GCT CTT WAW CAT 3′
Lepto 16S R1	5′-CCT TRC GGT TRR SW RAC C 3′
Lepto 16S F2	5′-AGTTAAGAATCTTGCTCAATGG 3′
Lepto 16S F3	5′-GCT CAC CAA GGC GAC GAT CG 3′
Lepto 16S R2	5′-ATC CCG TTC ACT ACC CAC GC 3′
Lepto 16S R3	5′-TAAACC ACA TGC TCC ACC GC 3′
Lepto 16S R4	5′-GCT CAT CTC CGA GCA ATA 3′

using Megalign with those from the VLA panel and isolates for comparison of VLA sequences and those already in the database.

2.6. PCR for D-HPLC

To produce PCR amplicons for D-HPLC analysis the Lepto 16S F1 and Lepto 16S R4 PCR primers were used (to obtain a 181 bp product). The PCR mixture contained: 3 mM $MgCl_2$, 0.2 mM dNTP (Amersham Pharmacia), 100 pmol of primers and 100 ng of bacterial genomic DNA or DNA isolated from tissues. The following parameters were used for PCR amplification: 1 cycle at 94 °C for 5 min; 33 cycles at 94 °C for 30 s, 58.8 °C for 45 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. Positive control PCR reactions were performed using 100 ng of *Leptospira interrogans* Copenhageni strain Wijinberg (AM050565) DNA; negative control PCRs included 500 ng of DNA from uninfected kidney.

2.7. PCR sensitivity for D-HPLC

The MagNA Pure LC instrument was used to prepare DNA from kidney tissue previously found to be *Leptospira* PCR negative and this was used with DNA from *Leptospira borgpetersenii* Hardjobovis Sponslee, to determine the sensitivity of the PCR used for D-HPLC analysis. Leptospiral DNA was diluted in the kidney DNA over a range 8 pg to 100 ng/μl and used as template (1 μl per 50 μl reaction), in the PCR reaction as outlined above.

2.8. D-HPLC

D-HPLC was performed as previously described using an Agilent series 1100 HPLC system and a Varian HelixTM DNA column (**Randall et al., 2005**; Eaves et al., 2002). The melt temperatures of the D-HPLC column were determined using the D-HPLC melt program (htpp://www.insertion.stanford.edu/melt.html) and temperatures 57 °C, 58 °C and 60 °C, were used. Amplified PCR products from the reference strains *L. interrogans* Bratislava isolates and from infected animal tissues were mixed with an equal amount of PCR product from the *L. interrogans* Copenhageni strain Wijinberg (AM050565) which was selected as a 'reference' strain and heteroduplexes were detected at 260 nm. The ChemStation software (Agilent) was used to overlay chromatograms produced by the *L. interrogans* Copenhageni strain (AM050565) against each test strain. Initial results indicated that there was difficulty in distinguishing some *L. interrogans* strains including *L. interrogans* Copenhageni strain Wijinberg (AM050565), from *Leptospira inadai* Lyme (AY631896) and *Leptospira broomii* strain 5399 (AY792329). When *Leptospira weilii* Celledoni (AY631877) sequence was compared with the strains that were difficult to distinguish between, different sites and numbers of SNP's existed when compared with *L. interrogans* Copenhageni 'reference' (AM050565). Hence, it was expected that the heteroduplex DNA produced when each test strain was mixed with a *L. weilii* Celledoni (AY631877) 'reference' would also vary and D-HPLC would yield differing elution profiles, enabling distinction between the different strains where sequence differences existed. Therefore an additional 'reference' *L. weilii* Celledoni (AY631877) was used in place of the *L. interrogans* Copenhageni 'reference' (AM050565). Amplified products for *L. inadai* Lyme (AY631896), *L. broomii* strain 5399 (AY792329) and *Leptospira fainei* Hurstbridge (AY631885) were each mixed with amplified products of *L. weilii* Celledoni (AY631877) 'reference' and the chromatograms of this 'reference' was overlaid with the chromatogram from each of these test strains. *L. fainei* Hurstbridge (AY631885) was included due to the indistinct D-HPLC profile and its sequence similarity with the *L. inadai* and *L. broomii* strains.

2.9. Sequence comparison of D-HPLC fragments with published sequences

Sequences for all reference strains and isolates used for D-HPLC and also the sequenced strains *L. interrogans* Lai (NC004342) and *L. interrogans* Copenhageni (NC005823) were trimmed to 171 bp (bases 11–181 of the fragment), aligned using Mega 4, and a phylogenetic tree was constructed using MEGA version 4 (**Tamura et al., 2007**). The Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm was used for phylogenetic analysis with 10,000 bootstrap solutions. Sequences for the full 181 bp of the D-HPLC fragment were unavailable for all strains selected and hence all strains were trimmed for comparison.

2.10. D-HPLC reproducibility

The D-HPLC reproducibility within and between batches was determined at 57 °C and 60 °C by following the elution profile of homoduplexes of the VLA *L. interrogans* Copenhageni (AM050565). Reproducibility was determined from repeat injections ($n = 10$) of same the PCR product; from different PCR reactions ($n = 10$); and the same PCR products in different batch runs ($n = 10$).

3. Results

3.1. Nucleotide sequencing and analysis

Sequencing of the VLA diagnostic panel belonging to the 3 species *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii* showed some variations (**Table 3**). Some variations were shared by each strain within the species whilst others were unique to an individual strain within that species group.

Comparison of the 16S rDNA sequence from the VLA panel of strains with all other *Leptospira* 16S rDNA sequences available from GenBank (**http://www.ncbi.nlm.nih.gov/**), showed the majority of sequences to compare well, with 0 to 1 base pair variation (**Table 4**) but one strain of *L. interrogans* Hardjo prajitno (AY461867) had 2 base changes from the published sequence. The entire 16S rDNA sequence fragment was sequenced and the sequences were aligned in ClustalW. A high degree of homogeneity was shown with each strain showing 99.4–100% identity. The variability that was identified was at the 5′ end which is in accordance with the findings of Postic et al. (**Postic et al., 2000**). The DNA sequences were deposited in GenBank and the Accession numbers are given in **Table 1**.

A phylogenetic tree (not shown) was constructed based on 1.3 kb of the 16S rDNA sequences from the 3 species: *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii*, generated 3 clusters that reflected exactly their speciation. As expected strains from previous work (**Hookey et al., 1993**; **Morey et al., 2006**), *L. interrogans* and *L. kirschneri* were more closely related to each other than to *L. borgpetersenii* strains.

3.2. Speciation of Leptospira by D-HPLC in control and test strains (57 °C and 58 °C)

DNA sequence predicts *in silico* what the likely differences are that should be detected by D-HPLC (**Fig. 1**). The PCR and D-HPLC protocol was devised (see Materials and methods) to detect those differences over 181 bp at the 5′ end of the gene. A phylogenetic tree of this region is shown in **Fig. 2**. The PCR and D-HPLC was applied to a wide selection of species. *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii* were differentially clustered whilst other groupings were generated that reflected the sequence similarities of the other species included. The two non-pathogenic *Leptospira*

Table 3
Variation of the 16S rDNA sequence from the consensus on comparison of the VLA panel of *Leptospira* strains by ClustalW.

Base position	Consensus sequence	Variation	Serovars sharing variation	Species
134	T	C	Hardjobovis, Ballum, Sejroe, Tarassovi, Javanica, Mini	*L. borgpetersenii*
134	T	C	Mozdoc, Gripotyphosa	*L. kirschneri*
161	A	G		
176	G	A		
187	A	G	Hardjobovis, Ballum, Sejroe, Tarassovi, Javanica, Mini	*L. borgpetersenii*
193	A	G		
236	T	C		
261	A	T		
436	G	A		
459	A	G		
1269	T	G		
191	T	C	Zanoni, Australis, Canicola, Hebdomadis, Pomona	*L. interrogans*
191		C or T	Hardjo	*L. interrogans*
379	-	G (insertion)	Pomona	*L. interrogans*
631	G	A	Tarassovi	*L. borgpetersenii*
1233	G	A	Hardjobovis	*L. borgpetersenii*
1252	G	A	Sejroe	*L. borgpetersenii*
		No variation	K5A Bratislava, Autumnalis, Bataviae, Bratislava BE, Copenhagenii, Icterohaemorhagiae, Bratislava HB (Hardjo)[a], Bratislava BP	*L. interrogans*

The above sequences of VLA strains have been compared between 48-1365 bp of the 16S rDNA. The 16S rDNA numbering system presented above is from the published genome sequences for serovar Lai (Ren et al., 2003) and Copenhageni (Nascimento et al., 2004).
[a]If T at position 191.

species (*L. meyeri* and *L. wolbachii*) clustered together as did the 3 intermediate species (*L. fainei*, *L. inadai* and *L. broomii*).

The reproducibility of the D-HPLC is shown in Table 5. The data showed the coefficient of variation (CV) between batches to be high at both 57 °C and 60 °C. In contrast the CV within a batch was low, whether 10 separately amplified PCR products were injected or the same PCR product was injected 10 times. Therefore, to ensure reproducibility for each D-HPLC run, and to compare between runs, the *L. interrogans* Copenhageni PCR was included both at the beginning and end of each batch run.

Melt temperatures at 57 °C and 58 °C gave similar profiles, but at 58 °C clearer profile patterns were obtained. When a melt temperature of 58 °C was used for D-HPLC, only 5 profile patterns were generated. Whilst species sharing the same sequence gave the same D-HPLC profile, there were strains with different sequence composition that shared the same D-HPLC profile at this temperature. For example, *L. borgpetersenii* strains Hardjobovis, Ballum, Sejroe, Tarassovi, Javanica and Mini share the same 5 SNPs within the 181 bp fragment but generated a profile shared by *L. alexanderi*, *L. kmetyii* and *L. santarosai*. Similarly *L. interrogans* strains Copenhageni, Autumnalis, Bataviae, Bratislava BP, Icterohaemorrhagiae and the Bratislava isolates K5A, HB and BE which have the same sequence, shared the same profile as for *L. kirschneri* Mozdoc, *L. Kirschneri* Grippotyphosa and the intermediate strains *L. broomii* strain 5399, *L. inadai* Lyme and *L. fainei* Hurstbridge. Whilst there was only one sequence difference between *L. interrogans* Copenhageni strain and *L. kirschneri* Mozdoc and *L. kirschneri* Grippotyphosa, there were 18–20 sequence differences between *L. interrogans* Copenhageni and *L. broomii* strain 5399, *L. inadai* Lyme and *L. meyeri* Ranarum. Interestingly 2 profiles were given by the *L. interrogans* group. One profile was given by *L. interrogans* Copenhageni, which was shared by other species, as outlined above and the second profile was given by *L. interrogans* serovars Zanoni, Australis, Canicola, Hebdomadis and Pomona, which share a SNP at base position 191 from the consensus and by *L. interrogans* Hardjo subtype Hardjoprajitno (Table 3).

3.3. Speciation of Leptospira by D-HPLC in control and test strains (60 °C)

To improve differentiation between the different species, the melt temperature was increased to 60 °C and examples of the profiles are shown in Fig. 3. The *L. interrogans* group still generated 2 distinct profiles, one for *L. interrogans* Copenhageni, Autumnalis, Bataviae, Bratislava BP, Bratislava isolates K5a, HB, BE and Icterohaemorrhagiae, and another for *L. interrogans* Australis, Zanoni, Canicola, Hebdomadis, Hardjo subtype Hardjoprajitno and Pomona (Fig. 3a). At this temperature, strains with the same sequence composition demonstrated the same D-HPLC profile. At this temperature, *L. kirschneri* Mozdoc and *L. kirschneri* Grippotyphosa had the same profile (*L. kirschneri* Mozdoc only shown) and this could be distinguished from *L. interrogans* Copenhageni standard (Fig. 3a) and the intermediate strains *L. inadai* Lyme (Fig. 3b), *L. broomii* strain5399 and *L. fainei* Hurstbridge (Fig. 3c). However, as before,

Table 4
Similarities and differences in the 16S rDNA sequence between published and VLA strains from the same serovar or strains, aligned using ClustalW.

Serovar	Accession no.	Base position of sequence used for comparison[a]	Identity with VLA strain sequence	VLA strain used for comparison (Accession no.)	Total number of base pair differences
Australis	AY996794	48-1358	99.9%	AM050579	1
	FJ154556	48-1365	99.9%	"	1
Autumnalis	AF157069	48-363	100%	AM050580	0
	AY461864	75-1254	100%	"	0
	FJ154543	48-1365	100%	"	0
Ballum	AY631884	48-1365	100%	AM050581	0
	AY461856	75-1254	100%	"	0
Bataviae	AY461865[b]	75-1255	99.9%	AM050582	1
	DQ991469	48-1347	100%	"	0
Bratislava	AY461863	75-1077	100% with Bratislava HB, BE, K5A & BP	AM050584 (HB) AM050585 (BE) AM050586 (K5A) AM050583 (BP)	0
	FJ154547	48-1360	100% with Bratislava HB, BE, K5A & BP	AM050584 (HB) AM050585 (BE) AM050586 (K5A) AM050583 (BP)	0
Canicola	AY461866[b]	75-1255	100%	AM050566	0
	X17547[b]	48-1365	99.9%	"	1
Copenhageni	AY461869[b]	75-1255	99.9%	AM050565	1
	AF0058231TS1[b]	48-1365	99.9%	"	1
	AF0058231TS2[b]	48-1365	100%	"	0
Grippotyphosa	AF157067	48-362	99.6%	AM050567	1
	AY46877[b]	75-1255	100%	"	0
Hardjo prajitno	AY461867	75-1256	99.4%	AM050568	2
	FJ154553	48-1365	99.9%	"	1
	AY996796	48-1365	100%	"	0
	AY996797[b]	48-1365	100%	"	0
Hardjobovis	CP000348	48-1365	100%	AM050569	0
Hebdomadis	FJ154551	48-1365	100%	AM050570	0
Icterrohaemorrhagiae	AY631894	48-1379	99.9%	AM050571	1
	AF157084[b]	51-355	100%	"	0
	AF157073[b]	48-364	99.7%	"	1
Javanica	AY461862	76-1256	100%	AM050572	0
	Z21630	48-1365	99.9%	"	1
	FJ154600	48-1365	100%	"	0
Mini	AY461859	75-1255	100%	AM050573	0
	FJ154592	48-1365	100%	"	0
Mozdoc	AY461879	75-1255	100%	AM050574	0
Pomona	AY461872	390-1269	100%	AM050575	0
	AY996800	48-1365	100%	"	0
	FJ154544	48-1365	100%	"	0
Sejroe	AF157071	48-1365	100%	AM050576	0
	FJ154593	48-1365	100%	"	0
Tarassovi	AY461861	75-1255	100%	AM050577	0
	FJ154595	48-1365	100%	"	0
Zanoni	DQ991473	48-1365	100%	AM050578	0

[a] Numbering of the base position is from alignment with the published genome sequences from serovar Lai (NC004342, (**Ren et al., 2003**)) and Copenhageni (NC005823, (**Nascimento et al., 2004**)) for which the whole rrs genes have been sequenced.
[b] Published strain has different nomenclature or strain number to the VLA strain used for comparison, but both belong to same serovar.

there was no distinction between the D-HPLC profile given by *L. interrogans* Copenhageni and between that given by *L. broomii* strain 5399 (**Fig. 3c**) and *L. inadai* Lyme (**Fig. 3b**). When the *L. weilii* Celledoni 'standard' was used in place of the *L. interrogans* Copenhageni 'standard' at melt temperature of 60 °C, the resulting profiles (**Fig. 3d**) were different for *L. interrogans* Copenhageni, *L. inadai* Lyme, *L. weilii* Celledoni and *L. fainei* Hurstbridge. Profiles for *L. inadai* Lyme and *L. broomii* strain 5399 were similar, as expected, as the sequence for the D-HPLC fragment for these 2 strains is identical.

3.4. Application of PCR and D-HPLC to clinical material

DNA was extracted from 19 samples (kidney and foetal) submitted to the VLA that were shown positive for pathogenic *Leptospira* using a PCR previously described by Fearnley et al. (**Fearnley et al., 2008**). The PCR and D-HPLC protocol was applied to the extracts and all produced profiles at 58 °C and at 60 °C. In the case of one animal (2 samples), a profile consistent with that given by *L. interrogans* serovars Australis, Zanoni, Canicola, Hebdomadis, Pomona, or Hardjoprajitno was generated. For all other cases the profiles were consistent with those given by *L. interrogans* Copenhageni, Autumnalis, Bataviae, Bratislava, Hardjoprajitno or Icterohaemorhagiae. *L. interrogans* Hardjoprajitno strains produce both D-HPLC profiles, confirmed by sequence analysis, and therefore may be ambiguously interpreted. *L. borgpetersenii* was not detected in any of the samples. In three instances, duplicate kidney samples were available from an infected animal and for each replicate the D-HPLC results were identical, showing the method to be reproducible.

4. Discussion

The aim of this study was to identify regions of single nucleotide polymorphism in the 16S rDNA sequence (the *rrs* gene) from

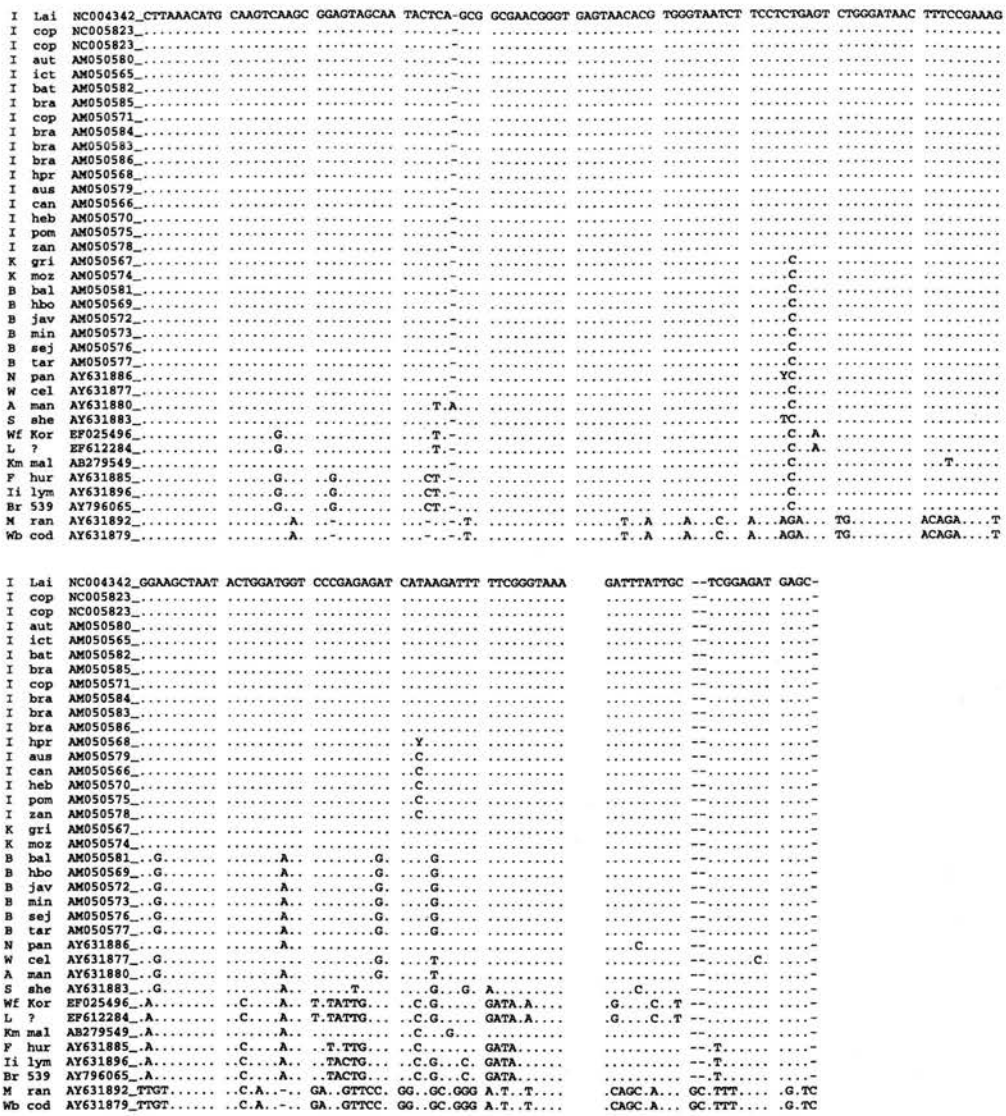

Fig. 1. Mega 4 sequence alignment of 171 bp of D-HPLC fragment (bases 10–181). Species: I, *interrogans*; K, *kirschneri*; B, *borgpetersenii*; N, *noguchii*; W, *weilii*; A, *alexanderi*; S, *santarosai*; Wf, *wolffi*; L, *lisceriae*; km, *Kmettyi*; F, *fainei*; Ii, *inadai*; Br, *broomii*; M, *meyeri*; Wb, *wolbachii*. First 3 letters of serovar and accession number indicated.

three of the pathogenic species *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri* that are of veterinary significance and, given the zoonotic nature of *Leptospira*, of relevance to human infection also. Once SNPs were identified within the 16S rDNA, D-HPLC was used to rapidly speciate *Leptospira* isolates. Low heterogeneity of the 16S sequence enabled us to use this approach where each species was divided into a distinct group showing little intraspecies variation, except in *L. interrogans*. Subsequently, particular D-HPLC profiles were attributed to each species and subspecies, based on the SNP variation. To enable wider application of this technique, *Leptospira* from other species, including non-pathogenic, intermediate and other pathogenic species were included. Inclusion of these species demonstrated the importance of selecting an appropriate D-HPLC melt temperature, as the lower temperature was unable to distinguish between some species. Inclusion of appropriate controls was also shown to be important for assigning species.

The strains from the VLA included for characterisation of SNPs within the 16S rDNA compared well with strains from the same serovars present in GenBank. Interestingly, the 16S sequence separated *L. interrogans* into 2 distinct subgroups: one with no variation and another with variation at base position 191. This was also confirmed by the D-HPLC work. Speciation of *Leptospira* using the *ompL1* gene (**Reitstetter, 2006**) also resulted in 2 distinct subgroups for *L. interrogans* serovars. The serovars within each subgroup matched our work. One explanation for variation between strains from the same serovar could be due to high rates of mutation within the gene, although this would be unexpected in a highly conserved gene such as the *rrs* (**Lane et al., 1985**). Postic

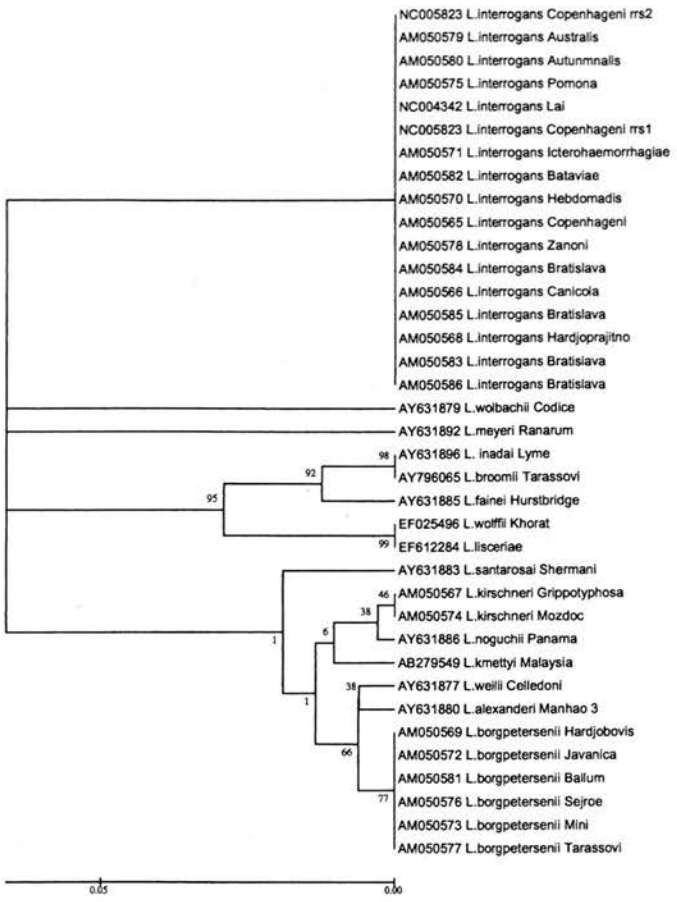

Fig. 2. A phylogenetic tree of the 16S rDNA sequences. The tree was drawn using UPGMA algorithm. The reliability was assessed using bootstrapping analysis and support for key nodes is indicated in percentage.

Table 5
Reproducibility of D-HPLC elution profiles between batches (runs) and within a batch using repeat PCRs and repeat injections. The mean (*M*) difference in the retention time; the standard deviation (SD) and coefficient of variation (CV) was calculated for each parameter; the number of repeats is given by *N*.

Parameter	Temp (°C)	Retention time (min)			CV
		N	SD	*M*	
Batches	57	10	0.6394	5.5060	11.6135
	60	10	0.5455	4.5328	12.0347
PCRs	57	10	0.0143	5.7534	0.2487
	60	10	0.0848	4.675	1.8139
Injections	57	10	0.0124	5.839	0.2127
	60	10	0.0172	4.2403	0.4064

et al. (2000) have noted similar discrepancies between several reference strains from different laboratories using the 16S rDNA sequence. They have suggested that possible heterogeneities exist within culture collections due to mislabelling, cross-reactions or even due to the original culture being mixed isolates. We concur with their suggestion and this data highlights the imperative to confirm strain identity by molecular means.

The *Leptospira* species identified by D-HPLC in the clinical samples were generally consistent with the case histories and other laboratory findings, including MAT serology (where undertaken) and full details of one case have already been published (Gaudie et al., 2008). The bovine abortion samples tested were taken from herds in various parts of England, but surprisingly we did not detect any *L. borgpetersenii* serovar Hardjo (hardjo bovis) which has long been considered to be endemic in England and Wales (Ellis, 1986). Further studies are needed to investigate this.

Most molecular approaches for *Leptospira* speciation use PCR followed by a sequencing step, and are generally based on culturing of *Leptospira* (Ahmed et al., 2006; Majed et al., 2005; Morey et al., 2006; Reitstetter, 2006; Wangroongsarb et al., 2007). Our approach of using PCR that amplifies a short fragment of *Leptospira* DNA isolated from tissues followed by D-HPLC makes our method rapid, sensitive and accurate and circumvents cultivation, thus expediting the process of speciation. The use of D-HPLC allows rapid speciation as the column can accurately run 99 samples in a single run, with each sample taking approximately 20 min. In addition D-HPLC chromatograms provide distinct profiles that are easy to interpret. Another advantage of using D-HPLC for speciation is that new species or strains that have not been characterised can

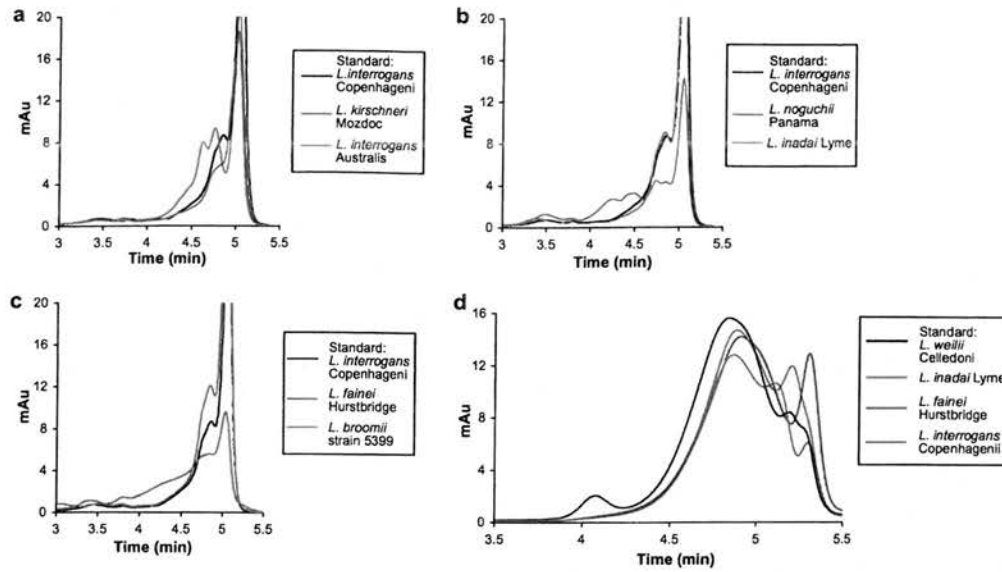

Fig. 3. DHPLC elution profiles of *Leptospira* species at 60 °C.

easily be detected by changes in the profile of the chromatogram which can subsequently be characterised by sequencing. However, all clinical samples used in this study were isolated from kidney and fetal tissues which may contain relatively high concentrations of *Leptospira*. In the future it will be useful to test the sensitivity of our approach using other samples such as urine, blood and CSF where the concentration of *Leptospira* is generally very low. Characterisation of other *Leptospira* species and serovars that are pathogenic to both humans and animals, using our approach, will also broaden its applicability in future.

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Unexplained sporadic milk drop in dairy cows

1.57

SIR, – With increasing frequency veterinary investigation centres receive reports from cattle practitioners of a sporadic milk drop syndrome in dairy cows. The potential causes of milk drop are legion. *Leptospira hardjo* and infectious bovine rhinotracheitis virus are recognised causes of epidemic disease and we have some evidence that bovine respiratory syncytial virus may act similarly. Seroconversion to any one of these agents, or to parainfluenzavirus-3, is associated with sporadic cases of milk drop in some herds; and colleagues with the Veterinary Investigation Division have investigated the possible involvement of chlamydia. We are aware of herds where traumatic reticulitis was responsible for several cases of milk drop and where the diagnosis was only reached after postmortem examination of culled cases. However, in many herds with sporadic milk drop, no infectious or metabolic cause can be found.

By definition, sporadic milk drop occurs sporadically, with only one or two cows affected at any one time. However, over time, the accumulated number of cases within a herd may be quite considerable and this is the cause for concern. In individual cases the classic sign is sudden onset agalactia. Some cows have total milk loss but partial loss is more usual. It occurs at any stage of lactation but is perhaps more common during the first three months.

Accompanying signs are variable but most cases are dull and inappetent, and weight loss may be observed. In some affected herds cows are non-pyrexic, while in others high fevers are usual; but typically a mild fever (39·0-39·5°C) is the norm. Increased respiratory rate and mucopurulent nasal discharge are sometimes observed but these are inconsistent signs. Neutrophilia and elevated acute phase proteins, for example, haptoglobin, have been detected in some of the blood samples examined. Recovery within seven to 10 days, with or without treatment, is usual although relapses are a significant feature in a few herds. There is no age predilection. Sporadic milk drop syndrome is typically a problem of the winter months but may occur in the summer. It may occur in successive years on the same farm.

In some cases inappetence, and therefore milk drop, may be due to indigestion, and there is a suggestion that there is a nutritional component to the problem. In most affected herds cattle are fed maize silage, usually as part of a mixed ration, and the possibility of transient acidosis has been raised. The use of computerised milk recording on some farms may have revealed cases that might otherwise have gone undetected.

There are some marked differences between herds in the clinical histories – for example, pyrexia versus no pyrexia, respiratory signs versus no respiratory signs, recurrent cases versus no recurrence – and it therefore seems highly unlikely that the sporadic milk drop syndrome is a single entity. However, within any one affected herd the majority of cases do seem to have a fairly consistent clinical presentation and may have a common aetiology.

We would value the observations and comments of colleagues elsewhere.

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1.58

Bovine respiratory syncytial virus infection in lactating cows

SIR, – In Britain, bovine respiratory syncytial virus (BRSV) is mainly recognised as an important pathogen involved in pneumonia of young calves but it can also cause acute, often fatal, pneumonia in older calves and yearlings. Occasionally, veterinary investigation centres encounter outbreaks of disease in cows that appear to be associated with BRSV infection and, as mentioned in the recent letter by Gunning and Pritchard (*VR*, May 3, p 488), it has been implicated as a cause of milk drop. A recent survey of antibodies in bulk milk samples by the Veterinary Laboratories Agency indicated a very high prevalence of BRSV infection in dairy herds in Britain. Elvander (1996) has described outbreaks of severe respiratory disease and milk drop in dairy cows in Sweden. Here, we wish to report a similar, albeit clinically milder, outbreak in a closed 120-cow Holstein Friesian dairy herd in southern England that was attributed to BRSV infection.

In February 1997, herd milk yield began to fall and several cows were reported to be eating only part of their concentrate ration in the parlour. A walk around the cubicle house indicated that about 50 per cent of the adult cows were coughing. During the course of the next few days eight cows showed more severe clinical signs – anorexia, pyrexia, dullness and purulent nasal discharge with conjunctivitis. These were injected with amoxycillin (Amoxypen; Intervet UK) and made a good recovery. Within a week after commencement of the outbreak the herd milk yield had fallen by about 12 per cent (300 litres/day). Two weeks later the situation had improved markedly and milk yield returned to its expected level.

The diagnosis was initially attributed to infectious bovine rhinotracheitis (IBR) virus infection, but nasopharyngeal swabs examined with the fluorescent antibody test (FAT) all gave negative results. The FAT for BRSV was not undertaken on the swabs because, to be successful, it usually demands lung tissue or tracheal washings. Haematological findings were unremarkable. Convalescent sera from eight affected cows were completely negative for antibodies to IBR virus, *Mycoplasma bovis*, *Chlamydia psittaci* and *Haemophilus somnus* infections, all of which were considered to be possible alternative diagnoses.

Three cows were seropositive for bovine viral diarrhoea virus but all eight samples were strongly seropositive for both BRSV and parainfluenzavirus-3 (PI3). When the convalescent sera were tested alongside sera taken at the acute stage, one cow exhibited a significant increase (>0·2) in optical density for PI3 virus. More significantly, all of the cows either seroconverted or showed a similar significant increase in optical density for BRSV, clearly demonstrating active recent infection.

In addition, because of the widespread coughing in the herd, the convalescent sera were tested for lungworm (*Dictyocaulus viviparus*) antibodies with ELISA. One cow was seropositive and another gave an inconclusive positive result. However, the cows had had no access to pasture since the previous October and it was considered that these findings reflected incidental historical exposure only.

In conclusion, the findings reported here strongly indicated that BRSV infection was the cause of the outbreak described. Colleagues in general practice should be aware that BRSV infection may be a cause of milk drop and upper respiratory disease in adult cattle in Britain. Diagnosis can be achieved by paired serology, as demonstrated here, or with fluorescent microscopy using tracheal washings from acute cases. Vaccination offers a potential control option.

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Reference

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Emergence of fasciolosis in cattle in East Anglia

G. C. PRITCHARD, A. B. FORBES, D. J. L. WILLIAMS, M. R. SALIMI-BEJESTANI, R. G. DANIEL

Liver fluke (*Fasciola hepatica*) infection caused weight loss, diarrhoea, decreased milk yield and occasionally death in cattle in East Anglia during the winters of 2001 to 2003. The condition had previously been limited mainly to stock imported into this part of Britain from endemically infected areas. In composite faecal samples collected by 16 farm animal veterinary practices in Norfolk, Suffolk and Essex, fluke eggs were found in 15 (28·8 per cent) of 52 previously unaffected suckler herds and 10 (16·7 per cent) of 60 dairy herds. Antibodies to *F hepatica* were detected by ELISA in 32 (53·3 per cent) of the bulk milk samples from these 60 dairy herds, including the 10 in which fluke eggs were found. The emergence of fasciolosis in East Anglia was attributed to recent higher summer rainfall, which favoured the intermediate snail host *Lymnaea truncatula* and the free-living stages of *F hepatica*, the increased influx of sheep from endemic fluke areas for seasonal grazing, and the wetter grazing conditions associated with the Environmentally Sensitive Area scheme.

LIVER fluke disease (fasciolosis) is caused by infection with the liver fluke *Fasciola hepatica*, and results in serious economic loss to cattle and sheep producers in Great Britain and Ireland (Hope Cawdrey 1984). It also affects other herbivorous species, including goats and deer. The acute disease, primarily in sheep, results in anaemia and death; in chronic cases, illthrift, weight loss, diarrhoea and secondary metabolic or infectious disease occur. In cattle, a subclinical infection may adversely affect milk yield, body condition and reproductive performance (Ross 1970, Oakley and others 1979). Veterinary Investigation Diagnostic Analysis (VIDA) data from the Veterinary Laboratories Agency (VLA) indicate that the incidence of fasciolosis has been increasing during the past decade. Most cases previously occurred in the wetter, western areas of the British Isles (Froyd 1975), but the disease is now increasingly occurring elsewhere, including the east of Scotland (Mitchell 2002).

This paper describes the emergence of fasciolosis in East Anglia – a drier region of Britain where previous diagnoses had been largely confined to sporadic incidents in seasonally grazed store lambs or cattle bought in from endemically infected areas; it also includes a prevalence survey.

MATERIALS AND METHODS

Local disease surveillance and VIDA data

General information on the occurrence of liver fluke infection and associated clinical disease in 2001 to 2003 in East Anglia (Norfolk, Suffolk, Essex and Cambridgeshire) together with Bedfordshire and Hertfordshire – the region covered by VLA – Bury St Edmunds – was obtained from the samples of carcases, faeces and blood submitted to the laboratory and from discussions with local veterinary practitioners.

VIDA diagnoses 1993 to 2003

Diagnoses of clinical fasciolosis (excluding the detection of fluke eggs in faecal samples submitted for screening purposes from apparently healthy cattle) that were based on submissions to VLA – Bury St Edmunds between 1993 and 2003 were recorded on the VIDA database (Hall and others 1980). These diagnoses were confirmed according to strict diagnostic criteria and depended on the general awareness of the disease and the submission of appropriate samples by veterinary practitioners.

Faecal sample survey

Sixteen of the main farm animal veterinary practices in Norfolk, Suffolk and Essex participated in a survey, using composite faecal samples, to assess the prevalence of subclinical (unrecognised) infection with *F hepatica* in their clients' cattle herds. The criteria used to select the herds included the requirements that they had at least 20 cows that had grazed on the farm for at least four months before the samples were collected, that they had no history of liver fluke disease, and that no flukicide treatment had been administered during the previous six months.

Between November 2002 and the end of February 2003, samples of approximately 50 g of freshly voided faeces were collected from 10 randomly selected cows in each herd and mixed in a plastic container; the numbers of fluke eggs in 50 g subsamples were counted by a standard sedimentation technique adopted by the VLA laboratories (Anon 1986). In addition, a similar composite sample was collected from 10 heifers in the dairy herds where they grazed separately from the milking herd. Ten cattle at least one year old were similarly sampled in beef suckler herds. Samples of bulk milk in preservative were obtained from the participating dairy herds as described by Pritchard (2001).

Data collection

Farm details In addition to the farms' addresses and county parish holding (CPH) numbers, the presence of sheep on the farms or at seasonal grazing was recorded to determine whether it was a significant risk factor for cattle acquiring fluke infection. Contact with other potential herbivorous hosts such as deer, rabbits and hares was not recorded because

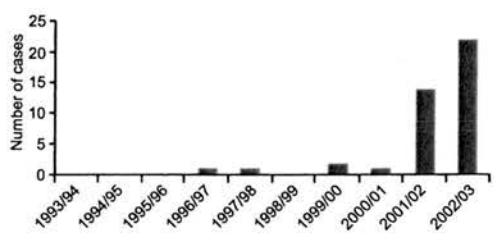

FIG 1: VIDA diagnoses of fasciolosis in cattle by Veterinary Laboratories Agency – Bury St Edmunds between March 1993 and March 2003

Veterinary Record (2005) **157,** 578-582

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TABLE 1: Results of the survey of composite samples of faeces for fluke eggs and the ELISA for antibodies to *Fasciola hepatica* in samples of bulk-tank milk

County	Number of veterinary practices	Suckler herds Tested	Suckler herds +ve eggs (%)	Dairy herds* Tested	Dairy herds* +ve eggs (%)	Dairy herds* +ve ELISA (%)
Norfolk	9	22	7 (32)	37	9 (24)	21 (57)
Suffolk	4	20	5 (25)	14	1 (7)	7 (50)
Essex	3	10	3 (33)	9	0 (0)	4 (44)
Total	16	52	15 (29)	60	10 (17)	32 (53)†

* Includes 34 herds from which samples of bulked faeces from heifers were also examined. In the positive herds, all the eggs were found in samples from milking cows apart from one herd in which only uncalved heifers were positive. Samples from heifers and cows were both positive in two herds

† Includes all 10 herds in which fluke eggs were found

these species were widespread and any assessment would have been too subjective. On completion of the survey, the location of all the infected and uninfected herds was mapped by using Geographical Information Systems (GIS), to explore the spatial relationship between wetland management under the Environmentally Sensitive Area (ESA) scheme operated by DEFRA and the presence of fluke infection; ESAs, such as the Norfolk Broads, Suffolk River Valleys and Essex Coast (all included in the survey area), were introduced in 1987 to offer incentives to farmers to adopt agricultural practices to safeguard and enhance parts of the country of particularly high landscape, wildlife or historic value.

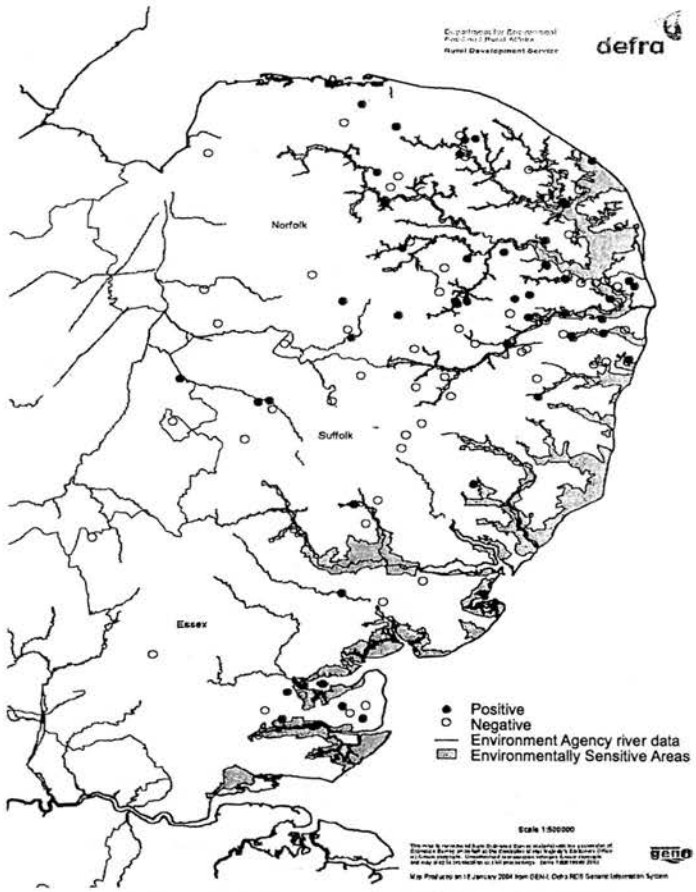

FIG 2: Geographical Information Systems map showing fluke-positive and fluke-negative herds recorded in the survey between November 2002 and the end of February 2003

Meteorological information The average temperature and rainfall statistics for East Anglia between 1971 and 2000 were obtained from the Met Office. These were used in conjunction with data for 1993 to 2002 from Coltishall and Ditchingham (stations at the centre of the study area) for comparative purposes. The number of days in each year when the temperature exceeded 10°C (10-degree days) – the minimum temperature for several key life cycle processes of *F hepatica* and *Lymnaea truncatula*, the intermediate snail host (Mitchell 2002) – was calculated from the 10-year dataset, together with the monthly average rainfall.

Milk antibody testing

A bulk milk antibody test was adapted by Salimi-Bejestani and others (2003) from an ELISA previously developed by Salimi-Bejestani and others (2001) to detect serum antibodies to *F hepatica*. The ELISA plates were coated with 0·5 µg/ml of *F hepatica* excretory/secretory antigens; phosphate-buffered saline/Tween containing 2 per cent skimmed milk powder was used to block the plates and as a diluent. The bulk milk tank samples were tested undiluted. Bound antibodies were revealed by using a monoclonal anti-bovine immunoglobulin G conjugated to horseradish peroxidase and hydrogen peroxide/tetramethylbenzidine (TMB) as the substrate/chromogen system. The results were expressed as the per cent positivity, that is, the ratio of the test sample's optical density (OD) to the mean of the ODs of four replicate positive control wells included on each plate. A cut-off value of at least 27 per cent positivity was used to indicate that 25 per cent or more of the milking herd had been exposed to the parasite (Salimi-Bejestani and others 2003). With this cut-off value, the diagnostic sensitivity of the test was 93 per cent (95 per cent confidence interval [CI] 83 to 100 per cent) and its diagnostic specificity was 81 per cent (95 per cent CI 67 to 96 per cent).

RESULTS

Local disease surveillance

During the winters of 2001/02 and 2002/03, fasciolosis was observed at various times in cattle, sheep, goats, red deer (*Cervus elephus*) and alpacas (*Lama pacos*). Most of the incidents occurred on farms with no previous history of the disease and where it had not previously been considered a potential threat. Outbreaks occurred in Bedfordshire, Cambridgeshire, Hertfordshire and Essex, but the disease was particularly prevalent in Norfolk and Suffolk. In sheep, its effects ranged from sudden death, with an affected group mortality of up to 15 per cent from the acute disease, to more chronic wasting with submandibular oedema, affecting between 10 and 30 per cent of the flock. Fluke infection was also recorded as an incidental finding in parkland red deer at slaughter. Fluke eggs were found in the faeces of scouring, unthrifty alpacas. There were field reports by practising veterinary surgeons of adult cattle severely affected with acute-onset disease leading to death or prolonged recumbency. In other cattle, including dairy cows, inappetence, weight loss, diarrhoea and high serum activities of liver enzymes were reported, but no abortions were reported.

VIDA data

The marked increase in the incidence of fasciolosis in cattle in the area served by VLA – Bury St Edmunds during 2000 to 2003 is shown in Fig 1. These data were based on confirmed VIDA diagnoses of clinical disease from submissions received from veterinary practitioners.

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Faecal survey

F hepatica eggs were found in 15 (28·8 per cent) of 52 beef suckler herds and 10 (16·7 per cent) of 60 dairy herds surveyed (Table 1). A further 22 dairy herds were positive only by the milk antibody test, giving an overall prevalence of 32 (53·3 per cent) in the dairy herds. Thirty herds (12 dairy and 18 suckler) had contact with sheep; of these, 16 (53·3 per cent) – eight dairy and eight suckler herds – were positive by either test, compared with 32 (39·0 per cent) of 82 herds that were positive but did not have contact with sheep. Although these data suggested a possible association between liver fluke in cattle (particularly suckler herds) and contact with sheep, the difference was not statistically significant.

GIS mapping

The GIS map (Fig 2) shows the distribution of the herds that were fluke-positive or negative by either test, in relation to river valleys and ESAs. Although fluke infection was not confined to ESA grazings, the pattern suggested that it was more common in these areas.

Meteorological data

Between 1993 and 2002 there was an average of 253 days in each year when the temperature equalled or exceeded 10°C, with a range from 207 days in 1996 to 281 in 2002, and there was no obvious trend during the decade. An average of approximately 200 days with temperatures of at least 10°C was recorded between April and October inclusive, allowing ample opportunity for the development of *F hepatica* in the snail *L truncatula* and its transmission and survival on pasture. Over the same period the average annual rainfall was 673·9 mm, with a range from 498·7 mm in 1996 to 859·2 mm in 2001; on average, 60 per cent of the rain fell between April and October. The annual number of 10-degree days and the total annual rainfall over the years 1993 to 2002 (Fig 3) indicated that the weather patterns over this period were conducive to fasciolosis.

In comparison with 1971 to 2000 (Fig 4), the monthly average maximum temperatures were consistently higher over the years 1993 to 2002. Fig 5 shows the average monthly rainfall recorded in East Anglia between 1971 and 2000, between 1993 and 2002, and between 2001 and 2002, the years during which there was an increase in the number of cases of fasciolosis. Between 1971 and 2000 the average annual rainfall was 605·6 mm, but between 1993 and 2002 it was 673·9 mm, and between 2001 and 2002 it was 781·1 mm. The increases were particularly marked in February, March, July, August and November.

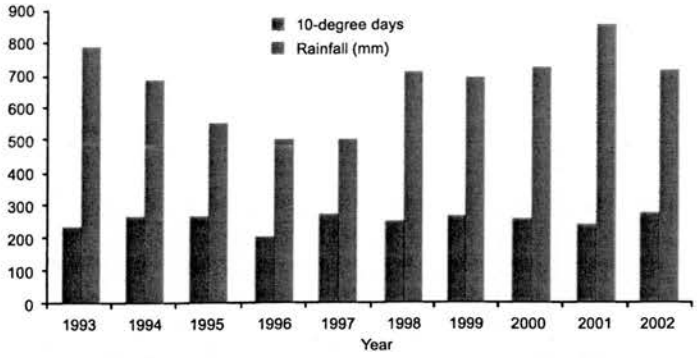

FIG 3: Annual numbers of 10-degree days and annual rainfall in East Anglia between 1993 and 2002

DISCUSSION

Daniel and Mitchell (2002) warned of an increased risk of fasciolosis in Britain in 2002. The subsequent increase was particularly apparent in East Anglia, where the condition had been diagnosed only very sporadically since the 1960s (Holmes 1962), and almost invariably on low-lying, seasonally rented marshland grazing in animals from endemically affected areas. In fact, most livestock farmers in the region and their veterinary surgeons were taken by surprise and, not having previously encountered the condition, were unfamiliar with its treatment and control. By 2003/04, the number of clinical cases had decreased markedly as a result of heightened awareness and the introduction of preventive measures, including the strategic use of flukicides and management practices such as the removal of stock from the wettest fields in the autumn and winter.

The survey method of using composite samples of faeces to detect fluke eggs was surprisingly successful and provides a reasonably sensitive, low-cost monitoring system, particularly for suckler herds. A similar method has been advocated for gastrointestinal nematodes in sheep (Nicholls and Obendorf 1994) and cattle (Ward and others 1997). The bulk milk antibody ELISA provided a much more sensitive method of identifying infected dairy herds. Although its specificity (81 per cent) is much lower than counting fluke eggs, it should provide a useful addition to the range of bulk milk antibody tests that have become available in recent years (Pritchard 2001), and be particularly useful for identifying the infection in naive herds. The low specificity of the test resulted in the prevalence of infection in dairy herds being overestimated by nearly 20 per cent.

Although the survey was not random, it appeared to represent the main distribution of the cattle population in the region adequately. It included 60 (26·4 per cent) of the 227 dairy holdings and 52 (17·7 per cent) of the 294 beef suckler holdings identified by the June 2002 DEFRA national statistics census (unpublished data, DEFRA) as being large enough to be included. The GIS mapping of the fluke-affected farms suggested that they were associated with ESA agreements, which supported observations by Daniel and Mitchell (2002). However, such an association is inevitable in East Anglia because most cattle production, particularly dairying, is based around low-lying ESA areas, such as the Waveney valley, which are unsuitable for cultivation but very valuable for wildlife if grazed and managed appropriately.

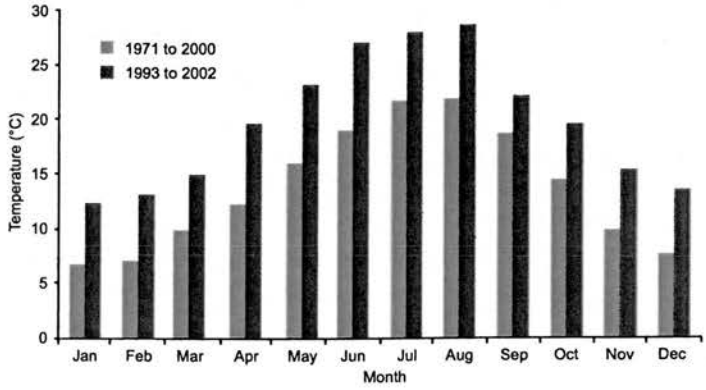

FIG 4: Average monthly maximum temperatures in East Anglia between 1971 and 2000 and between 1993 and 2002

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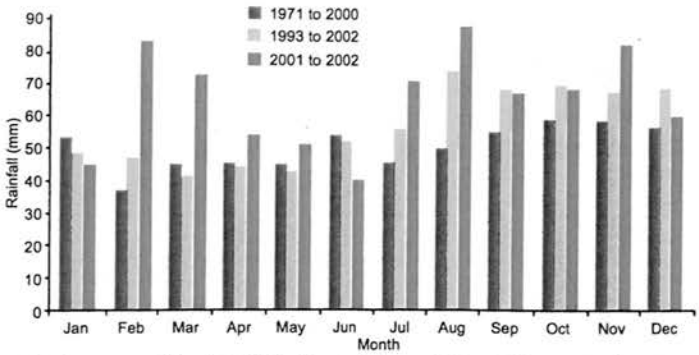

FIG 5: Average monthly rainfall in East Anglia between 1971 and 2000, 1993 and 2002 and 2001 and 2002

Agri-environment schemes, such as the ESA, prescribe land management systems to encourage wetter grassland conditions for breeding and migrating birds and invertebrates; they also maintain suitable habitats for endangered flora. The maintenance of water flow in drainage ditches and dykes, and the associated accumulation of surface water and 'ponding' on adjacent grazing pastures, inevitably favours the reproduction and survival of *L truncatula* and hence *F hepatica*, where it is present. This is particularly significant for herds practising organic methods of production – and increases their reliance on chemotherapeutic rather than husbandry methods of disease control.

Liver fluke infection requires the presence of the parasite, a suitable mammalian host and the intermediate amphibious snail host. The snail population is temperature and moisture-dependent and changes in either factor could affect the availability of snails as potential intermediate hosts. Data produced by the Conchological Society of Great Britain and Ireland (Kerney 1999) indicate that *L truncatula* is widespread throughout East Anglia (Fig 6) and hence its presence is unlikely to be a limiting factor. Temperatures above 10°C and adequate moisture are particularly crucial for the hatching of fluke eggs, the survival of the motile miracidia in search of snail hosts and the movement of cercariae from the snail to the vegetation. There were ample 10-degree days in East Anglia for these processes to take place. The increase in overall and summer rainfall in 2001 to 2002, particularly during July and August (Mitchell 2002), probably led to increased numbers of infective metacercariae on the pastures, and hence an increased risk of infection and disease. The optimal range of temperatures for the development of *F hepatica* on the pasture and in the snail is from 15 to 20°C; the higher temperatures recorded over the past decade may therefore have further contributed to the more rapid development and increased populations of the parasite.

Among domestic stock, sheep and cattle are the parasite's main hosts, and sheep appear to favour most the survival of the adult flukes and their reproduction (Mitchell 2002). During recent years, there has been a distinct increase in the seasonal grazing of sheep rather than cattle on ESAs in East Anglia, and although the changes are not statistically significant from the limited data available, the influx of sheep from the more endemic fluke areas of the country was probably the main means by which the grazing pastures of East Anglia acquired fluke infection. Some wildlife species can also be infected and act as reservoir hosts: the most common examples that may graze livestock pastures are rabbits and deer, both of which are common in East Anglia. Such species may be able to maintain the infection in the absence of cattle or sheep, but it is unlikely that they would account for more than minor local dissemination. *F hepatica* was found in coypu (*Mycocaster coypus*) – now eradicated – in riverside marshland grazings in east Norfolk in the early 1960s (Holmes 1962). It was thought they had acquired the infection when grazing with store cattle, which were then more common than sheep on these marshes.

The potential for fasciolosis to develop in this drier region of Britain has undoubtedly been present for many years. However, the changing weather patterns, the increased seasonal grazing of sheep from endemic fluke areas and the introduction of the ESA schemes appear to have combined to make it a significant problem.

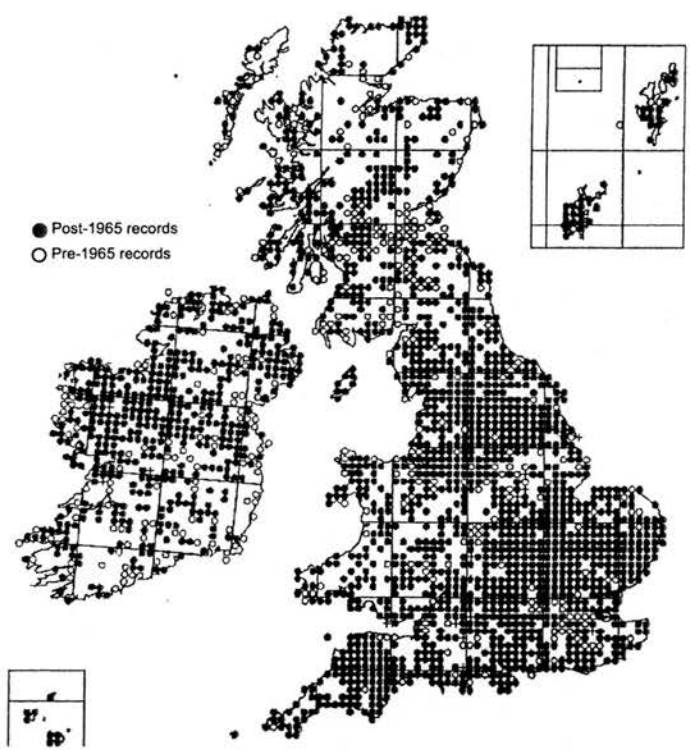

FIG 6: Distribution of *Lymnaea truncatula* in Britain and Ireland. Reproduced from the Atlas of the Land and Freshwater Molluscs of Britain and Ireland by kind permission of Michael Kerney and Harley Books

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance and co-operation of farmers and veterinary surgeons in providing material for this survey, particularly Mike Bardsley who initially drew attention to the problem of fasciolosis in East Anglia. They also thank Richard Scorer, CERA, VLA – Weybridge and the DEFRA RDS East GI Unit, Cambridge, for the GIS map. Bill Nickson, senior adviser of the Broads and Breckland ESA scheme, provided useful comments. Statistical analyses were performed by Robin Sayers, CERA, VLA – Weybridge. Meteorological data were provided by the UK Met Office. The work was jointly funded by Merial Animal Health and by DEFRA through VLA surveillance project ED1000 into endemic disease in cattle.

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Letters

1.60

'Goitre' in goat kids

From Mr G. C. Pritchard

SIR, — I wish to alert colleagues to a condition in goat kids that may be tentatively diagnosed as goitre but which is, in fact, due to a proliferation of thymus tissue.

Affected kids are normal at birth but by 10 to 14 days old they develop a softish, palpable, non-painful, oedematous swelling in the thyroid region of the neck. This shows progressive enlargement and may reach 5 to 6 cm in diameter before spontaneous regression occurs – usually at about four months.

Twenty of 25 Anglo-Nubian and Toggenburg cross kids born during 1987 in a herd in west Norfolk were affected with this condition. Elsewhere, cases of an apparently similar condition in a variety of breeds have recently been described to me by goatkeepers or veterinary surgeons. Because of the small size of most goat herds, cases have mainly involved only one or two animals, but the condition has also been seen in a high proportion of kids in a large herd of Saanens and Angora crosses (A. C. Winter, personal communication). The owner of the Norfolk herd considered that affected kids were quieter than usual and had swallowing and vocalisation difficulties but such clinical features have not been noted elsewhere.

In the affected herd in Norfolk, the condition was initially attributed to goitre but when it proved unresponsive to iodine supplementation a male kid aged about three months was euthanased and necropsied at the veterinary investigation centre, Norwich. The thyroid gland was normal and in fact somewhat difficult to find, but the thymus was grossly enlarged and most of the extrathoracic portion – weighing in excess of 200 g – was located in the upper neck region.

Goitre seems to be fairly rare in ruminants in Britain unless goitrogens are present although Dunn (1982) illustrated a case in a goat kid which appears identical to the condition described here. There are several other references in the literature to goitre in goat kids but, as pointed out by Baxendell (1980), enlarged salivary or thymus glands could be mistaken for enlarged thyroids.

My inquiries lead me to suspect that many cases of goitre diagnosed in goat kids in Britain and perhaps elsewhere may also have been thymic hyperplasia. Since the thymus regresses by about four months of its own accord, it may appear that the resolution has been due to iodine supplementation. Such goats are not normally ill and it is unlikely that post mortem evidence will be available for purposes of differential diagnosis. I would be most interested to hear from colleagues who have encountered similar cases and who have views about this condition.

Investigations are currently in progress, with the cooperation of colleagues at the Moredun Research Institute, Edinburgh, to determine whether any pathological processes are involved in the condition. The thymus tissue examined to date has been histologically normal and so far all laboratory examinations have produced negative findings.

During the course of the present investigations it became apparent that proprietary rations for goats often contain iodine concentrations in excess of suggested requirements (Agricultural Research Council 1984). Also, as in the affected herd in Norfolk, goats are often supplemented with extra iodine in the form of mineral licks or additives. Hence, it is of some concern that additional unnecessary administration of iodine could lead to iodine toxicity.

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Norwich

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A22

THYMIC ENLARGEMENT IN GOAT KIDS

G C Pritchard,* Veterinary Investigation Centre, Norwich.

Twenty of the 25 kids born in a West Norfolk goat herd in 1987 developed a softish palpable non-painful, oedematous swelling in the upper neck or submandibular region by 10-14 days of age. The worst affected kids experienced vocalisation and swallowing difficulties. The swellings regressed - apparently spontaneously - at about four months of age although slight swelling persisted for several months afterwards in some kids. Necropsy of affected goats revealed gross enlargement of the thymus gland, particularly the extra-thoracic portion. Histologically the thymus was normal although there appeared to be some nodular expansion in the medullary areas. Further enquiries suggested that this, or an apparently similar, condition, is quite common in kids in Britain. It is usually tentatively diagnosed as "goitre" and iodine supplementation is often provided. Excluding the Norfolk herd, the condition appears to be of no clinical significance but is of concern to owners particularly if valuable animals, such as Angoras, are involved. It is also of concern that excessive iodine supplementation in such cases could result in toxicity problems. Extensive laboratory investigations have so far failed to indicate the aetiology of the condition.

ACKNOWLEDGEMENTS

Colleagues at the Moredun Research Institute, Edinburgh collaborated in the investigations; additional laboratory support was provided by Newcastle Veterinary Investigation Centre and various departments at the Central Veterinary Laboratory, Weybridge.

THROAT SWELLINGS IN GOATS

1.62

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INTRODUCTION

This paper is essentially a case history concerning a form of throat swelling currently under investigation in a herd in Norfolk (Pritchard, 1987) however reference is also made to the differential diagnoses of throat swellings in goats.

THE HERD

Mid-Norfolk smallholding with 10 adult female goats (mainly Anglo-Nubian but including Toggenburgs and Saanens) and an Anglo-Nubian stud male. A total of 25 kids were born during 1987 to either February/April kiddings or to two later kiddings in July. Feeding and management were conventional and of a high standard. A proprietary coarse ration was fed together with crushed oats, bran, flaked maize, beet pulp, hay and mineral blocks. Seasonal grazing. The goats were kept in a wooden purpose-built boarding with separate pens.

DISEASE HISTORY

There were no significant disease problems until 1985 when three of five male kids all showed an enlargement of the neck region from about seven days of age which persisted until they were slaughtered at 6-9 months. No kids were born during 1986 but in May 1987, 18 of 20 kids were seen to have developed similar lesions. The swellings showed spontaneous regression at about four months of age. Twin kids born to the July kiddings were also affected but triplets born the same month were normal.

CLINICAL FINDINGS

All affected kids were normal at birth but subsequently developed a softish palpable non-painful oedematous swelling – generally bilateral – in the thyroid region of the neck from about 7-10 days of age. The swelling showed progressive enlargement and in the worst affected cases appeared to result in swallowing difficulties, altered vocalisation and general depression. Regression almost invariably occurred – apparently spontaneously – at about four months of age and most of the oedema subsided leaving loose skin under the neck where the swelling had been. Figure 1 shows the typical appearance of affected kids aged about three months.

DIFFERENTIAL DIAGNOSES OF THROAT SWELLING IN GOATS

The main differential diagnosis for throat swellings in goats – such as was considered for this present condition – is presented below:-

1. **BACTERIAL INFECTION** – including vaccination abscesses and caseous lymphadenitis due to *Corynebacterium ovis* (very rate or? not seen in Britain).

2. **THYROID ENLARGEMENT** – due either to iodine deficiency (goitre) or iodine excess (Driscoll and others 1978).

3. **LYMPHOSARCOMA** (Higgins and others 1984).

4. **SALIVARY GLAND LESIONS**

5. **THYMUS ENLARGEMENT – Thymoma** (Moulton and Dungworth 1978) Thymic hyperplasia/non regression of the thymus.

IODINE ESTIMATIONS

The condition seen in the Norfolk herd appeared grossly similar to the thyroid enlargement illustrated by Dunn (1982) and was initially tentatively attributed to iodine deficiency goitre. Potassium

iodine solution was adminstered by mouth but in view of lack of response the owner's veterinary surgeon sought assistance from the Veterinary Investigation Centre, Norwich. Subsequently blood and milk samples were taken for iodine estimations together with a sample of the coarse goat ration. The findings (Table 1) clearly indicated that there was no evidence of iodine deficiency although an excess appeared to be a possibility.

TABLE 1: IODINE ESTIMATIONS

	FEED (mg/kg/dm)	MILK (μ/1)	SERUM THYROXINE (Total T4) nmol/1
Concentration Found	Coarse goat mix – 4.46 (3.5 declared)	850, 970	80 – 130 (most >100)
Normal or suggested*	(Herbage is usually 0.2 – 0.3 Outside – 0.15 Inside – 0.5	40 – 50	43 – 90
Concentrations (mainly cattle & sheep data)	Goitrogens Present – 2.0 (maximum suggested safe levels 8 – 20 mg/kg)	(if >25 in cattle advise supplementation	

*ARC (1984) & MAFF/DAFS/UKASTA/BVA WORKING PARTY

POST MORTEM FINDINGS

The cause of the condition remained unresolved until the owner agreed to cull one of the affected male kids. At necropsy, the throat swelling was found to be due to gross enlargement of the thymus (Figure 2) with most of the extra thoracic portion – weighing in excess of 200 grams – being located in the upper neck region. The thymus tissue was histologically normal.

Two other affected kids were subsequently post mortemed and both were found to have similar proliferation of thymus tissue. One of those examined at about four months of age – after apparent regression – had somewhat less thymus tissue than the first case examined but, including the intrathoracic portion, it still weighed nearly 170 grams. A three month old kid necropsied from the later kiddings had 115 grams of thymus tissue of which 31 grams was located ventral to the lower jaw.

The anatomical distribution of thymus tissue in affected kids post mortemed during the investigation is represented in Figure 3.

LABORATORY EXAMINATIONS

Further examinations were performed on post mortem material and on blood samples collected from animals of all ages in the herd. These included bacteriology, virology, haematology, biochemistry and serology (caprine arthritis encephalitis, enzootic bovine leucosis, Border disease, Infectious Bovine Rhinotracheitis and *Toxoplasma gondii*) all with **negative** results. Further tests are currently in progress.

DISCUSSION

At the time of writing (November 1987) the cause of the enlarged thymus condition (thymic hyperplasia) is unresolved and of necessity this is only an interim account.

During recent months, numerous colleagues and goat owners have informed me that they have encountered an apparently similar condition. In most cases the condition is tentatively attributed to goitre and there is an apparent response to iodine supplementation. However, since the thymus normally regresses by about four months of age of its own accord it is evident that the resolution could be wrongly attributed to iodine supplementation. However, since the thymus normally regresses by about four months of age of its own accord it is evident that the resolution could be wrongly attributed to iodine supplementation.

Investigations are currently in progress with the assistance of colleagues at the Moredun Research Institute, Edinburgh, to determine whether any pathological processes are involved. I would be pleased to hear from any veterinary surgeons or goat owners who have encountered the condition or who have any useful comments to add.

During the course of the present investigations it was apparent that proprietary rations for goats often contain iodine concentrations in excess of suggested requirements (Agricultural Research Council 1984). Also, as in the affected herd in Norfolk, goats are often supplemented with extra iodine in the form of mineral licks or additives. Hence it is of some concern that additional unnecessary administration of iodine could lead to toxicity.

ACKNOWLEDGEMENTS

The assistance of the owner and veterinary surgeon concerned in the case is gratefully acknowledged. Newcastle Veterinary Investigation Centre carried out the Thyroxine (T4) estimations; the feed and milk iodine estimations were performed by the Nutrition Chemistry Department of ADAS, Trawsgoed. The photographs at Figures 1 and 2 were taken by Mr Chris Gilbourne who also assisted with on-farm investigations.

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Postcript

Since writing this paper, I have received further reports of what appears to be the same condition occuring in goat herds throughout Britain, with no obvious breed predisposition.

Investigations are still in progress with the assistance of colleagues at the Moredun Research Institute, Edinburgh to determine whether any pathological processes are involved. Thymic hyperplasia has been observed as a "normal" occurence over the past two years in the Moredun goat herd of Anglo-Nubian and Anglo-Nubian X Toggenburg goats. 1-3 week old kids of both sexes develop a soft linear tissue swelling around the trachea which extends from the thoracic inlet to the submandibular area. Despite the submandibular swelling being more pronounced in some kids than others, no clinical signs of dysphaga or altered vocalisation have been observed. Samples of the tissue have the histological appearance of normal thymic tissue which spontaneously regresses when the kids are 6-9 months of age.

Editor's note

A similar condition occurs in British Alpine kids.

Figure 1 Goat kids with thymic hyperplasia

Figure 2 Post mortem appearance of enlarged thymus tissue

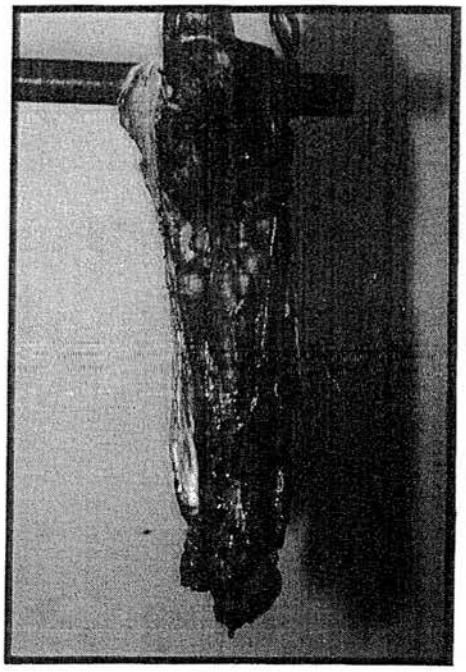
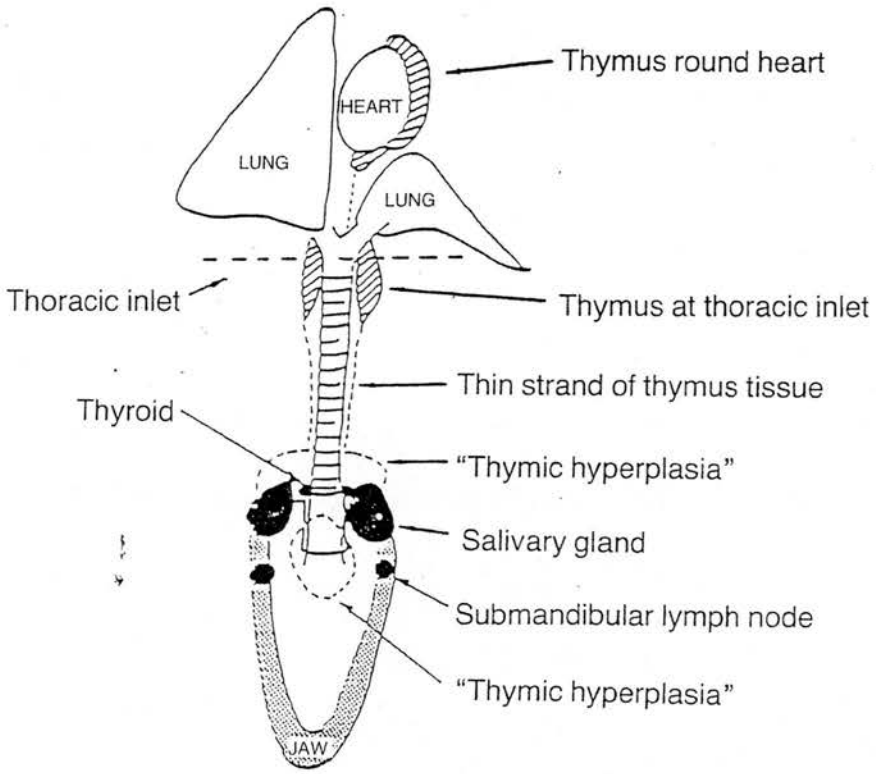


Figure 3 Diagram to show the location of thymus tissue in affected goat kids



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FUNGAL MASTITIS IN A GOAT DUE TO INFECTION WITH *PAECILOMYCES VERIOTI*

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This is a summary of the paper presented at the meeting. A unilateral mastitis in a British Alpine goat caused by the fungus *Paecilomyces varioti* is described.

The goat was served at about 17 months of age and for a month or two before service the affected half of the udder had yielded a little milk which occasionally contained clots. The affected udder was treated several times with intramammary antibacterial antibiotics but the clots in the milk persisted and increased during the three months before kidding and firm palpable nodules appeared in the affected half. A bacteriological examination of the milk during that period did not yield a pathogen. The mastitis increased in severity immediately after kidding and infection by *P. varioti* was demonstrated in several subsequent samples of milk even though the clinical abnormality declined progressively over the ensuing four to five months without any specific treatment.

P. varioti is a common saprophyte on silage and other vegetable material and it infects animals or man very rarely. Also, mycotic mastitis has been recorded in the goat only rarely although mastitis caused by filamentous fungi from the environment has been demonstrated on many occasions in cattle. In bovine cases, the course of disease usually has been similar to that seen in this goat:- prolonged, but with a slow spontaneous resolution of the focal lesions until these become clinically insignificant. The infection may remain active for months or even years, however, and the fungus can still be detectable in the milk.

Laboratory confirmation of this type of mastitis cannot be based reliably upon culture alone. A direct, and sometimes lenghty, microscopic examination of the milk is essential. Samples from uninfected animals are contaminated easily by the air-borne spores of potentially pathogenic fungi and these may grow in culture. Conversely, some infected samples fail to yield an isolate as the clinical disease becomes less acute. Fungi may be slow to grow on some media or be inhibited by the presence of some bacteria. *P. varioti* grew poorly on blood agar and was evident only when the cultures were incubated for more than two days. However, the use of appropriate techniques may disclose more instances of fungal infection among cases of mastitis in the goat for which no other cause can be demonstrated.

The route of infection and the factors which may lead to its occurence were discussed briefly. When intramammary administrations of antibiotics preceded laboratory demonstration of fungal infection , it is uncertain whether the initial mastitis was mycotic or whether the mycotic infection was introduced in the process of treatment and enhanced by the antibacterial preparation used.

OSSIFYING FIBROMA IN AN ANGLO-NUBIAN GOAT

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In November 1983, the head of a five-year-old Anglo-Nubian female goat was submitted to the Veterinary Investigation Centre, Norwich, for examination. The goat had been destroyed because of the rapid growth of a soft, solid, unilateral swelling affecting the maxilla. Apparently the lesions had developed over a period of only two to three weeks. There had been no response to antibiotic medication and the goat had begun to experience difficulty in mastication.

Dissection of the skin and tissue over the swelling revealed a sharply demarcated, solitary tumour about the size of a tennis ball . The tumour was of soft fibrous consistency and pinkish-white in colour. When removed it weighed approximately 300 gms . The tumour mass had replaced much of the bone of the upper jaw and the teeth were embedded within the rubbery mass. There was an area of ulceration in the mouth associated with displacement of the teeth.

Histopathology showed the lesion to consist of numerous osteoid spicules randomly dispersed in loose fibrous tissue. Osteoblast differentiation was evident around some spicules and there was focal osteoclast differentation. The lesion was diagnosed as an ossifying fibroma. Pool (1978) described this lesion as a rare tumour of horses, ruminants and possibly other animal species.

It was of interest, although probably of no significance, that the goat's four-month-old kid had been euthanased two months previously having developed what was diagnosed clinically as lymphosarcoma. Apart from this there is no other familial history of neoplasia.

Acknowledgements

The author wishes to thank Mr. R.A. Clarke, M.R.C.V.S., for submitting this specimen. Histological examinations were carried out by Dr. I.F. Keymer, Veterinary Investigation Centre, Norwich, and by Mr. G.A. Well, Pathology Department, Central Veterinary Laboratory, Weybridge.

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Papers and Articles

Natural scrapie in goats: case histories and clinical signs

J. N. L. Wood, S. H. Done, G. C. Pritchard, M. J. A. Wooldridge

Veterinary Record (1992) **131**, 66-68

The case histories of the 20 goats affected with natural scrapie which have been examined since 1975 at the Central Veterinary Laboratory, Weybridge, are reviewed. Their ages ranged from two to seven years (median three years, four months) and 85 per cent of them were between two and four years old. The most common clinical signs were hyperaesthesia, ataxia and pruritus. The histories indicated that scrapie can occur in goats which have not been in contact with sheep.

SCRAPIE is one of the group of diseases collectively referred to as subacute transmissible spongiform encephalopathies that includes kuru (Hadlow 1959), transmissible mink encephalopathy (Burger and Hartsough 1965) and chronic wasting disease of mule deer and Rocky Mountain elk (Williams and Young 1982). Since 1986, cattle and several species of ungulates in zoos in the United Kingdom have also been affected with a spongiform encephalopathy which has been attributed to the feeding of meat and bone meal contaminated with a scrapie-like agent (Wells and others 1987, Wilesmith and others 1988, Kirkwood and others 1990).

Although scrapie has been recognised in sheep for more than 200 years (Parry 1983) it was not until 1942 that its natural occurrence was first reported in goats (Chelle 1942). Since then only a few reports of natural scrapie in goats have appeared. Most of them record contact with scrapie-affected sheep (Chelle 1942, MacKay and Smith 1961, Hourrigan and others 1969, Stemshorn 1975, Toumazos and Alley 1990) but some do not (Harcourt and Anderson 1974, Hourrigan and others 1979, Fankhauser and others 1982, Garcia del Salon and others 1991) and scrapie has occurred in goats isolated from sheep (Hourrigan and others 1979).

The similarity of the results of assays of infectivity in the extraneural tissues of sheep (Hadlow and others 1982) and goats (Hadlow and others 1980) suggest that the scrapie agent might be passed from goat to goat in the same way that it can be passed from sheep to sheep, or from sheep to goat.

This paper describes the histories and clinical signs of all the cases of scrapie in goats diagnosed at the Central Veterinary Laboratory, Weybridge, during the last 30 years. Possible sources of infection and the literature on the epidemiology of scrapie in the goat are briefly discussed.

Materials and methods

The 20 submissions of caprine central nervous systems received at the pathology department since 1975 which recorded a diagnosis of scrapie were reviewed histologically. No cases of scrapie in goats were submitted between 1960 and 1974. The pathological features and case definitions are described in a separate paper (Wood and Done 1992).

Whenever possible, the cases were followed up through the referring veterinary investigation centres, veterinary surgeons and owners. Information was sought concerning the clinical signs, the fate of close relatives and any other goats in the same herd, and the feeding histories and other possible sources of infection, such as the presence of scrapie in any in-contact sheep or goats. In some cases, the time lapse and multiple ownership meant that a complete follow-up was not possible, and the records were often less detailed than those required for a full investigation.

Results

Clinical features

The diagnosis of scrapie was reconfirmed in all 20 cases, which originated from 18 herds. The goats died when they were between two and seven years old (median three years and four months), and 85 per cent of them were two to four years old (Table 1). The duration of clinical signs ranged from two to 24 weeks (median eight weeks). The frequencies of the different clinical signs are shown in Table 1. In one goat (J) the only obvious clinical sign was regurgitation of ruminal contents. Objection to being milked was often the first observed behavioural change in the goats that were lactating, although the number lactating was not known. Some practitioners suggested that hyperaesthesia was more pronounced in goats than in sheep.

The ages at death, the sex and herd descriptions of individual cases are shown in Table 2. There were many different breeds and crossbreeds of goat. If movement between herds had occurred, but details of the earlier herds could not be determined, then 'not known' was recorded. Where contact with sheep could not be excluded, but was not known to have occurred, 'not known' was recorded. Contact with sheep is recorded as 'negative' only if no contact with sheep took place during the goat's life, either directly or indirectly through the pasture. Scrapie in in-contact goats was recorded as 'positive' only if it was confirmed by a histopathological examination of the brain; it was recorded as 'probable' if any animal had a clinical history which was suggestive of scrapie, but the condition had not been confirmed post mortem.

Additional information

Goat C was the sixth member of a family line to die, each having shown clinical signs of scrapie when more than two years old. They died over a period of 10 years.

Goats D and E were two of a litter of triplets; the third littermate and the dam both died showing clinical signs of scrapie, but their brains were not examined microscopically. The dam died five months after the kids were born.

The dam and sire of goat I (both eight years old) and its twin sibling (five years old) were all alive and healthy some two years after goat I died. It never had contact, either directly or indirectly, with any sheep, or with any goats with clinical signs suspicious of

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TABLE 1: Frequencies of different clinical signs among 20 goats with natural scrapie

Clinical sign	Frequency
Ataxia	15
Visual impairment	3
Difficulty in milking	4
Dribbling/regurgitation	7
Hyperaesthesia	13
Loss of condition	7
Muscle fasciculation/tremor	4
Pruritus	11

TABLE 2: Epidemiological features of 20 goats with natural scrapie

Goat	Year of death	Age (years, months)	Sex	Contact with sheep	Scrapie in in-contact goats
A	1975	NK	F	NK	NK
B	1984	4	F	NK	NK
C	1984	2	F	NIK	Probable
D	1984	3,4	F	NK	Yes
E	1984	3,5	F	NK	Yes
F	1984	2,6	F	Yes	NK
G	1985	5	F	NK	NK
H	1987	4	F	NK	NK
I	1989	3,9	F	No	No
J	1989	2,6	F	Yes	No
K	1989	4,6	F	No	Probable
L	1990	2,10	F	No	NK
M	1990	7	F	NK	Probable
N	1989	2,11	M	No	Yes
O	1990	2,7	F	No	Yes
P	1990	3,6	F	Yes	NK
Q	1990	3	F	No	NK
R	1989	4	F	NK	NK
S	1989	NK	F	NK	NK
T	1990	3	F	No	Yes

F Female, M Male, NK Not known

scrapie. It had been fed a concentrate ration which included meat and bone meal from three months of age in March 1986 until July 1988.

The sire of goat J was killed when five years old and its dam was killed when 14 years old; neither of them showed signs of scrapie. All the other traceable siblings were healthy. Indirect contact with sheep had occurred in the first six months of goat J's life, through pasture only. It had been fed a concentrate containing meat and bone meal during its first six months of life, which began in February 1987.

The dam of goat K died of dystocia without showing signs of scrapie; no other goats in the breeding unit ever showed clinical signs suggestive of scrapie. The goat had never had contact with sheep. Its only animal contact from three months of age was with an unrelated goat of the same age. This in-contact goat died, having been ataxic for two months, shortly before goat K became sick, but no diagnosis was made. From May 1985, when they were both two months old, they were fed a concentrate containing meat and bone meal for more than two years.

Both parents (now aged over six years) of goat N were still alive 20 months after it died. At no time was it in contact with sheep, or with any other goat showing signs of scrapie. From its birth in the summer of 1986 until July 1988, goat N was fed a concentrate containing meat and bone meal.

The dam of goat O is alive and five and a half years old. Its sire was goat N. Its twin sibling died of an unrelated disease. The goat had no contact with sheep. It was fed a concentrate containing meat and bone meal from weaning in the spring of 1988 until July 1988.

In the cases for which definitive information could be obtained direct or indirect contact with sheep had occurred in three of seven goats. Contact with goats with suspected or confirmed scrapie had occurred in eight goats, and seven of these in-contact goats were related.

Discussion

In contrast to the extensive literature on scrapie in sheep, very little has been published on the naturally occurring disease in goats. In the 30 years that cover this series, 12 of the 20 cases occurred in 1989 and 1990. There has recently been an increase in the numbers of goats kept in the United Kingdom (MAFF June Agricultural Census, 1988 to date) and efforts were made to obtain material for this study; it is not clear whether the apparently high incidence in these two years reflects a real increase in the incidence rate.

The clinical signs shown by the goats were broadly similar to those previously described (Hadlow and Eklund 1968); hyperaesthesia, ataxia (especially of the pelvic limbs) and puritus were the most common. Regurgitation of ruminal contents was the only obvious sign in goat J, and this has been recorded previously only as a late sign of the disease in goats (Hadlow and Eklund 1968). In lactating goats, the first sign was often a reluctance to permit milking. Similar milking difficulties are encountered in dairy cows with bovine spongiform encephalopathy (BSE) (Wilesmith and others 1988). Severe loss of weight appeared to be uncommon, whereas loss of weight is common in sheep with scrapie.

The ages of the goats in this study have been combined in Fig 1 with those reported in the literature (Chelle 1942, MacKay and Smith 1961, Hourrigan and others 1979, Fankhauser and others 1982, Toumazos and Alley 1990, Perrin and others 1991, Veterinary Investigation Service 1991). The age distribution is similar to that reported in sheep (Parry 1983).

Scrapie is an infectious disease, and in sheep maternal transmission may play a major role in its spread (Kimberlin 1990). However, in sheep there is a genetic influence on the incubation period, and the disease may appear to behave as a purely hereditary condition controlled by a homozygous recessive gene (Parry 1983). In contrast, in experimentally infected goats, no genetic influence is apparent (Pattison and others 1959, Hunter and others 1989). There are no reports of maternal transmission of scrapie in goats, although goats may be affected with scrapie even when kept apart from sheep (Hourrigan and others 1979). Hadlow and others (1980) concluded that scrapie could maintain itself in goats kept apart from sheep. Other postulated sources of infection are intermediate hosts (Pàlsson 1979) or subclinically infected carriers (Kimberlin 1990).

The oral route has been shown to be effective in the transmission of scrapie to goats (Pattison and Millson 1961) and this route has been proposed for both BSE (Wilesmith and others 1988, 1991) and transmissible mink encephalopathy (Burger and Hartsough 1965). Goats can be infected by close contact with sheep or goats suffering from naturally occurring scrapie (Hourrigan and others 1979). A similar lateral spread of scrapie in sheep has been reported to occur after animals have been confined closely together, as when they are housed during the winter (Kimberlin 1990), and infection through contaminated pasture has also been observed (Greig 1950). Other routes of infection have

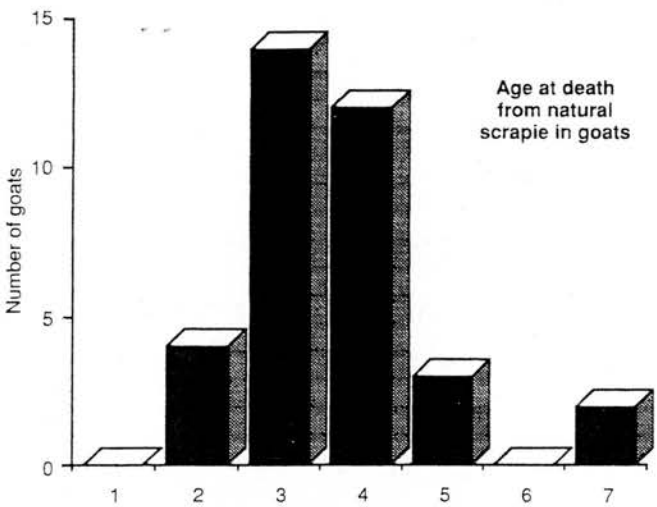

FIG 1: Age at death from natural scrapie in goats

also been reported, for example, by the administration of a contaminated vaccine (Gordon 1946).

Goats I and N had not been in contact with any sheep or scrapie affected goats but both of them had been fed from 1986 to July 1988 on feed containing meat and bone meal. During this period meat and bone meal has been implicated in the BSE epidemic (Wilesmith and others 1988, 1991) and also in the disease in zoo ungulates (Kirkwood and others 1990). It is therefore possible that these two goats were infected by eating meat and bone meal. It has been established that goats are susceptible to the agent of BSE (J. D. Foster and H. Fraser, personal communication).

In 13 of the 20 goats it was not possible to exclude sheep as a source of infection. Of the remaining seven goats, I and N are discussed above, and for L and Q no further information was available; goats K and O were also fed meat and bone meal and full information was not available for the last of the seven.

The authors consider that further studies are necessary on the epidemiology of natural scrapie in goats, especially to investigate the mode of contagion. Furthermore, attempts should be made to sequence the PrP gene of goats suffering from natural scrapie and compare it with the gene in healthy controls, as has been done with great benefits in the study of Creutzfeldt-Jakob disease (Brown and others 1991).

Acknowledgements. — Colleagues in the Veterinary Investigation Service, other veterinary surgeons and the goats' owners were very helpful in this investigation. Especial thanks are due to Dr W. J. Hadlow for encouragement and for much help. Thanks are also due to Mr J. W. Wilesmith, Mr R. Bradley, Professor R. M. Barlow, Dr H. Fraser and Dr R. H. Kimberlin for help with the manuscript.

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1.66

COMMON DISEASES ARE COMMON

A View from the Veterinary Investigation Centre

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INTRODUCTION

The information presented here is based on the Veterinary Investigation Diagnosis Analysis Database (VIDA) for the period 1993 - 1995. VIDA has been operating since 1975 and includes data from the Veterinary Investigation Centres (VICs) in England and Wales, and those in Scotland. In 1993 the VIDA codings for goats were separated from the miscellaneous species grouping and a range of new diagnoses codes were added, which were not previously available in goats. The total numbers of submissions recorded by VIDA represent only the material submitted to the participating laboratories for investigation and this is influenced by many factors including, for example, the particular clinical presentation of a suspected disease, the level of awareness of a disease and its perceived importance, the value of the animal or animals affected and the general economic climate. Despite the in-built bias VIDA provides useful information on disease trends particularly for those conditions that lend themselves to laboratory investigation

As shown in the histograms presented, diagnoses are subdivided into specific categories in which the relative frequency of each diagnosis can be readily demonstrated. It should be borne in mind that the diagnostic criteria are not clearly defined so the results can only give a general indication and may not stand up to detailed retrospective scrutiny. Since an insufficient number of diagnoses were made to show any meaningful annual trends between 1993 and 1995 the findings have been combined for this presentation.

GENERAL COMMENTS

Relative numbers of submissions by type for goats – overall the number of submissions received by VICs from goats is relatively small and dominated by serological examinations for caprine arthritis encephalitis (CAE) virus infection. Faeces samples, for parasitology and possibly Johne's disease examinations represent the next most common submission.

Systemic disease and those not readily classified organically – this category is dominated by hypocupraemia/hypocuprosis and staphylococcal infection. Surprisingly, perhaps, it is not uncommon to detect low blood copper concentrations in goats and swayback is also occasionally diagnosed. Clostridial disease is encountered frequently but it is more relevant to consider this under diseases of the digestive system. Compared with other species neoplasia (cancers) is a not infrequent diagnosis and reflects the fact that many goats examined are pets and may be kept until a later age than other farm animal species.

Diseases of the digestive system – as might be expected, the main diagnosis here is parasitic gastroenteritis followed by *Clostridium perfringens* type D enterotoxaemia, coccidiosis and Johne's disease. Johne's is certainly a common problem in goat herds.

Diseases of the respiratory system – pasteurellosis, predominantly associated with *P.haemolytica* infection, is the most commonly diagnosed cause of pneumonia in goats, closely followed by parasitic infection.

Diseases of the urinary system – there have only been very few incidents, most of which have been classified as 'nephrosis not otherwise specified'. Urinary tract disease is fairly uncommon in goats and it is difficult to reach any conclusions from the VIDA data.

Disease of the musculo-skeletal system – again there are very few diagnoses in this category which is dominated by CAE. Whether these all represent clinical cases of CAE or whether they are goats – possibly lame – which are seropositive for CAE is not clear. White muscle disease is the next most common diagnosis in this category but again numbers of cases are very low.

Diseases of the nervous system and organs of special sense – scrapie, swayback and cerebrocortical necrosis (CCN) are all diagnosed occasionally, again in small numbers. The most common diagnosis in this group however is encephalitis associated with *Listeria* sp. infection. It would be interesting to know the feeding practices in affected herds and whether it is mainly associated with silage feeding as in sheep.

Diseases of the skin – ectoparasitism, particularly chorioptic mange, is the main diagnosis here. It is interesting to note that ringworm appears to be more common in goats than sheep.

Diseases of the mammary system – mastitis associated with *Staphylococcus aureus* infection is not unexpectedly the most commonly diagnosed condition followed by the isolation of coagulase negative staphylococci and staphylococci not specified. *Pasteurella haemolytica,* which is a common cause of mastitis in the ewe, does not appear to be as prevalent as staphylococcal mastitis.

Diseases of the reproductive system – abortion associated with toxoplasmosis is by far the most common condition, followed by abortions associated with chlamydia and *Listeria monocytogenes* infection. There seems little doubt that toxoplasmosis is very common in goats, probably reflecting husbandry conditions and the close relationship with cats which is often seen.

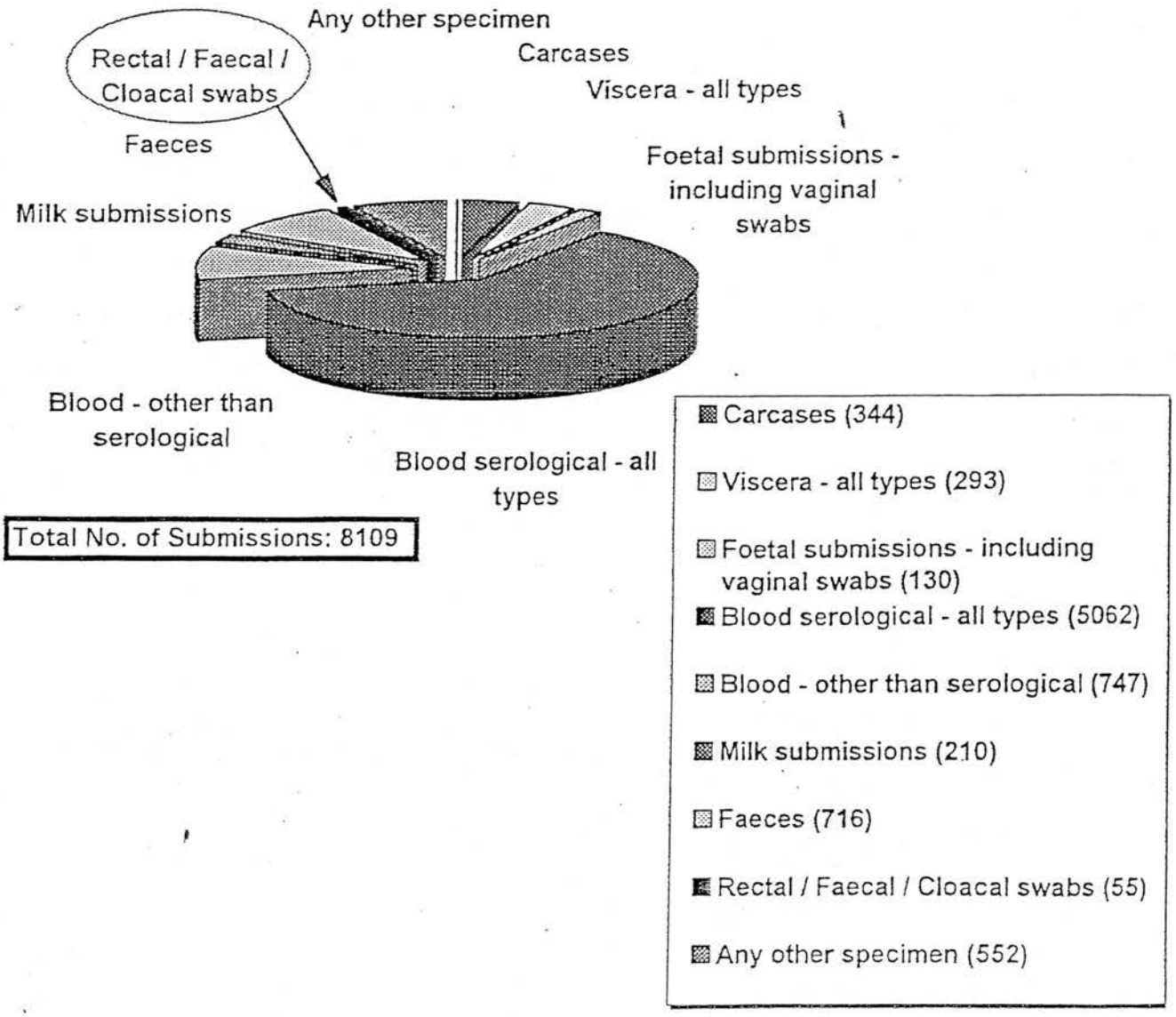
CONCLUSIONS

Whilst VIDA provides only very limited and somewhat biased data concerning the relative incidence of goat diseases it nevertheless gives a reasonable indication of what diseases are commonly diagnosed in the laboratory. The findings detailed here should be of value to veterinary surgeons when confronted with the conditions falling within the categories listed by VIDA – remember the truism 'common things are common'.

ACKNOWLEDGMENTS

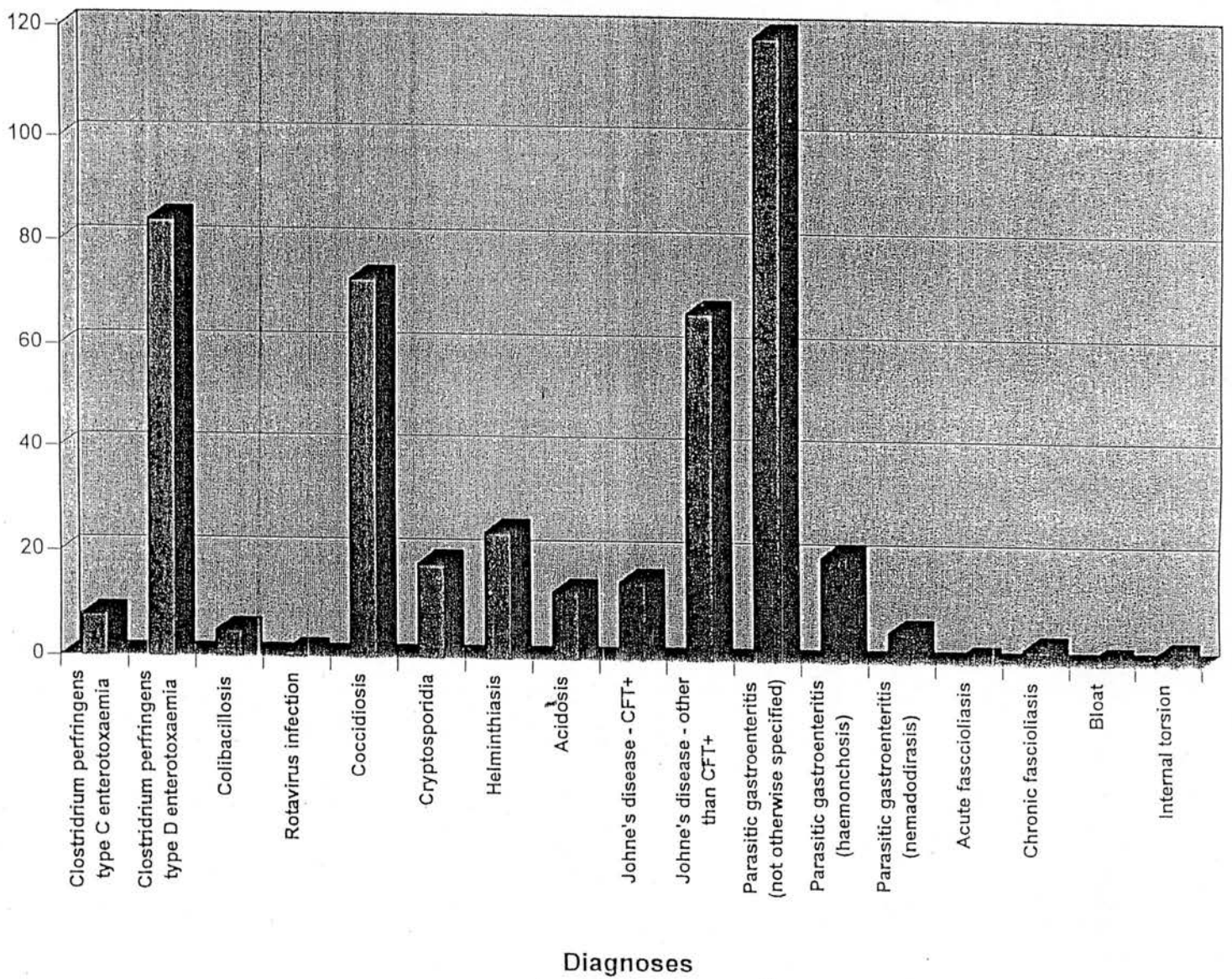
I acknowledge the help of Peter Warren from the Epidemiology Department at the Central Veterinary Laboratory in providing the VIDA data and figures for this presentation.

RELATIVE NUMBERS OF SUBMISSIONS BY TYPE FOR GOATS (VIDA 1993-95)

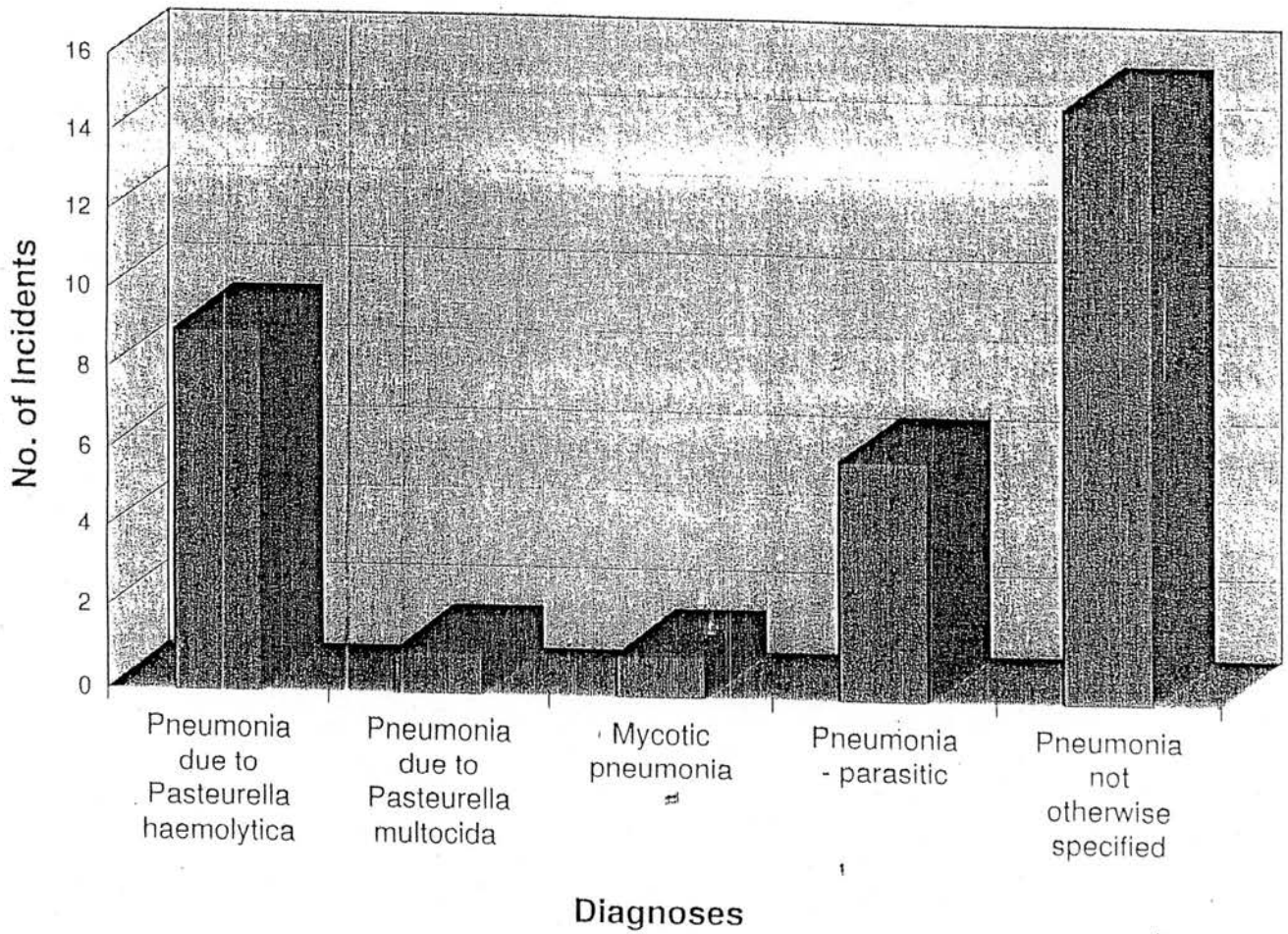


RELATIVE INCIDENCE OF SYSTEMIC DISEASES AND DISEASES NOT READILY CLASSIFIED ORGANICALLY IN GOATS (VIDA 1993-95)

RELATIVE INCIDENCE OF DISEASES OF THE DIGESTIVE SYSTEM IN GOATS (VIDA 1993-95)



RELATIVE INCIDENCE OF RESPIRATORY DISEASES IN GOATS (VIDA 1993-95)



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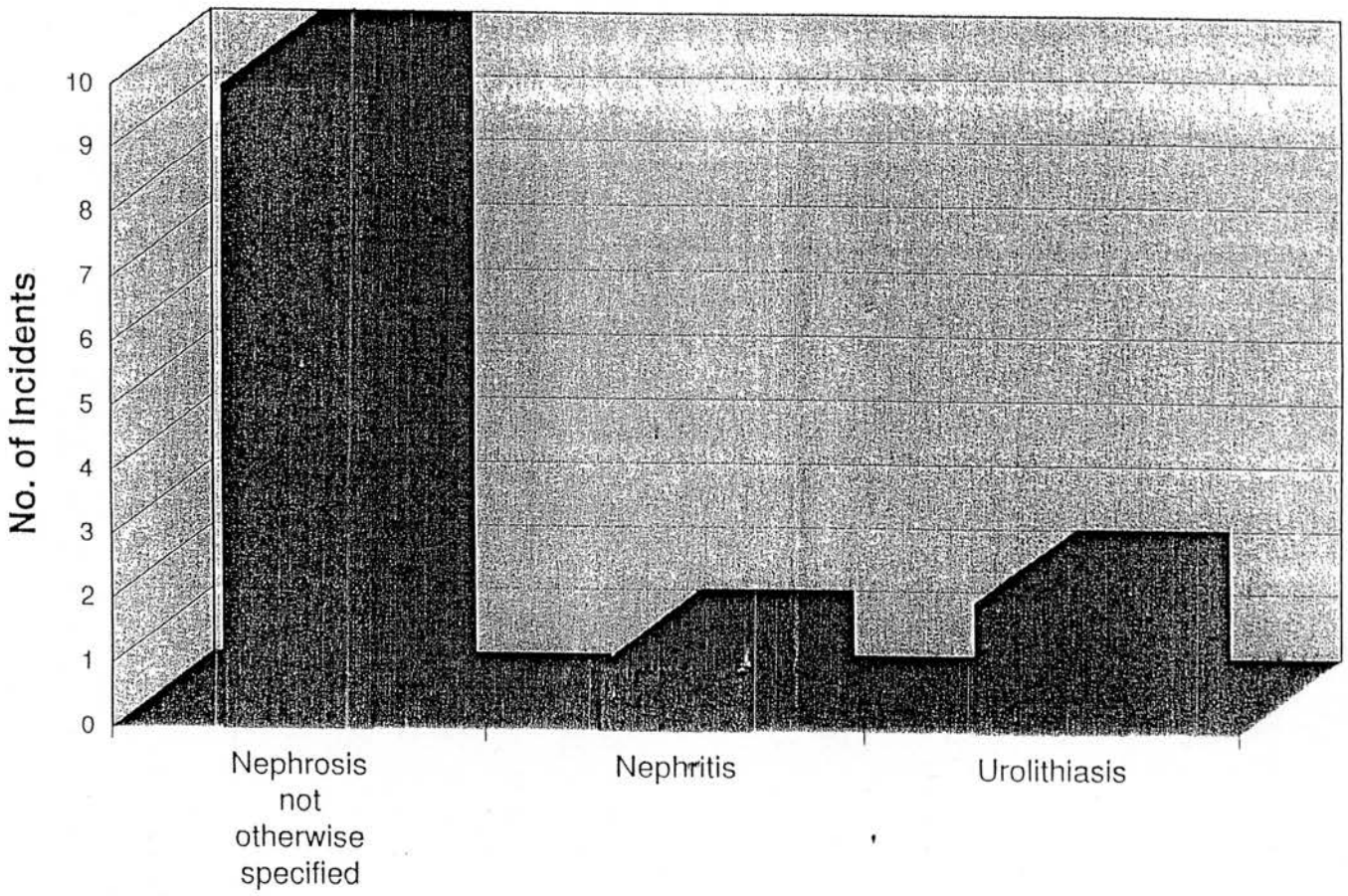
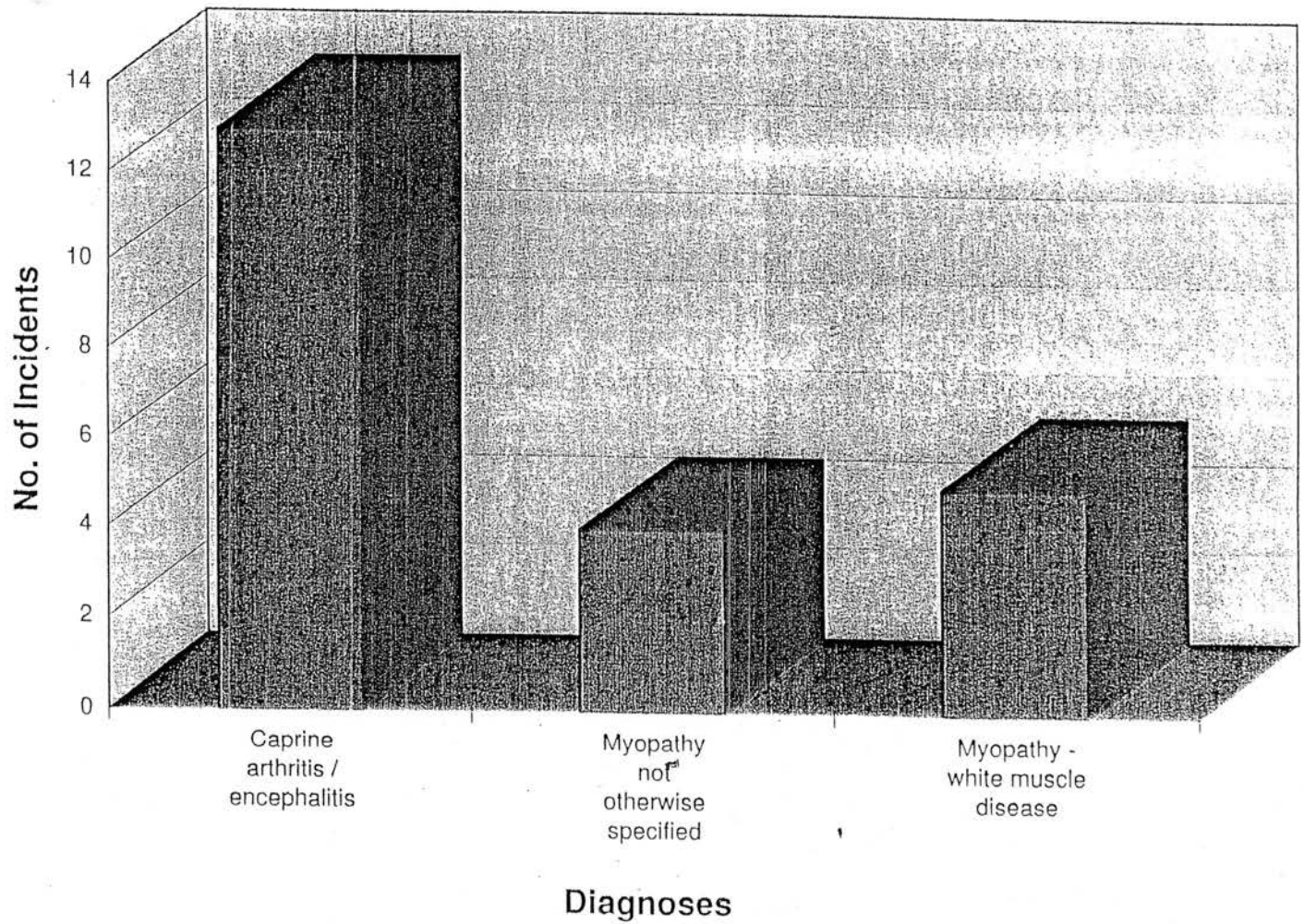
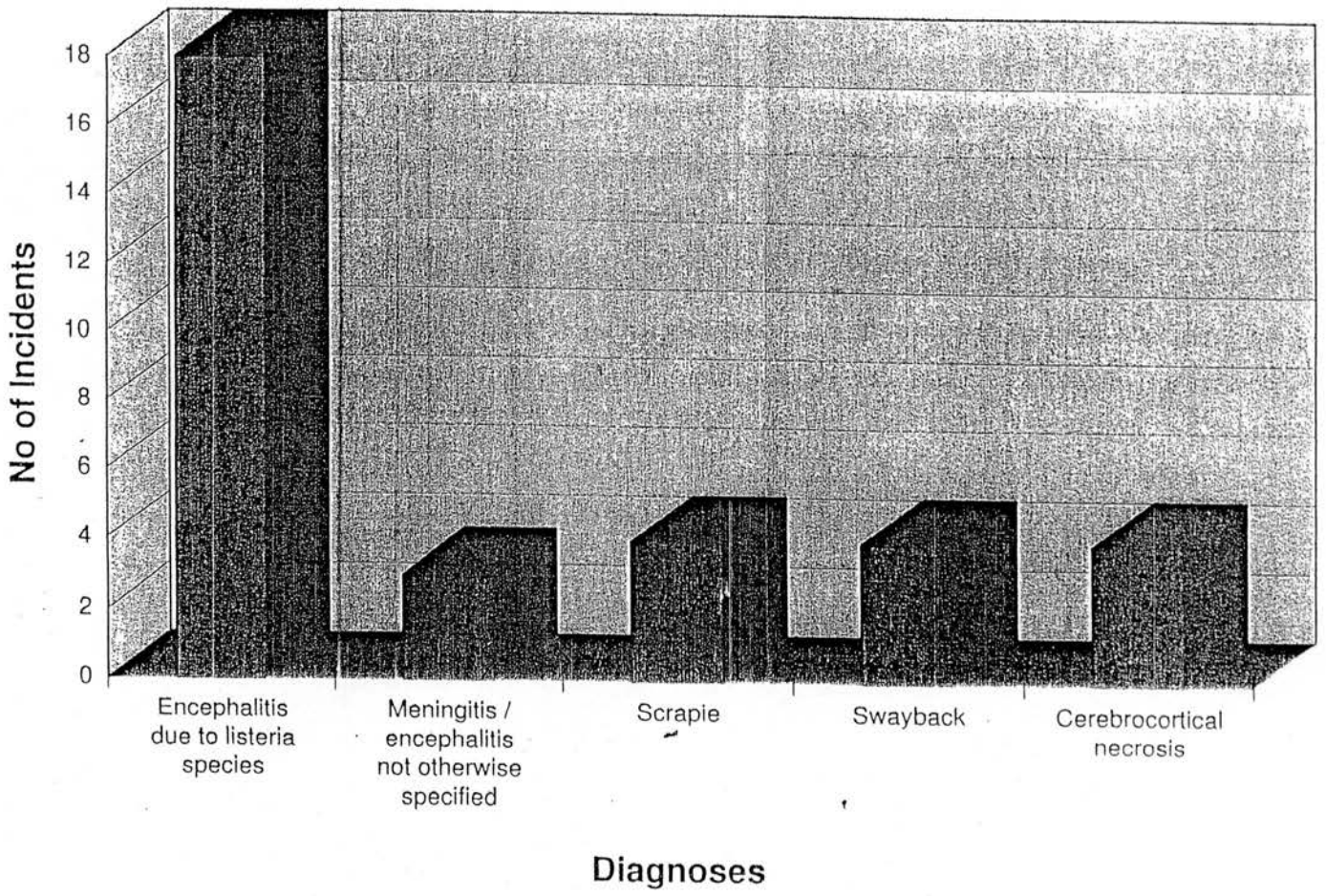


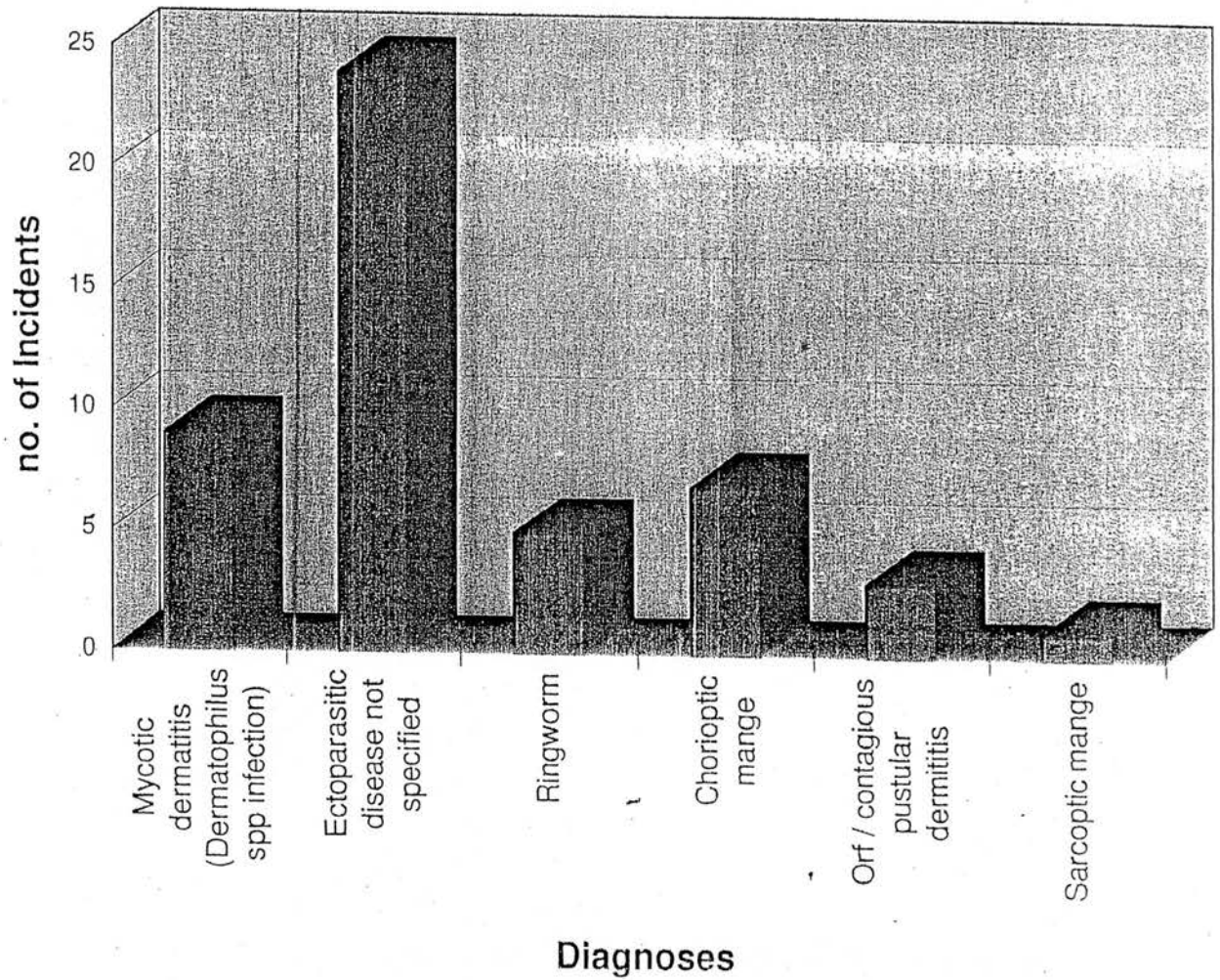
RELATIVE INCIDENCE OF MUSCULO-SKELETAL DISEASES IN GOATS (VIDA 1993-95)



RELATIVE INCIDENCE OF DISEASES OF THE NERVOUS SYSTEM AND ORGANS OF SPECIAL SENSE (VIDA 1993-95)

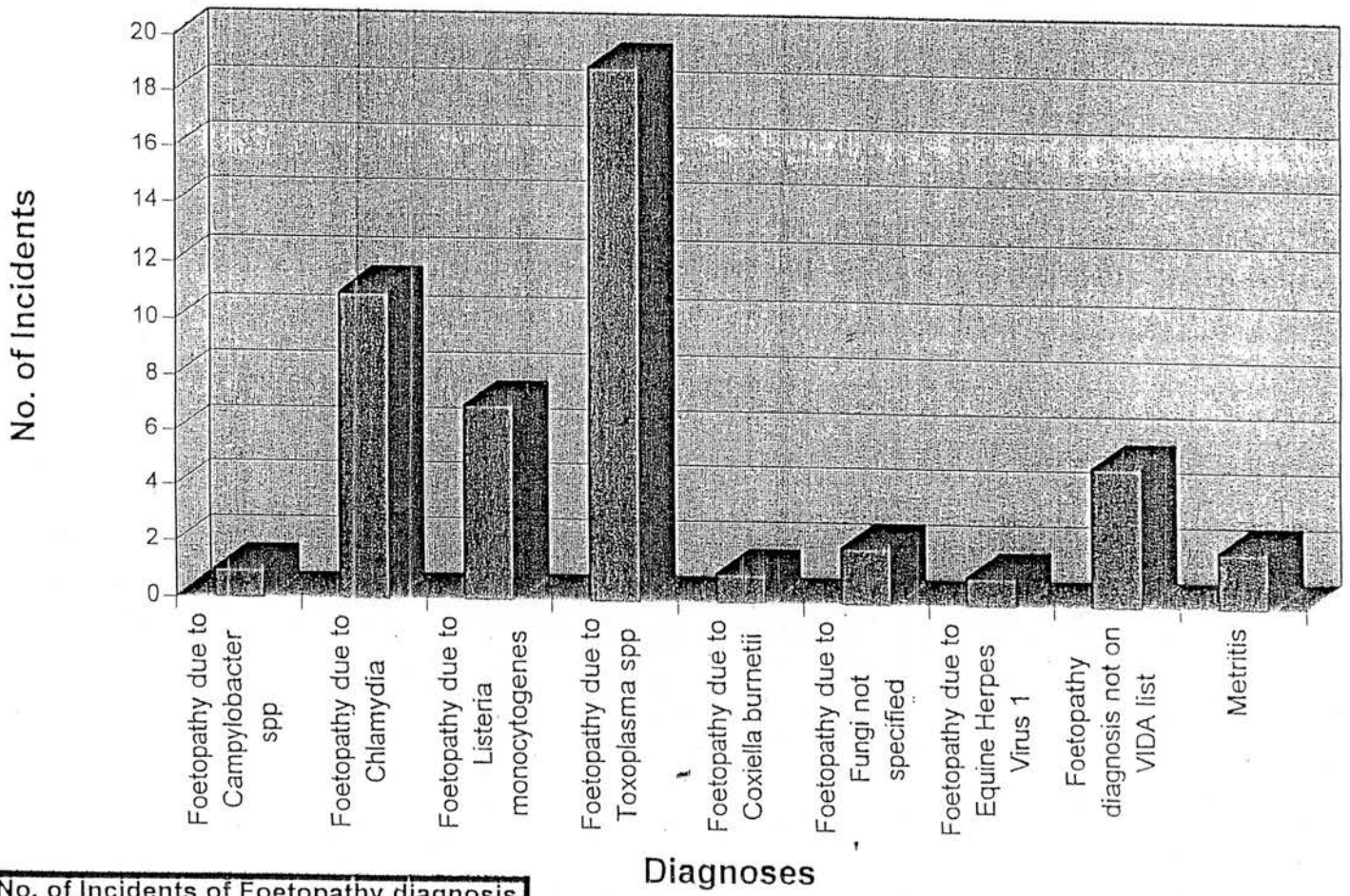


RELATIVE INCIDENCE OF SKIN DISEASES IN GOATS (VIDA 1993-95)



RELATIVE INCIDENCE OF DISEASES OF THE MAMMARY SYSTEM IN GOATS (VIDA 1993-95)

RELATIVE INCIDENCE OF DISEASES OF THE REPRODUCTIVE SYSTEM IN GOATS (VIDA 1993-95)



Please note: No. of Incidents of Foetopathy diagnosis not reached was: 112

1.67

CASEOUS LYMPHADENITIS IN GOATS

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INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic contagious bacterial infection of sheep and goats caused by *Corynebacterium pseudotuberculosis (ovis)* which leads to abscesses, primarily affecting superficial lymph nodes. There is also a small zoonotic risk through skin infection. The condition occurs in many regions of North and South America, Australia, New Zealand, Europe and South Africa. It was first reported in Britain, in 1990, in a herd of dairy goats in Buckinghamshire which had been in contact with imported goats (Lloyd and others 1990, Harker 1990). Since then there have been reports of CLA in goat herds elsewhere in Britain as well as a number of reports in sheep including that of Rizvi and others (1997). CLA is not notifiable and not usually fatal but is of particular significance to those engaged in showing and selling pedigree breeding stock. The incidence of infection in sheep and goats in Britain is still unclear but in order to obtain more information on the occurrence of the condition the Veterinary Laboratories Agency have offered to carry out cultural examinations for *C.pseudotuberculosis* free of charge from suspected cases during 1998. Not surprisingly however, owners are often unwilling to disclose the problem because of the economic implications.

CLINICAL FEATURES

CLA in sheep classically causes abscesses in the superficial lymph nodes, particularly prescapular (in front of the shoulder), precrural (flank) and inguinal (groin). However, most of the descriptions of the condition in both sheep and goats in Britain have involved the head and neck. In the infected goat herd investigated by Harker (1990) 80 percent of the enlarged lymph nodes were either parotid, submandibular or prescapular. One or more lymph nodes can be affected in individual animals. Affected lymph nodes vary in size up to a small apple and contain yellowish green or creamy coloured pus which, as the condition progresses, becomes a mass of cheesy material which can develop a dry laminated appearance. CLA can also occasionally affect deeper lymph nodes resulting in suppurative lesions in lungs, liver, kidney or brain. This generalised form of the condition, which has been described recently in sheep in Britain (Laven and others 1997) is a particular concern because it may not be evident on clinical examination.

Although CLA is rarely fatal, the abscesses are unsightly and inevitably restrict exhibition of valuable show animals and render affected animals unsaleable (assuming the purchaser is aware of the significance of the lesion). Carcases may also be condemned at meat inspection.

DIAGNOSIS

Most cases of CLA, particularly in sheep, are detected incidentally, for example, whilst blood sampling for maedi-visna. Diagnosis is based on a history of abscesses in one or more animals, poor response to antibiotic treatment and a tendency to recur. Clinically, other bacterial infections, including vaccination abscesses, represent the main differential diagnosis. Confirmation is achieved by bacteriological culture of pus from suspect lesions. There is no serological diagnostic test currently available in Britain. An ELISA test has been developed in the Netherlands but was found to be unreliable, particularly in sheep, although it has apparently been used with some success in an eradication programme for goats (Dercksen and others 1996).

TRANSMISSION

Large numbers of *C.pseudotuberculosis* organisms are present in the pus within abscesses. It is a very resistant bacterium which can remain viable in the environment for several weeks, or months, depending on conditions and can also survive in commercial sheep dips for at least 24 hours. Not surprisingly, therefore, the main route of spread is through contamination of small breaks in the skin or mucous membrane, typically following events such as shearing or fighting. It can also be transmitted by contaminated needles, tattooers or surgical instruments. Allowing an abscess to rupture in a pen with other animals present will inevitably increase the risk of spread. There is also evidence that respiratory infection can occur via the aerosol route from lung abscesses, particularly under intensive housing conditions. There is a protracted incubation period of up to four months.

TREATMENT AND CONTROL

Surgical treatment involves either draining or surgically removing the abscessed lymph nodes. According to Smith and Sherman (1994) surgical removal of the encapsulated abscess offers the advantages that the treated animal does not need to be quarantined afterwards and spread to other nodes is less likely to occur. However, this approach is only likely to be suitable for the most valuable animals. Long term antibiotic therapy is generally unrewarding. No vaccine is available in Britain although effective vaccines are used elsewhere, including Canada and Australia.

PREVENTION

It is important to prevent the infection being introduced into a flock or herd by carefully checking proposed purchases and rejecting those with suspicious lesions or scars. Adopt strict hygiene measures at lambing or kidding and during docking or castration. With sheep it is essential that all clipper blades are disinfected thoroughly before shearing commences and between animals. Dress cuts and abrasions promptly with an antiseptic preparation. Once established in a group the condition is difficult to eradicate and owners must be prepared to cull affected animals. Infected animals should be isolated and always handled last to prevent spread. If the environment becomes contaminated with pus this may act as a source of new infections for several weeks or months thereafter unless thoroughly cleaned and disinfected.

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The distribution of Mycoplasma meleagridis in normal turkey carcases and those affected with air sac disease

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Veterinary Record (1980) **106.** 285-287

***Mycoplasma meleagridis* was isolated from both normal and diseased air sacs of turkeys examined and graded for air sacculitis on the evisceration line in a processing plant. It was also cultivated from wing bone cavities of turkeys with severe air sac lesions but not from leg bones or from samples of deep muscle. A further investigation, using material taken randomly from the condemned meat container, confirmed that *M meleagridis* was recoverable from wing bones.**

AIR sacculitis is a common manifestation of respiratory disease in turkeys. Although the condition is frequently subclinical and not detectable from flock health records or ante mortem examination it may be important in relation to carcase judgement and suitability for human consumption under the Poultry Meat (Hygiene) Regulations 1976. This work represents part of an investigation into air sacculitis in turkeys which was carried out to examine the relationship between the microbiological status of affected carcases and the assessment of gross appearance during inspection on a processing line. Since mycoplasmas have frequently been implicated in avian respiratory disease (Vlaovic and Bigland 1971, Jordan 1975), they were included in the investigation in order to complete the appraisal of total carcase microbiology even though avian *Mycoplasma* spp are not regarded as pathogenic for man. Tests for mycoplasmas were carried out as a separate study described here. Bacteriological and mycological findings will be considered in a later paper.

Materials and methods

SELECTION AND GRADING OF CARCASES

Between February and December 1978, 31 turkeys of both sexes, originating from a number of rearing sites in East Anglia, were removed from the processing line at the poultry meat inspector's point on the evisceration line. The factory was usually visited once a week and one or occasionally two birds selected at each visit. Twenty-eight of the birds were in the age range 12 to 24 weeks and three were end-of-lay hens (64 weeks old). The carcases, which included eight apparently normal birds, were selected to give a range of severity of air sac disease from slight cloudiness of air sacs to gross thickening and opacity. The condition of the air sacs was briefly assessed from their gross appearance, especially that of the anterior thoracic air sacs which are particularly conspicuous in the turkey. The viscera were then detached from the carcase and both were packed in crushed ice and taken without delay to the laboratory. Definitive grading of the air sac lesions was not attempted until the air sacs had been opened to disclose the full extent of the pathological change. To avoid contamination of cultures this was deferred until sampling had been completed.

The following categories of disease were recorded.

(1) Normal, thin, glossy, translucent air sacs ("normal").
(2) Air sacs showing a slight degree of opacity, not obviously due to fat deposition ("cloudy").
(3) Opaque thickened air sacs with underlying mucopurulent exudate adherent to the underlying surface of air sac wall ("opaque").
(4) As 3, but more severe and with caseous or inspissated material free or attached within the air sacs ("severe")

In a separate investigation, 50 wing bones were sampled for *Mycoplasma* spp. These specimens were taken randomly on three occasions from a bin for condemned portions and were mainly affected with post mortem machine damage.

SAMPLING TECHNIQUE

Sites selected for the attempted isolation of mycoplasmas were the anterior thoracic air sacs, including any inspissated contents, wing and leg bone cavities, liver and deep muscle from leg, wing and pectoral regions.

Contamination difficulties initially encountered in sampling the air sacs and contents were overcome by using a short sterile stainless steel tube inserted obliquely from the exterior of the carcase into the thoracic air sac with the bird lying in dorsal recumbency. The undersurface of the air sac was swabbed vigorously through the tube for about 20 seconds. The swab was then withdrawn and broken off into a sterile universal container. The humerus and femur were broken at mid-shaft with bone forceps and the medullary cavities swabbed aseptically. Deep muscle samples, weighing 3 to 5g each, were taken from breast, wing and leg muscle aseptically using either a stainless steel cork borer or scalpel and forceps, after searing the surface of the tissue. A sample of 3 to 5g of liver was similarly taken with scalpel and forceps as was 0·5 to 5g of any inspissated material found in the air sacs when these were opened later. The muscle, liver and caseous air sac material were prepared for culture by homogenising the samples in 20 ml of 0·1 per cent peptone water using a stomacher (Colworth 80).

MYCOPLASMA MEDIA AND CULTIVATION METHODS

The medium used for isolation and subcultures was one modified from that of Bradbury (1977) and consisted of Bacto PPLO broth plus heat-inactivated swine serum (15 per cent v/v), aqueous yeast extract (10 per cent v/v), nicotinamide adenine dinucleotide (0·01 per cent w/v), glucose and arginine (each 0·2 per cent w/v), plus thallous acetate (0·025 per cent w/v) and ampicillin (150 μg per ml). The solid medium also contained Oxoid purified agar (1 per cent w/v) and was normally poured in plastic petri plates (50 mm). A diphasic medium, consisting of a 1 ml slope of solid medium overlaid with 2 ml of fluid medium, was contained in 5 ml screw-cap bottles. Media were checked for efficacy with reference strains of *M gallisepticum, M meleagridis* and *M synoviae.*

The swabs and prepared suspensions were received at the mycoplasma reference laboratory within two hours of sampling. About 0·25 ml of each suspension was inoculated into diphasic medium to provide an initial dilution and two further 100-fold dilutions were made in the same medium. Each of these was inoculated on to petri plates of solid medium and both sets of cultures were incubated. Swab specimens were each streaked on an agar plate and then rinsed in a bottle of diphasic medium which was then treated in the same manner as the initial dilution of tissue suspensions. All cultures were incubated at 37°C for at least 10 days, the plate cultures being maintained in an atmosphere of 5 per cent carbon dioxide/95 per cent nitrogen. The diphasic broths were examined every one to two days for a colour (pH) change indicative of mycoplasma growth and the plates every two to three days for mycoplasma colonies. Confirmatory subcultures were made on to solid medium. Any mycoplasmas cultivated were maintained by subculture on solid medium and were identified by standard biochemical and serological techniques (Boughton and Thorns 1978).

TABLE 1: Isolation of *M meleagridis* from turkey carcases

Air sac appearance	Air sacs		Wing bones	
	No of carcases tested	No of carcases with positive results	No of carcases tested	No of carcases with positive results
(1) Normal	8	3	8	0
(2) Cloudy	4	2	3	0
(3) Opaque	3	1	3	0
(4) Severe	16	5†	10	5*†

*In two carcases from both wing bones
†In two carcases isolations were obtained from wing bone and air sac

TURKEY SERA

The serological status of the turkeys examined was assessed by collecting blood samples at the killing point from five birds selected at random from the same consignment. Twenty-six of the 31 consignments involved were sampled in this way, giving a total of 130 samples which were tested for antibodies to *M gallisepticum, M synoviae* and *M meleagridis* by plate agglutination tests, using the respective stained mycoplasma antigens (Wellcome Reagents).

Results

CULTURAL EXAMINATIONS

M meleagridis was isolated from 13 (42 per cent) of the 31 carcases examined. No other mycoplasma species were recovered.

As shown by Table 1, *M meleagridis* was isolated from the air sacs of 11 (35 per cent) of 31 turkeys and from the wing bone cavities of five (21 per cent) of 24 of these carcases. No isolations were obtained from any of the muscle samples examined. There was also one apparent isolation from a liver sample but this was unconfirmed by repeat tests on the original suspension. The numbers of mycoplasmas detected in most specimens were small. Over two-thirds of the original plate cultures produced less than 10 colonies per plate and only two produced more than 1000. Positive specimens usually contained some bacteria in numbers insufficient to spoil plate cultures but causing complete overgrowth of broth cultures. Hence, most mycoplasma isolates were derived from the original plate cultures and only two from diphasic broth cultures.

Cultural examination of the medullary cavities of the 50 wing bones taken from the condemned meat bin yielded *M meleagridis* from 0/19, 6/19 and 1/12 swabs, giving an overall isolation rate of 14 per cent. No other mycoplasma species were isolated.

SEROLOGICAL EXAMINATIONS

Thirty-two (25 per cent) of the 130 blood samples gave a positive reaction with the *M meleagridis* antigen, with 16 (62 per cent) of the 26 batches tested showing a positive reaction in at least one of the five samples tested. There were no positive results for *M gallisepticum* or *M synoviae*.

Discussion

The overall recovery rate of *M meleagridis* from turkey carcases was somewhat higher than expected because the average age of the birds examined was about four months, which is well beyond the usual stage for acute and evident infection (Vlaovic and Bigland 1971). The results of the plate agglutination tests established the presence of infection in most of the batches of turkeys examined but the actual number of positive blood samples was fairly low. This conforms with the findings of Snell and Cullen (1978) who noted a substantial decrease in the number of reactors to the rapid serum agglutination test three months after infection with *M meleagridis*.

The air sacs were the most common site for recovery of mycoplasmas which were isolated with similar frequency from both normal and diseased air sacs, but not from caseous exudate. In the main investigation the sampling of wing bones was not started until bird 8, but *M meleagridis* was isolated from 50 per cent of severely diseased birds subsequently examined. The organism was not recovered from the wing bones of birds with normal or merely cloudy air sacs. The recovery of *M meleagridis* from wing bone cavities was confirmed by the results of further investigation using condemned wing portions, but unfortunately no information was available as to the appearance of the air sacs of these birds.

Many studies on the distribution of *M meleagridis* in turkeys—mostly with experimentally inoculated birds or embryos—have revealed its presence throughout the respiratory tract, including the nasal sinuses, air sacs, lungs and trachea (Rosenfeld and Grimes 1972, Rhoades 1971, Arya and others 1971). Other workers have isolated the organism from a range of sites including the oesophagus, bursa of Fabricius and the genital tract (Yamamoto 1967, Yamamoto and others 1965, Jordan and others 1978), but we do not know of any previous report of its presence in the wing bones of turkeys. Moreover as far as we are aware this is the first reported isolation of any *Mycoplasma* spp from the bone cavity of birds.

The recovery of mycoplasmas from aerated avian bones is not surprising since these cavities connect with the main respiratory tract where the primary infection occurs. In turkeys, air sac diverticulae from the cervicoclavicular air sac enter the medullary cavity of the humerus, whereas the femur is not pneumatised (Cover 1953). This was supported by our examination of sampled bones using the absence of medullary content as the main criterion for pneumatisation. However, pneumatisation of the humerus appears not to be constant in turkeys—about 5 to 10 per cent of those examined were not pneumatised or only partially so. None of the femurs was pneumatised. *M meleagridis* was not recovered from any of the obviously unpneumatised wing bones.

The presence of *M meleagridis* in pneumatised turkey wing bones probably represents a surviving population derived from the retraction of an initially more extensive area of infection within the respiratory system. It seems unlikely that the presence of mycoplasmas in the bone cavities has any great pathogenic significance for the infected turkey, although it could represent a persistent focus for potential flock infection inaccessible to immune reactions or antibiotic treatment.

It is suggested that *M meleagridis* may be a common inhabitant of the air sacs of turkeys without necessarily causing disease and that mycoplasmas may persist in the aerated wing bones of turkeys that have recovered from an episode of air sacculitis. Certainly wing bone sampling ought not to be overlooked in any future examinations in turkeys or other avian species with respiratory disease.

Acknowledgements.—The authors wish to acknowledge the cooperation provided by the poultry processing industry and poultry meat inspectors. Mrs K. Riley provided valuable technical assistance with the mycoplasma isolations. Mr S. Line and Dr G. C. Mead gave helpful advice on sampling technique and Dr J. Bradbury on media formulation. Thanks are also due to colleagues and to the staff of the Veterinary Investigation Centre, Norwich, for general help and support.

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Ionophore toxicity in turkeys

1.69

SIR, – Each year, the rearing of turkey poults for the Christmas trade brings with it incidents of ionophore toxicity. In England and Wales, these are investigated as potential food safety incidents by the Veterinary Laboratories Agency on behalf of the Veterinary Medicines Directorate and the Food Standards Agency. Most incidents appear to occur on non-specialised poultry premises where existing buildings are exploited opportunistically and where farmers and their veterinary practitioners may be unaware of the potential risks.

Ionophores, such as salinomycin, narasin, lasalocid and monensin, are used at varying concentrations in poultry feed as anticoccidial agents. Depending on the product, they may be licensed for use in turkeys up to 16 weeks old. Toxicity varies with species and age: turkeys are particularly susceptible from around nine weeks of age. Clinical signs, which can develop within 24 hours, comprise muscular weakness with collapse, gasping respiration (mouth breathing) and death. Morbidity and mortality are often very high. In a recent outbreak in East Anglia, 178 (24 per cent) of 750 turkeys aged 12 to 18 weeks died or were euthanased during a period of a few days shortly before Christmas. Postmortem findings are invariably inconclusive, particularly during the early stages. Histological examination of skeletal musculature (particularly adductor muscles of the legs) and cardiac muscle may reveal myopathy, but this is not specific and may not be evident in acute cases. Creatinine kinase enzyme estimations on serum are more useful and usually exceed one million U/litre. In view of the high morbidity and mortality, combined with the respiratory distress, conditions such as avian influenza (fowl plague), need to be considered in the differential diagnosis. The latter is a notifiable disease and, if suspected, must be reported to the local divisional veterinary manager of MAFF. However, in most cases of ionophore toxicity, the history indicates a recent change in feed, and the incident rapidly subsides after its removal.

The main situations under which ionophore toxicity occurs in turkeys are: inadvertent use of broiler or other feed containing ionophore; continuing to feed rations containing ionophore to older birds (either accidentally or through lack of awareness); and the accidental inclusion of ionophore in feed due to contamination at the mill. Depending on the individual circumstances, it is usually necessary to impose restrictions on the sale of the birds for human consumption until ionophore residues have been eliminated following removal from the source of contamination.

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1.70

Suspected necrotic enteritis in wild swans

SIR, – The unfortunate deaths of at least 30 whooper swans (*Cygnus cygnus*) on a wetland wildlife reserve in East Anglia during a six-day period in November 2003 was reported in the November 2003 surveillance report of the Veterinary Laboratories Agency (VLA) (*VR*, January 3, p 8). In view of the high mortality and general interest in the incident, we thought it appropriate to provide further details.

The swans, which were in a flock of about 2200 birds, had recently arrived from Iceland and were congregating on the River Ouse Washes in Cambridgeshire, where they fed in surrounding arable fields during the day and roosted at night on the water. Following a dry summer, the Washes were less flooded than previous years but there was sufficient water in the main rivers, and a large man-made lagoon, for drinking, washing and preening. As is usual for the time of year, whoopers and about 3300 Bewick's swans (*Cygnus columbianus*) and other waterfowl on the reserve were offered wheat to supplement their diet.

Virtually all the deaths were in the whoopers, with only two or three Bewick's affected. Most losses occurred overnight; the few sick live birds seen were described as 'drowsy and wobbly'. Four swans submitted for postmortem examination had died in the previous 12 to 24 hours. They were in good body condition with adequate fat deposits and well-developed musculature. They all had a severe and very haemorrhagic necrotic enteritis extending from the proximal small intestine to the cloaca, which was distended with watery bloody contents. The intestinal lumen of all the birds contained unusual soft, haemorrhagic, gelatinous cores which were easily removable like lengths of rope. The livers were haemorrhagic, friable and two had ruptured. The hearts were congested. One bird had prominent urate deposits in the renal pelvis.

Gram-stained smears from intestinal mucosa revealed numerous clostridia-like bacteria; *Clostridium perfringens* alpha toxin was identified by ELISA in the intestinal contents of two of three birds tested. There were no other significant bacteriological findings and, apart from a few flukes, no enteric parasites were seen. No viruses (such as duck virus enteritis virus) were isolated following passage in embryonated eggs and in Vero-cell cultures at VLA – Weybridge; a PCR for West Nile fever virus was also negative. There was no biochemical evidence of lead toxicity. Cyanobacteria were not found in water samples collected from the vicinity. Although hampered by autolysis, histopathological examinations of the alimentary tract indicated loss of superficial epithelium, crypt attenuation, dilation and deficit. There was also mild, multifocal lymphocytic/plasmacytic cholangiohepatitis and pericarditis. Although the cause of these deaths was not fully established, the overall findings suggested that necrotic enteritis was the most likely cause.

Necrotic enteritis is well documented in domestic fowl although the lesions are more those of a velvet-like diphtheresis. Wobeser (1997) described the condition in migrating geese in the USA, and MacDonald and others (1987) blamed it for sporadic losses in swans in Britain. The VLA and the Wildfowl and Wetlands Trust have reviewed six similar suspect necrotic enteritis incidents since 1997 involving whooper and mute swans (*Cygnus olor*) at four locations in England. Postmortem findings were similar to those described here. The total recorded mortality was 65 whoopers and 14 mutes, with both adults and juveniles affected. All occurred between November and January – a time when migratory whooper swans are winter visitors to Britain. In five incidents, the swans had access to supplementary wheat, and in several incidents environmental changes were also noted. For example, in 2003 a pond used by three affected mute swan cygnets had dried out. In another incident involving mute swans, in which 11 birds died, most of the available water surface had frozen. Pennycott (1999) suggested that the presence of fishing line in the intestine may also predispose birds to the condition.

In view of difficulties in achieving a definitive diagnosis of necrotic enteritis, particularly in autolysed carcases, this condition may be poorly recorded as a cause of mortality in swans. Although only speculative, it seems possible that changes in environment and diet – particularly fermentable carbohydrates in the form of wheat or potatoes (readily available in surrounding arable fields) – may trigger the onset of this disease.

These examinations were carried out under the VLA: Diseases of Wildlife Project (Duff 2003) funded by DEFRA to investigate unusual mortality in wildlife.

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2.1a

Investigation of farms as potential sources of human VTEC O157 cases

Johanne Ellis-Iversen, Richard Smith, Tom Cheasty, Geraldine Smith & Geoff Pritchard

- VTEC O157 case detected by Health Protection Agency
- Epidemiological investigation identifies farm as potential source

→ **Public health authority requests a FARM INVESTIGATION from Veterinary Laboratories Agency**

Farm investigation

Participation in farm visit
- Veterinary Investigation Officer
- Local public health authority
- The farm manager/owner
- Occasionally CCDC/HSE

Faecal Sampling
- Statistically derived sampling Strategy that ensures detection of VTEC O157 if the prevalence of positive animals within the group is at least 10% with 95% confidence.

Farmer specific advice
- Avoidance of transfer from animals to humans
- Reduction of VTEC in animals
- Farm specific issues arising from the farm visit

Laboratory analyses
- Pre-enrichment (BPW)
- IMS
- CT-SMAC
- Latex agglutination confirmation
- PCR to confirm VT genes (1+2)
- Phage-typing (PT)
- PFGE to match to human isolates.

Standardised data collection
- Instructions to Veterinary Investigation Officer
- Forms and questionnaires

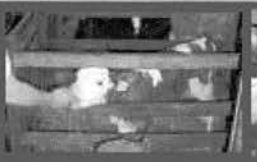
Outcome of previous investigations

Open farms/public amenities 1997-2007

- 31 amenities were investigated
- Amenity types:
  - 26 typical open farms:
    - 14 with shop or cafeteria; 2 with campsites and 3 with associated commercial farms.
  - 5 livery stables or farm campsites premises with a number of animal species to which the public had general access
- 61% (19) were VTEC O157 positive
- Prevalence of VTEC O157 positive samples originating from cattle was 29%, sheep: 24%, donkeys: 15%, pigs: 14%, horses: 12% and from goats: 10%
- Groups containing younger cattle more likely to be VTEC O157 positive than older cattle groups (p=0.05).
- Adult pig groups more likely to be VTEC O157 positive than groups including young pigs (p=0.01)
- 52% (16 farms) had a PT-match to the human case
- 50% of the farms matched PFGE profile of case

Commercial farms 1998-2007

- 36 farms were investigated
- Main enterprises:
  - 19 dairy
  - 10 beef
  - 5 sheep
- 67% (24) were VTEC O157 positive
- 55% (20) had a PT-match to the human case
- Cattle farms were more likely to be VTEC O157 positive than sheep farms (p=0.03)
- Dairy farms were more likely to be PT-match to human case than beef (p=0.05) or sheep farms (p=0.03)



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2.1b

Investigation of animal premises in England and Wales as potential sources of human Verocytotoxigenic *E. coli* O157

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Sporadic cases and outbreaks of VTEC O157 infection have been linked to direct or indirect contact with farm animals or their environment. In England and Wales, potential associations with farm animals are investigated by the Veterinary Laboratories Agency at the request of human health professionals. The veterinary investigations include on-farm inspection, faecal sampling of animals and provision of advice on husbandry and hygiene aspects relevant to the control of VTEC O157. Epidemiological information is also collected on standardised forms and entered onto a database for subsequent analysis.

A comprehensive structured sampling approach is applied to the premises investigated. Each epidemiological group of animals is sampled to ensure a 95% confidence of detecting VTEC O157 if the prevalence of positive animals within the group is at least 10%. Faecal samples are examined for VTEC O157 using the established techniques of pre-enrichment, immunomagnetic separation and inoculation onto CT-SMAC followed by *E. coli* O157 latex agglutination testing of non-sorbitol fermenting colonies. PCR is used to confirm the presence of Vero cytotoxin (VT) genes 1 and 2 and strains are phage typed (PT) and profiled by pulsed field electrophoresis for comparison with the human isolates.

During the period 1997-2007, a total of 31 farms with general public amenity access (open farms) and 36 commercial cattle and sheep farms were investigated at the request of public health authorities because of putative associations with human infection. VTEC O157 was isolated from 61% of the open farms and 67% of the commercial farms. The most common PTs detected were PT2 and PT21/28, which were similar to the types most commonly causing human infections during this time period. On 86% of the investigated farms, at least one animal sample harboured VTEC O157 of the same PT as the human case. On open farms a variety of animal species were found to shed VTEC O157.Groups of young cattle were more likely to shed VTEC O157 than older cattle and older pigs housed without piglets presented a higher risk than younger or mixed groups of pigs. On the commercial farms, multivariable logistic regression analyses revealed that sheep farms were less likely to be VTEC O157 positive than cattle farms. VTEC O157 of the same PT as that causing human disease were significantly more likely to be present on dairy farms than on sheep or beef farms.

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Prevention and control of zoonoses on farms open to the public

Geoff Pritchard

Geoff Pritchard graduated from Edinburgh in 1973 and has worked for the former Veterinary Investigation Service and Veterinary Laboratories Agency (now AHVLA) since 1980. Based in Bury St Edmunds, Suffolk, he has been the VLA project leader for non-statutory zoonoses since 2004, a post from which he retires in July 2011. Working in collaboration with human health professionals, he has been involved in many investigations into outbreaks of zoonotic disease, including those on farms open to the public, particularly infection with verocytotoxigenic *Escherichia coli* O157 and *Cryptosporidium parvum*. He is a Fellow of the RCVS.

Faced with falling profit margins from conventional livestock production, farmers have sought to diversify into new ventures to increase farm income. Despite a setback during the 2001 foot-and-mouth disease epidemic, farm attractions have been an important growth area in recent years and help to bridge the gap between urban living and the agricultural industry. Livestock farms that open to the public are now a well established part of the tourism industry in Britain and serve useful leisure, entertainment and educational roles. Close contact with animals or their environment may occasionally result in injury or zoonotic illness in humans. This article discusses the major zoonoses associated with farm livestock and highlights the role of veterinary surgeons in preventing and controlling disease on public farms.

Balancing the risks and benefits

Outbreaks of zoonotic disease can have considerable impact, resulting in major public concern and intense media scrutiny. These may affect the viability of the enterprise and impact on the wider rural economy as schools and parents become increasingly concerned about the potential health risks from farms and farm visits. Visitors, especially those in family groups, may underestimate or be unaware of the hazards and are intent on having a fun time with as few restrictions as possible. If young children are poorly supervised it can increase the risk of exposure to zoonotic hazards in this particularly vulnerable group. Farm operatives have the difficult task of providing visitors with an enjoyable experience while protecting them from hazards. In an increasingly litigious society, adequate public liability insurance is also an important consideration.

The likelihood of acquiring zoonotic illness is very small in relation to the total number of farm visits (variously estimated at between five and 15 million visits annually) and is reduced further by adopting risk management measures to protect human health, particularly adequate hand washing. The public needs to be aware of the hazards and risks in relation to the undoubted benefits. Veterinary surgeons can play a useful role by being conversant with zoonotic hazards on farms and helping their clients to adopt appropriate measures to reduce the occurrence of zoonotic infections in their livestock. They should also be aware of the relevant Health and Safety legislation and sources of advisory information available to farmers.

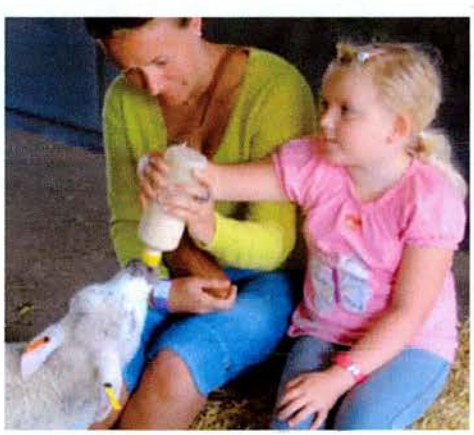

Fig 1: Opening up farms to the public can provide a useful income stream for the livestock industry, but visitors must be made aware of the potential risks of zoonoses

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Box 1: Main zoonotic pathogens on farms in Great Britain

- *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*
- *Chlamydophila abortus* (enzootic abortion)
- *Coxiella burnetii* (Q fever)
- *Corynebacterium pseudotuberculosis* (caseous lymphadenitis)
- *Cryptosporidium parvum*
- *Leptospira icterohaemorrhagiae* and *Leptospira hardjo*
- *Mycobacterium bovis*
- Orf (parapox) virus
- Dermatophytes (ringworm)
- *Salmonella* species
- *Toxoplasma gondii*
- Verocytotoxigenic *Escherichia coli* (VTEC) O157, VTEC O26 and other strains
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Zoonotic disease associated with farm livestock

Zoonoses are defined by the World Health Organization (WHO) as diseases and infections that are naturally transmitted between vertebrate animals and humans. This occurs either via the foodborne route or from environmental exposure to animals or their environment. Person-to-person spread can also occur with some zoonoses. Exposure to zoonotic hazards is an occupational risk for workers on livestock farms and, to a lesser extent, for their families and personal visitors. There is a significantly increased potential for large outbreaks of zoonotic disease to occur once a farm opens up to the general public. For many visitors, the farm encounter is their first significant exposure to zoonotic infections (Box 1). This threat can come from unsuspected sources, as even apparently normal healthy animals (especially ruminants) can shed important pathogens such as *Cryptosporidium parvum* (Box 2), verocytotoxigenic *Escherichia coli* (VTEC) O157 (Box 3), *Coxiella burnetii*, which causes Q fever (Box 4), and *Campylobacter jejuni* or *Campylobacter coli*.

Outbreaks of zoonotic disease (mainly VTEC O157 infection and cryptosporidiosis) from visits to farms and other animal attractions have been reported worldwide in recent years, including a significant number in Britain. Many of these have been investigated by the former Veterinary Laboratories Agency (VLA) (now Animal Health and Veterinary Laboratories Agency [AHVLA]) working in collaboration with the Health Protection Agency (HPA). The potential scale of zoonotic disease in a susceptible population exposed to a hazardous pathogen should not be underestimated. In April 2009, an outbreak of cryptosporidiosis affected about 50 per cent of 380 school children who visited a dairy farm in Cumbria containing infected calves and goats. Outbreaks of cryptosporidiosis have also occurred in veterinary schools in Britain. In September 2009, Godstone Farm in Surrey experienced the largest ever outbreak (93 cases) of VTEC O157 infection in Britain related to direct contact with animals; similarly large outbreaks have been reported in the USA and Canada. In addition

Box 2: Cryptosporidiosis

- Cryptosporidiosis (due to *Cryptosporidium parvum*) is probably the most common zoonosis arising from farm visits
- *C parvum* is a protozoan parasite with resistant oocysts that can persist in the environment
- Cryptosporidiosis affects a wide range of animal species, especially calves and lambs, causing diarrhoea and dehydration
- Clinical disease in animals is usually diagnosed by examining stained faecal smears
- Subclinical infection is common but detection requires a sensitive fluorescent antibody test on faeces
- Human cases occur following faeco-oral contact with contaminated water or infected animals. Cryptosporidiosis causes diarrhoea and stomach cramps in people
- A very low infectious dose is required to cause disease (subclinically infected animals can shed sufficient numbers of oocysts to cause illness in people)
- Outbreaks occur if hand washing facilities are inadequate (alcohol-based hand gels are unsuitable)
- Bottle feeding of orphan lambs is a particular risk factor

Box 3: Verocytotoxigenic *Escherichia coli* O157

- Verocytotoxigenic *Escherichia coli* (VTEC) O157 is an enterohaemorrhagic strain of *E coli* belonging to serogroup O157, which is the main verocytotoxigenic strain of *E coli* found in Great Britain
- VTEC O157 is a commensal bacterium found in the gut of ruminants (cattle and sheep being the main reservoirs). It may also be present in faeces of a wide range of domesticated and wild animal species, but not small domesticated mammalian pet species such as guinea pigs or pet rabbits
- It is important to note that infection does not cause disease in animals but can potentially cause very serious illness in people
- Can cause haemolytic uraemic syndrome, which is the most common cause of acute renal failure in young children
- Infection is acquired via the faeco-oral route as a result of contaminated food, or due to contact with infected animals or their environment. Person-to-person spread also occurs very readily
- Most human cases are sporadic but large outbreaks can occur (the cause for which is usually foodborne in origin)
- Outbreaks can also occur on farms open to the public
- Risk to visitors depends on the degree of contact with animals (goats may present a higher risk because of their generally more friendly nature)
- A very low infectious dose is required to cause disease

Box 4: Q fever

- Q fever is caused by *Coxiella burnetii*, a bacterium that is similar to *Legionella pneumophila*. *C burnetii* was previously classified under the genus *Rickettsia*
- *C burnetii* produces a spore-like form resistant to desiccation and common disinfectants
- Infection is mainly subclinical but can cause abortions in cattle, sheep and goats
- Diagnosed by serology (using an ELISA or complement fixation test) and/or PCR on fetal material (eg, cotyledons, fetal fluids)
- Human infection causes flu-like illness but is often asymptomatic
- Potentially serious sequelae can occur in vulnerable people (eg, those with valvular heart disease, pregnancy)
- Contaminated bedding/parturition products (normal births and abortions) are main hazards
- A very low infectious dose is required to cause disease
- Large human outbreaks caused by windborne aerosol spread from infected farms
- Recent human outbreaks occurred in Cheltenham (2007) and Stirlingshire (2006). A major outbreak (>3000 human cases) occurred in the Netherlands (2007 to 2011), which was linked to large milking goat herds. Fewer large goat herds, lower disease prevalence and different demographics make this scenario unlikely to occur in Britain

Fig 2: Open farms come in various guises, ranging from city farms, petting zoos, farm parks to rare breed sanctuaries and mobile farms

to other concerns, such zoonotic outbreaks can impose a considerable financial burden on health care services, particularly for cases requiring hospitalisation. The purely monetary costs incurred in treating serious complications in the very young or elderly affected by highly toxigenic strains of VTEC O157 can be huge.

An example of what can happen in a worst-case scenario is the massive ongoing outbreak of Q fever in the Netherlands (2007 to 2011). This has been attributed to the exposure of a high density nearby human population to infected aerosols and contaminated dust particles from possibly the highest concentration of dairy goat herds worldwide. There were 2357 reported cases in humans in 2009 alone, mainly among those living within 5 km of infected farms. This serious public health problem triggered major veterinary interventions, including vaccination, animal movement restrictions, culling and hygiene measures.

Farm open days

Working livestock farms may decide to open their gates to the public for one or more days a year (eg, during lambing). These farm open days can supplement farm income or may primarily be for educational purposes to offer hands-on experience and improve awareness of farming activities. Farm open days may be organised on an ad hoc individual basis or as part of a national initiative such as the widely sponsored Open Farm Sunday annual event organised by Linking Environment and Farming (LEAF), which had the largest attendance of its five-year history in 2010 when 184,000 people visited 420 farms. Outbreaks of zoonotic disease (particularly cryptosporidiosis) have sometimes occurred at farm open days because the owner or manager was unaware of the potential hazards and did not know how to manage the risks.

Open farms

Open farms are commercial operations whose primary purpose is entertainment or leisure and they provide direct public access throughout most or all of the year. Open farms may be separate from commercial farms or may be an associated enterprise, perhaps to make use of redundant farm buildings or as a way of generating income from surplus lambs, goat kids or other animals. Open farms come in various guises, including city farms, petting zoos, farm parks (Fig 2), animal rescue or rare breed sanctuaries and mobile farms, which bring small groups of animals to establishments such as schools, nurseries and residential homes. They vary widely in size and facilities; some may regularly have more than 1000 visitors a day during school holidays, reaching to about 5000 on very large establishments at peak season bank holidays.

The range of species present on open farms includes domesticated farm livestock, rare or exotic species of mammals and birds, camelids, horses, donkeys, ponies and various small pet species. Most open farms have a shop, provide refreshments and offer a number of activities such as bottle-feeding of lambs, animal grooming, pony and tractor rides, adventure playgrounds, amusement parks and birthday parties involving contact with animals. Some open farms are included in a voluntary registration scheme operated by the National Farm Attractions Network (NFAN), which provides professional assistance and support to farm and rural attractions. Open farms invariably have some hand washing facilities for visitors and provide varying degrees of supervision and advisory information.

Other exposures to farm animals

Incidental exposure of the general public to zoonotic hazards such as VTEC O157 may occur in other recreational scenarios such as agricultural shows, farm cottage holidays on livestock farms, farm gate sales, campsites and other outdoor events, including music festivals, car boot sales and picnics on pasture grazed by livestock. Oral contact with the faeces of wildlife species, such as rabbits grazing pasture used by infected cattle, can also lead to human infection with VTEC O157. In addition, leisure pursuits involving the countryside and farmland carry the risk of exposure to other zoonotic hazards such as Lyme disease (from tick bites) or Weil's disease (from water contaminated with *Leptospira icterohaemorrhagiae*).

Management of zoonotic disease on farms open to the public

The prevention and control of zoonotic diseases should be an integral part of health plans for all livestock farms but especially those that open to the public. Keeping a closed herd is ideal but rarely achievable on many farms. The small possibility that visitors to farms might compromise general disease biosecurity and introduce new infections on their clothing or footwear if they have come directly from other livestock premises is often overlooked. The provision of protective clothing for visitors to farm open days should be considered and this might also help to prevent their clothing becoming contaminated with zoonotic pathogens. It is generally impractical to provide similar protective clothing for visitors to open farms because of the large numbers of people involved. Visitors should be encouraged to wear wellington boots when visiting farms, especially in wet weather.

Prevention

Preventing the introduction of new zoonotic diseases with incoming animals can be particularly difficult on open farms that rely on a constant supply of purchased lambs, goat kids or calves to meet the demands for bottle feeding, which is a popular visitor attraction. This practice could introduce new infections such as *Salmonella* species and may partly explain the higher prevalence of VTEC O157 seen in diverse animal species on open farms compared with the prevalence recorded from farm and abattoir surveys. Conversely, naive animal introductions may be susceptible to endemic diseases already present on the premises. The constant turnover of young animals and handling by visitors may act as stress factors leading to increased shedding of *Salmonella* species and possibly VTEC O157, although published evidence on this issue is sparse.

To reduce the risk of introducing new diseases, including zoonoses, replacement young animals should come direct from reputable local farms (not livestock markets) in batches of similar age and size, with assurances about colostrum intake. They should be inspected for evidence of disease (particularly diarrhoea and zoonotic skin infections) on arrival and isolated for as long as practicable, even if only a few days, before being exposed to the public. Very young animals should not be used for bottle feeding for welfare reasons. An 'all-in, all-out' approach to management should be adopted with strict cleaning and disinfection between batches using approved disinfectants at the correct concentration. Introductions of older and adult replacement animals, including breeding stock, should follow the established principle of a four-week isolation period, ideally after prior inquiries have been made about their health status from the farm of origin. During the isolation period, added stock should be inspected for evidence of clinical disease, with particular reference to zoonoses, and treated as appropriate. Relevant laboratory tests should also be undertaken, including serological examinations for *Leptospira hardjo* in the case of cattle.

Control

Controlling existing zoonotic pathogens depends on the availability of efficacious interventions or control measures, such as vaccination or medication. Unresolved disease outbreaks, particularly abortions (Fig 3, Figs 7a,b), diarrhoea (Fig 4) or skin lesions (Figs 5, 6), must

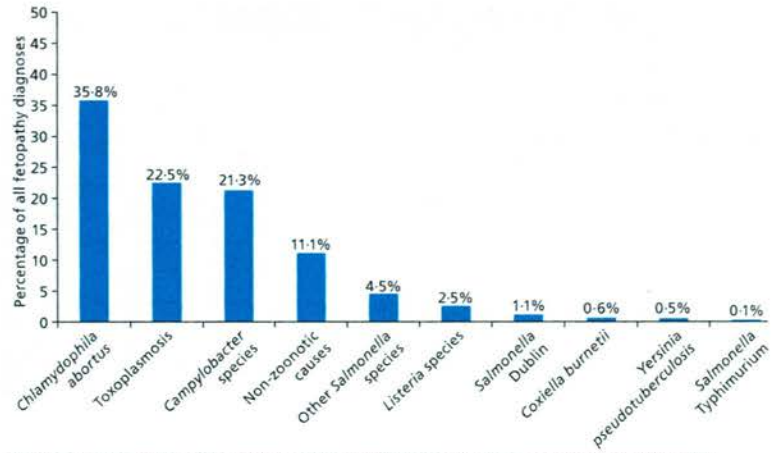


Fig 3: Graph showing all incidents of fetopathy in sheep and goats in Great Britain in 2010 as a percentage of all fetopathy diagnoses reached (n=959). Most abortions in sheep and goats are caused by zoonotic pathogens. Source, VIDA (2010)

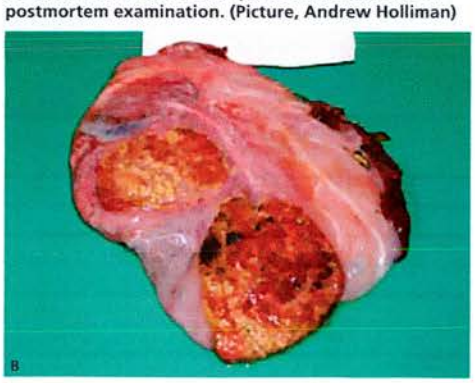
Fig 5a: Severe orf in a lamb. (Picture, Arthur Otter). Fig 5b: Small, less severe orf lesion in a goat kid. (Picture, David Harwood). Fig 5c: Typical orf lesions on the finger of a sheep handler. Orf is a common and grossly under-reported zoonotic skin condition. It can easily be introduced to farms by new animals brought in, particularly young lambs that are to be used for bottle-feeding. Infected scabs are resistant and stringent measures are required to eliminate infection from the environment

Fig 4: Calves (Fig 4a; Picture, Guda van der Burgt) and Angora goat (Fig 4b; Picture, David Harwood) with cryptosporidiosis. Cryptosporidiosis (caused by *Cryptosporidium parvum*) should always be suspected in cases of diarrhoea in young unweaned ruminants although subclinical infection is prevalent. Large numbers of resistant oocysts are shed into the environment from infected animals and can present a significant zoonotic hazard to visitors and farm staff, particularly due to the very low infectious dose required to cause human illness. Fig 4c: Calf with salmonellosis. Salmonellosis (involving various serotypes) is another major cause of diarrhoea, particularly in calves, although it appears to be a much less common cause of zoonotic illness in farm visitors than cryptosporidiosis. (Picture, Guda van der Burgt)

Fig 6: Ringworm in humans is a common (Fig 6a) but often under-reported zoonotic condition. It is mainly caused by *Trichophyton* or *Microsporum* species and occurs in many animals, ranging from cats and rabbits (often without apparent lesions) to farm livestock. Ringworm in calves is particularly prevalent and unlikely to be missed but it is much less common in sheep (lesions usually occur on the head and face) (Fig 6b; Picture, John Watkinson) and pigs (Fig 6c; Picture, Stan Done). In pigs, ringworm is mainly seen in older pigs and sows and presents as non-pruritic circular expanding hairless lesions that develop crusts

be investigated to determine whether zoonotic pathogens are involved, as they may present environmental hazards for visitors even without animal contact. After outbreaks of enteric zoonoses, it is especially important that the buildings are cleaned and disinfected thoroughly before the public is allowed onto the farm. Contaminated bedding should be disposed of hygienically, while ensuring that the public does not have access to manure or run-off water from muck heaps. Steam cleaning of buildings and equipment, including railings, is a useful adjunct to disinfection, particularly if there are concerns about resistant zoonotic organisms such as *Cryptosporidium* species.

Fig 7a: *Chlamydophila abortus* (enzootic abortion) in sheep and goats (and occasionally cattle) usually presents as a severe placentitis, seen here on postmortem examination. The infection is a well-known zoonotic hazard for pregnant women.
Fig 7b: *Coxiella burnetii* infection (Q fever) is also potentially serious in pregnant women and other susceptible groups. Most *C burnetii* infection in animals is subclinical but it can occasionally cause abortion and, in rare cases, placentitis, seen here on postmortem examination. (Picture, Andrew Holliman)



Many abortions in sheep and goats are caused by zoonotic infections that present particular risks to the public, especially pregnant women. These include *Chlamydophila abortus* and *Coxiella burnetii* (Fig 7). The latter can persist in a resistant spore-like form in bedding material for several months, and aerosols from areas used for ruminant parturitions (not necessarily abortions) present a significant hazard to farm workers and visitors. Advice on zoonotic risks to pregnant women from lambing, and for farmers dealing specifically with Q fever, is available on Health Protection Agency and Defra websites (see further information). Irrespective of the cause, groups of animals exhibiting any clinical signs of disease should always be kept isolated away from the visiting public. If zoonotic disease is diagnosed on farms that are open to the public, or those planning to host open days, it is advisable to contact the enforcing authority for health and safety legislation (see below) to allow risk assessment to be carried out and for precautions to protect human health to be adopted.

While commercial farms may be anxious to control zoonoses that cause significant animal disease, they will generally be less motivated to control zoonotic infections such as VTEC O157 that have no effect on livestock productivity. VTEC O157 is present in about 4·5 per cent of cattle and 40 per cent of cattle farms. While it is not practical to try and prevent this infection from entering a herd (see section on screening for zoonotic pathogens), good farm management, husbandry and hygiene practices can help to reduce the burden of infection with VTEC O157 and other pathogens such as *Salmonella* species, *C coli* and *C jejuni*. Most of the published work relates to cattle rather than the multiple species present on most open farms, and further studies are needed in this specific area. However, measures, such as maintaining a high level of general farm hygiene, operating a closed herd policy (not always possible on many open farms), maintaining stock in the same (preferably small) stable groups, improved water

trough hygiene, storing manure, slurry and dirty water for at least two to four weeks (with no additions) before spreading, and keeping the bedding clean and dry, are all likely to be beneficial for the control of VTEC O157 and other enteric organisms.

Screening for zoonotic pathogens

Since the mid-1990s, microbiological examination of epidemiological groups of livestock on farms (now most frequently open farms) for VTEC O157 have been undertaken by the VLA at the request of public health authorities investigating outbreaks with putative animal associations (Fig 8). Epidemiological groups of animals were sampled using a statistical approach and VTEC O157 isolates from animals were compared with those from affected people using phage typing and molecular techniques to help establish the source of infection. The findings were also used to help the enforcing authorities to adopt risk management procedures. Investigations on open farms have frequently identified VTEC O157 in a wide range of animal species in addition to cattle, including sheep, pigs, goats, horses, donkeys, ponies, deer, alpacas and llamas. Of these goats, cattle and sheep appear to be the most common sources of human infection.

When outbreaks of zoonotic illness such as VTEC O157 hit the headlines, the concept of routinely screening animals for such zoonotic pathogens is frequently raised in the minds of owners, farm managers, veterinary surgeons, human health practitioners and others. This is not a straightforward issue and test results need to be interpreted with caution. The cost–benefit implications must also be reviewed. For instance, one should consider whether there are any worthwhile interventions and whether the results will significantly affect risk management measures.

Routine disease screening can have a place with regard to zoonotic pathogens for which there is a defined course of action (eg, vaccination). For example, open farms with breeding cattle, dairy farms that open to the public or cattle farms linked to agricultural colleges should undertake regular testing (using blood or bulk tank milk samples) for *L hardjo* infection unless they are already fully vaccinated against this infection. A Q fever vaccine has been successfully used to help control the outbreak in the Netherlands and is a possible option for use (under licence) in infected flocks and herds in Britain.

Apart from the above, to undertake routine screening tests for common zoonotic infections such as VTEC O157, *Cryptosporidium* and *Campylobacter* species on livestock farms is not recommended for several reasons. Consider VTEC O157, for example – this does not cause animal disease but is shed intermittently in animal faeces, particularly from cattle, which are the main reservoir of infection. Infection is also often present in the faeces of other species, especially on open farms (possibly derived from direct or indirect contact with cattle). VTEC O157 also appears to be more common on larger premises containing many livestock species. As a result of these various issues, it would require frequent and costly testing of a large and statistically selected proportion of animals of different species to provide sufficient confidence in negative findings. The latter is essential to prevent a false sense of security and possibly a reduction in risk management procedures to protect human health. More significantly, perhaps, VTEC O157 can be introduced by infected added animals and various wildlife species such as rabbits. It can also persist in faeces on pasture for at least three to four weeks and possibly much longer (where it can constitute a zoonotic hazard for outdoor events, such as music or dance festivals on farmland). All such sources may lead to recolonisation and further shedding by livestock.

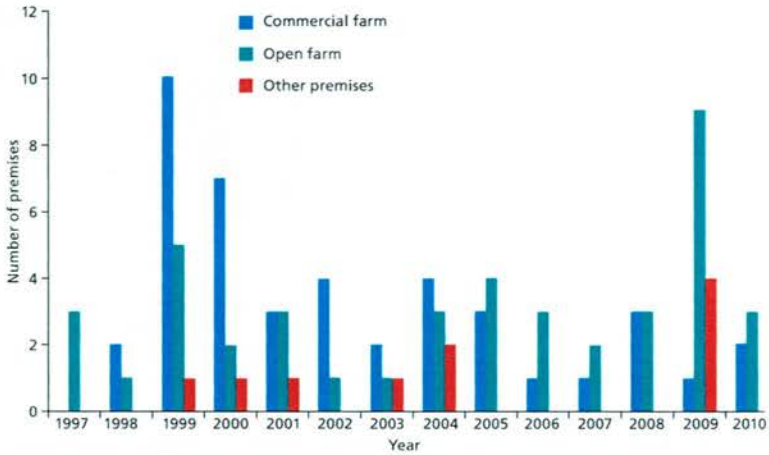


Fig 8: Graph showing the numbers of investigations into VTEC O157 outbreaks undertaken by the Veterinary Laboratories Agency at the request of public health authorities by type of premises from 1997 to 2010. From the AHVLA Centre for Epidemiology and Risk Analysis, Weybridge (unpublished data)

If VTEC O157 (or *Cryptosporidium* or *Campylobacter* species) is detected in one or more otherwise clinically normal animals, there are no obvious follow-up actions other than tightening up husbandry and hygiene measures as outlined earlier, and ensuring that adequate risk management measures are in place to protect human health. Most research into the shedding of VTEC O157 has concentrated on reducing foodborne risks from cattle rather than from direct contact with animals. A test-and-cull approach is not feasible and no intervention measures are currently available to eliminate infection at either an individual animal or farm level, although vaccination may be an option in the future. A positive result in an individual animal, such as a goat on an open farm, may also raise difficult ethical issues for the vendor if it is destined to be a family pet. It is far better to assume that any animal, especially a ruminant, is always likely to carry zoonotic infections such as VTEC O157 and therefore adopt good hygiene precautions as a matter of routine.

Health and safety legislation to control zoonoses

The primary health and safety legislation to protect the public and employees from injury and zoonotic hazards on farms is the Health and Safety at Work etc Act 1974. The Control of Substances Hazardous to Health (COSHH) Regulations 2002 made under this Act specifically includes biological hazards such as zoonotic

Box 5 : Health and safety regulations

The Health and Safety at Work etc Act 1974, and associated health and safety regulations, place duties on a wide range of people (duty holders). If you open your premises to the public, including for charity events, you have duties under health and safety law.

COSHH regulations

E coli O157 and other microorganisms that may cause ill health are subject to the Control of Substances Hazardous to Health (COSHH) Regulations 2002 (as amended). These regulations require an employer or self-employed person to:

- Assess the risks to employees, self-employed people and the public from exposure to hazardous substances, including microorganisms;
- Prevent, or, where this is not reasonably practicable, adequately control exposure to hazardous substances;
- Introduce and maintain control measures;
- Inform, instruct and train employees about the risks and precautions to be taken;
- Inform visitors about the risks and precautions to be taken;
- Regularly review the assessment and the effectiveness of control measures.

infections (Box 5). The enforcing authority is determined by the Health & Safety (Enforcing Authority) Regulations 1998. Under these regulations the Health and Safety Executive (HSE) is the enforcing authority for the legislation as it applies to open days on working commercial farms, including visits by school parties as planned occasional activities and schemes such as Learning Outside the Classroom, (LOtC), Farming and Countryside Education (FACE) and Countryside Education Visits Accreditation Scheme (CEVAS) and LEAF. No licence is required for farm open days and similar events but farmers must comply with the legislation and consult the HSE for advice if necessary. Farmers hosting open days may need to provide additional hand washing and toilet facilities to cater for visitors, the numbers of which can often be significantly more than anticipated.

Responsibility for enforcing the same legislation at open farms falls to the Environmental Health Departments of Local Authorities although delegation of responsibility is not always completely straightforward in unusual situations and local arrangements may vary depending on circumstances. Regardless of which body is the enforcement authority, the underlying regulatory framework under which control is exercised is the same for all visitor farm attractions.

Managing public health risks on farms

The microbiological risk of acquiring zoonotic illness depends mainly on:

- The number and type/age of animals;
- The number and type/age of visitors;
- The degree of contact between people and animals;
- The adequacy of control measures, particularly hand washing.

It must be assumed that endemic zoonotic infections are always likely to be present regardless of whether laboratory tests have been undertaken. Personal hygiene and other risk management precau-

Fig 9: Prominent well-worded signs are an important way to increase awareness and control the risks associated with zoonoses

Fig 10: Good, conveniently located hand washing facilities with hot and cold running water that are fully accessible to both children and adults is essential. Cleansing wipes or antibacterial gels are not an acceptable substitute. Although more expensive, knee- or foot-operated equipment is preferable and is probably more likely to be used at peak periods on open farms

tions, including avoiding contact with animal faeces, should therefore be adopted routinely.

The HSE provides an advisory document for managing the risks from VTEC O157 and *C parvum* on farms open to the public (Agriculture Information Sheet [AIS] 23). This has recently been comprehensively revised following a report into the VTEC O157 outbreak at Godstone Farm in 2009 (see further information), and includes a supplement for teachers for use for school visits. An industry code of practice is currently under development and, when finalised and agreed, will eventually replace AIS 23. AIS 2, another HSE leaflet, addresses other common zoonoses in agriculture. Guidance for zoo operators and other stakeholders on managing zoonotic risk in zoos and wildlife parks will be included in the Zoos Forum Handbook, and is available from the British and Irish Association of Zoos and Aquariums (see further information).

AIS 23 recommends that measures that are 'reasonably practicable' should be put in place to minimise risks from exposure to zoonoses acquired by the faeco-oral route. These include the provision of warning signs (Figs 9, 10), adequate supervision, avoidance of contact with animal faeces and the installation of strategically placed hand washing facilities (not medicated hand gels as these are ineffective against cryptosporidial oocysts) (Figs 9, 10). It is also incumbent on farm operatives (duty holders) to arrange the facilities and exhibits so that they minimise risks – for example, by keeping eating areas well away from animal contact areas. The most important point is that people should not come into contact with animal faeces.

Summary

There are currently no simple veterinary solutions for controlling major zoonotic pathogens such as VTEC O157 that may be present on farms, and traditional means of controlling infectious agents are not always feasible. The potential impact of zoonotic disease increases considerably when farms are opened to the general public and veterinary surgeons should help to minimise the zoonotic burden even if elimination of these hazards is not possible. The risks from farm visits need to balanced against the benefits and compared with other hazards of everyday life.

Acknowledgements

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- Guidelines for investigation of zoonotic disease, which provide general background information on dealing with zoonoses. www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Zoonoses/Guidelines/ (Accessed May 10, 2011)
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- Zoos Forum Handbook. ww2.defra.gov.uk/wildlife-pets/zoos/zoo-forums-handbook/
- Guidance on how to manage zoonotic risk in zoos and wildlife parks. www.biaza.org.uk/resources/library/images/zoonotic%20risk.pdf
- COSHH guidance. www.hse.gov.uk/coshh/index.htm?
- Advice to pregnant women during the lambing season. www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1191942128199 (Accessed June 6, 2011)

Useful websites

- www.defra.gov.uk
- www.defra.gov.uk/ahvla/
- www.hpa.org.uk
- www.hse.gov.uk/
- www.biaza.org.uk

2.3

Escherichia coli O157 incident associated with a farm open to members of the public

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Summary: *Three children, one who lived on an open farm and two who visited the farm in school parties, developed Vero cytotoxin producing* Escherichia coli *(VTEC) O157 infection in 1997. All had been in direct contact with animals. All were admitted to hospital with diarrhoea and two developed the haemolytic uraemic syndrome, one of whom developed severe neurological impairment.* E.coli *O157 phage type 21 that produced Vero cytotoxin 2 were isolated from the three children and from a goat paddock and two cows at the farm. The isolates were indistinguishable by molecular typing.*

The farm closed voluntarily for six weeks while recommendations to reduce the exposure of visitors to faecal contamination and to improve hygiene procedures (especially handwashing) were implemented.

More research is needed into all aspects of VTEC O157 on farms, including the natural history of carriage in animals, the organism prevalence and factors that affect infectivity to humans. Further consideration is needed about the role of open farms in zoonoses, and how open farms can be assisted in reducing risks for visitors. The public need to be educated about the risks and about their responsibilities, which include the need to wash hands thoroughly and to avoid hand to mouth contact when visiting farms.

Key words:
animals, domestic
Escherichia coli O157
haemolytic uraemic syndrome
hygiene
zoonoses

Commun Dis Public Health 1999; **2**: 22-6.

Introduction

Farm visits for recreational and educational purposes are popular in the UK[1]. About 250 open farms receive up to nine million visitors each year and several farms have more than 100 000 visitors annually[2]. The risk to visitors of contracting zoonotic infections is recognised[1,3,4]. Recent reports of Vero cytotoxin producing *Escherichia coli* (VTEC) O157 infections among farm visitors[2,5-8] have caused particular concern. The infectious dose of VTEC O157 is low[9] and those infected usually present with diarrhoea and severe abdominal cramps[9]. About half of these cases develop haemorrhagic colitis, which usually resolves within two weeks[9]. Between 2% and 7% of all cases in general develop the haemolytic uraemic syndrome (HUS)[10], but this proportion has risen to 21% in outbreaks[11] and, in one hospital study, was 34%[12].

Substantial gaps exist in current knowledge about the ecology of VTEC O157 in farms, including its distribution and the vehicles by which infection is spread[13]. VTEC O157 is known to occur in livestock, often without causing disease[9], and can survive for several months in soil samples[14].

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Incident report

A 7 year old boy who lived on an open farm and had access to all areas and livestock developed bloody diarrhoea on 10 May 1997 and was admitted to hospital for two days. VTEC O157 was isolated from his faeces. The boy often visited areas where pigs and birds of prey were kept and mucked out calf pens and helped with ear tagging of calves two or three weeks before becoming ill.

A 6 year old girl became unwell on 6 June 1997 and was admitted to hospital on 10 June with diarrhoea and vomiting. She made good clinical progress and was discharged after three days. Biochemical results later showed that she was developing HUS, but she responded to conservative management on a paediatric renal unit. VTEC O157 was isolated from her faeces. She had visited the open farm where the first case lived on 3 June (a dry and sunny day) as a member of a school party, accompanied by her mother. She touched several animals in the touching barn and washed her hands thoroughly before eating her packed lunch. After lunch, she touched most of the animals in the touching barn and then washed her hands before leaving the farm. She liked biting her finger nails.

When the local public health department was informed about the first case and then the second, an incident management team was convened to investigate and recommend control measures.

A 4 year old boy developed diarrhoea that contained blood on 30 June 1997 after visiting the open farm on 27 June, and was admitted to hospital on 2 July. He developed HUS with severe neurological impairment. VTEC O157 was isolated from his faeces. He too was a member of a school party and was accompanied by his mother on a visit in wet weather. He clambered on fences, looked at cattle, and stroked animals in the touching barn. He washed his hands in cold, running water before eating his packed lunch and then left the farm. He usually sucked his fingers when tired at bedtime. The identification of the third case prompted the incident management team to seek further expert advice. An ad hoc group, including team members, met once at the Department of Health.

TABLE 1 Results of farm sampling

Date (1997)	Nature of specimen	Number tested	Result
	Fresh environmental faeces		
20 June	horses	2	
	goats	2	*E. coli* isolated from goat paddock
	pigs	3	
	rabbits	2	
	chicken	4	
	sheep	4	
	guinea pigs	1	
	cattle	5	
	cattle muck scraper	1	
	milking parlour	1	
	pig slurry	2	
	cattle muck heap	2	
3 July	goats	5	*E. coli* isolated from goat paddock
14 July	goats	7	*E. coli* isolated from goat paddock
19 Aug	goat	1	
	Rectal faeces		
12 July	goats	4	
19 Aug	goats	6	
	horses	3	
	calves	4	
	lambs	1	
	piglet	1	
	sows	3	
	rabbit	1	
	homebred cows	12	*E. coli* isolated from one cow

Investigation

Epidemiological

The incident management team asked medical microbiologists in the North Thames region to examine all faecal specimens from children under the age of 10 years for *Escherichia coli* O157. Tertiary referral centres for cases of HUS in North Thames region were asked to report new cases. All isolates of VTEC O157 referred by medical microbiology laboratories within 50 miles of the farm to the PHLS Laboratory of Enteric Pathogens (LEP) in 1997 were identified in order to find cases retrospectively. Isolates of the same phage type as those from the three cases and the open farm (see below) were selected. Consultants in communicable disease control (CCDCs) were contacted and asked to enquire about possible links between those cases and the open farm.

All CCDCs were informed about the cases by electronic message (Epinet) and invited to consider reminding their local authorities and chief education officers about the risk of infection associated with educational and recreational farm visits. Local education authorities were reminded about their responsibilities to pupils visiting open farms.

Microbiological

Faecal specimens were plated on sorbitol MacConkey agar and sorbitol non-fermenting colonies were identified as putative *E. coli* O157 by agglutination with *E. coli* O157 antiserum[9]. The strain of VTEC O157 from the third case was identified by immunomagnetic separation and culture[15]. Samples taken from the farm environment and livestock were initially examined for VTEC O157 by similar methods[16]. All isolates underwent serotyping[17], phage typing[18], Vero cytotoxin (VT) detection[19], VT2 subtyping[20], and pulsed-field gel electrophoresis (PFGE)[20] at LEP.

Environmental

Inspection of the farm identified ways in which farm hygiene for visitors would be improved. Appropriate advice and recommendations were given.

Results

Epidemiological

Prospective surveillance identified no further cases. Eight cases of VTEC O157 phage type (PT) 21 (see below) were identified retrospectively, but none with known links to the open farm.

Microbiological

Isolates of VTEC O157 from the three cases were of PT 21, which hybridised with the VT2 gene probe but not with VT1. Subtyping of the VT2 genes showed that the strains possessed both VT2 and VT2c sequences. PFGE patterns of the strains were identical. VTEC O157 was detected in five of 89 samples taken at the farm (table 1). Isolates from the goat paddock and cattle were indistinguishable from strains isolated from the cases.

TABLE 2 Control measures

Recommendations	Measures taken
Reinforce precautions to be taken by visitors	Visitor leaflet updated; signposting improved; bootwashing facility installed
Reassess the provision of general hygiene facilities, especially those for handwashing and its supervision	More washbasins installed at strategic points and made more attractive by signposting or building features around them; guides instructed about hygiene by environmental health officers
Reassess the species type and age of animal kept in the touching barn	Animals and activities undertaken there reviewed; calves removed
Designate and segregate eating and drinking areas from animal touching areas	Identifiable zones created within the farm – eating zones, non-eating zones, play zones, etc; double fences installed between eating areas and adjacent animal paddocks
Avoid visitor exposure to faecal contamination, access to manure heaps, etc	Paths subject to faecal contamination brushed regularly; manure heaps resited
The farm trail used by visitors crossed a road used by dairy cattle going to and from the milking parlour. There was heavy faecal contamination in this area and an alternative route or means of access was needed	Removable bridge installed
Reinforce the 'no touch' policy in parts of the farm trail – eg, in the calf pen area	Additional signs installed and supervisors were told to discourage touching in any area not specifically designated as a touching area
Aid decontamination of the goat paddock (see table 1) by putting it out of use for six weeks and keeping the grass short	Goats removed and isolated; grass kept short
Be rigorous about detergent cleaning in areas of public access and in the appropriate use of disinfectants	Detergent cleaning/disinfection of huts and fences carried out
Install a barrier system to prevent contact between the public and cows/calves in the calving barn	No visitors within calving barn; additional barriers installed
Prohibit access of visitors to the milking parlour	Viewing gallery used

Environmental

The open farm was part of a business that included an adjoining commercial farm with a dairy herd of about 250 cows and a manufacturing premises (about a mile away) that produced high quality yoghurt and ice cream from milk produced at the farm. These products were sold in a cafe at the farm and also supplied to high street shops. The open farm had been operating for about nine years and opened 363 days a year, receiving about 112 000 visitors each year, including 30 000 schoolchildren. School parties arrived about 1030 and departed by 1430. Pupils brought their own packed lunches, which were left in a function room on arrival. Parties were prebooked and none had reported illness after visiting the farm. Up to 12 parties toured simultaneously, about 600 people daily. Visitors followed a predefined trail that took them past sheep, goat, and pig paddocks, a pets corner, and outdoor picnic areas. Visitors could watch milking at the adjoining dairy farm. A touching barn had been built during the winter of 1996/7, where visitors could enter wooden pens and touch animals. Signs were displayed about handwashing after animal contact and handwashing facilities were available in the touching barn, function room, and cafe. Teachers, parent helpers, and staff supervised. Visitors were allowed to eat or drink throughout the open farm. Faecal matter was present in visitor areas, especially on a road used by dairy cattle.

Control measures

Several recommendations were made to the farm after the second case occurred. All house rules were to be promoted; advice about hygiene at the farm was to be emphasised to staff and party organisers, especially the importance of thorough, supervised handwashing; and visitors should not be able to touch calves.

After the third case was reported on 7 July, the team recommended to the Health and Safety Executive (HSE) that the farm should close immediately to visitors, pending further expert advice. The farm closed voluntarily to visitors on 8 July. The farm management responded to additional recommendations by implementing several specific new or revised practices (table 2). Arrangements for school parties were also reviewed to ensure that schools received information before visiting and that guides instructed parties on arrival. Teachers were to be given revised and updated information and a checklist to confirm that all actions were taken. Handwashing was to be encouraged by introducing a 'fun' element. Children were to keep all food (including sweets) with their packed lunch and those aged under 12 years had to be supervised. Wellington boots or similar were to be worn on the farm, and children were to bring clean footwear to change into afterwards.

The farm reopened to visitors on 23 August, after the new or revised practices were implemented.

Discussion

Two of the three children infected with VTEC O157 in this incident developed features of HUS, and one suffered severe neurological impairment. VTEC O157 is an important cause of renal impairment[11,12,21]. Of 173 people affected in 18 reported outbreaks, 21% developed HUS and 3% died[11]. Two of these outbreaks were associated with contact with livestock and of the nine people who were ill, seven were children and four developed HUS. Twenty-nine of 85 children with VTEC O157 developed HUS in one study[12]. HUS is believed to be the commonest cause of acute renal failure in children in the United Kingdom and may be fatal in up to 10% of cases[9]. Those who recover may have long term renal damage[9]. Thus infections with this organism may have serious and distinct implications for public health.

The incident management team had to establish at the outset who had statutory responsibilities for the incident, particularly in relation to the HSE, Veterinary Investigation Centre (VIC; part of the Veterinary Laboratories Agency), and the local authority. Under the *Health and Safety (Enforcing Authority) Regulations 1989* the enforcing authority for the *Health and Safety at Work etc Act 1974 (HSW Act)* can, depending on the nature of a farm, be either the HSE or the local authority. At this farm, the HSE was the enforcing authority as the 'main activity' was agriculture, whereas an open farm that is primarily an exhibition or show farm rather than a working commercial farm would be enforced by the local authority. The *HSW Act* requires 'reasonably practicable' steps to be taken to protect employees and public alike. Under the legislation, the enforcing authority has powers to issue enforcement notices, although appropriate evidence is needed before an improvement or prohibition notice can be issued. Enforcement notices are invariably accompanied by a schedule that specifies the necessary remedial measures. In this incident, enforcement notices were not issued as the farm management closed the farm voluntarily and HSE considered that the farm was already doing all that was 'reasonably practicable' to fulfil its statutory duties and conform to existing guidance. Such issues need to be discussed by incident management teams.

The local VIC had the facilities and expertise to undertake appropriate sampling at the farm. It provided advice on farm hygiene and husbandry, and made observations to indicate likely risk areas. The local authority was responsible for ensuring that the food manufacturing premises and farm cafe complied with the legislation relating to food hygiene and had duties under the *Public Health (Control of Disease) Act 1984*. Press enquires about health were handled by the regional office of the NHS Executive.

The value of identifying strains of VTEC O157 was evident[2,5-7]. All isolates from the incident were indistinguishable, which provided evidence of an association between the human cases and the farm and helped to justify the recommendations made. Phage type 21 was identified in 15.9% of all VTEC O157 in England and Wales confirmed by LEP in 1997 (HR Smith, personal communication).

More basic research is needed on VTEC O157 in the farm environment. As sampling and its funding is problematic, the indications for doing it and the significance of positive and negative results need to be better understood. VTEC O157 infection usually causes no illness in animals, so farmers have no financial or animal husbandry incentives to seek it out[13]. The natural history of carriage in animals, the prevalence of the organism, and factors that affect infectivity to humans are poorly understood. More needs to be known about the relative risks of handling different animal species.

The most important vehicle of infection appears to be animal faeces from infected livestock[13]. We detected VTEC O157 initially in faeces obtained from a paddock that housed goats. Goats have previously been shown to carry the organism[5] and our follow up investigations concentrated on this species. The main reservoir of VTEC O157 is cattle[13], most commonly dairy calves[9], and we isolated the organism from two cows. One study found no excretion of VTEC O157 from a dairy herd during a seven month winter/spring period and a maximum prevalence of 14% in lactating cows and 56% in calves[22]. Seventy-four per cent of the animals that excreted did so on only one occasion[22], providing evidence that VTEC O157 in herds may be transitory and cannot be defined by testing a few animals at one time[9].

Farm visits are popular[1,2], but the educational and entertainment benefits need to be balanced against potential risks of zoonoses. Legislation relating to this is based on risk assessment, which led to our recommendations. Consideration should be given to how open farms, especially large scale enterprises that adjoin commercial working farms with livestock, can be helped to reduce risks to visitors. A hazard analysis of critical control points (HACCP) approach would be appropriate.

The infections in this incident occurred in a child resident on the farm and in two supervised party visitors. We are concerned particularly about how farms manage school visits. The Pennington report (1997) stated that the educational and other benefits of school visits to farms should not be lost, but stressed the need to ensure that appropriate hygiene measures were taken[13]. In practical terms, the quantity of faeces, often liquid, produced by livestock makes environmental contamination inevitable.

General measures need to be taken to improve hygiene on open farms and a national debate is needed about the public health implications of open farms and activities undertaken there. Guidance has been produced, recommending more handwashing facilities, supervision of children's handwashing, and separation of eating areas from animal areas[23]. People of all ages are encouraged to visit open farms and have close contact with the animals including watching them give birth. Visitors must be informed about the

risks and about their responsibilities, including the need to wash hands thoroughly and to avoid hand to mouth contact during visits.

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Verocytotoxin-producing *Escherichia coli* O157 on a farm open to the public: outbreak investigation and longitudinal bacteriological study

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Verocytotoxin-producing *Escherichia coli* (VTEC) O157 phage type 2 (PT2) was isolated from three calves and two goats on a farm open to the public. Phenotypic and DNA-based typing showed that the strains were identical or very closely related to those obtained from an outbreak of VTEC O157 infection in two separate family groups who visited the farm. No VTEC O157 PT2 was isolated again from the farm during a 12-month longitudinal bacteriological study undertaken after the infected animals had been removed. However, phenotypically and genotypically indistinguishable VTEC O157 PT21/28 strains were detected in two of 474 faecal samples collected at monthly visits from 15 species of animals of various ages. The two isolates were obtained from calves from different sources sampled 146 days apart, suggesting that the infection had persisted on the farm although it was not detected in the other species. The same strain was subsequently isolated from another calf housed in the same pen as one of the infected calves. The longest period during which the organism was excreted was seven days. No VTEC O157 was isolated either from 204 replacement animals (including 138 orphan lambs and 10 calves) brought in from various sources, and sampled while they were kept in isolation for two weeks before being introduced to the farm, or from environmental samples. During the study a visitor became ill with VTEC O157 PT2. However, the isolate was distinct from those recovered from the farm and there was no evidence to suggest that the visit was the source of the infection.

VISITS to farms for recreational or educational purposes have become an important part of the tourism and leisure industries. They are generally either to farms open to the public throughout the year (open farms) or to occasional open days on commercial working farms. Although there are potential risks to the visiting public from exposure to physical hazards, including traumatic injury by livestock, and from various zoonotic infections (Dawson and others 1995), the number of reported incidents is relatively small. However, the emergence of verocytotoxin-producing *Escherichia coli* (VTEC) O157, with its very low infectious dose and associated risks of serious human illness, has greatly increased the potential importance of zoonotic disease acquired from farm livestock, including those on open farms.

Although VTEC was first described in the late 1970s, it was not until 1982/83 that VTEC O157 was first recognised as a significant human pathogen. It is associated with a broad spectrum of human illness, including diarrhoea, abdominal cramps and haemorrhagic colitis (Griffin and Tauxe 1991) and there can be severe systemic complications, particularly haemolytic uraemic syndrome. The organism is transmitted mainly by foodborne infection (particularly undercooked minced beef and unpasteurised milk or cheese), by person to person spread or by direct or indirect contact with animals (Parry and others 1995, Trevena and others 1999). Cattle are the principal reservoir of VTEC O157 but it has also been isolated from other species including goats, sheep, deer, pigs, horses, dogs, chickens, flies and wild birds (Hancock and others 1998, Wallace 1999). Although VTEC O157 has been isolated (rarely) from calves with enteritis (Daniel 1999), experimental infections of gnotobiotic calves have failed to induce any significant disease (Woodward and others 1999).

Several workers, including Chapman and others (1997), Hancock and others (1997) and Mechie and others (1997), have undertaken longitudinal bacteriological studies to examine the variable prevalence and persistence of VTEC O157 in farm animals (mainly cattle) and their environment. There are several published accounts of outbreaks of human VTEC O157 infection associated with visits to open farms, most recently by Milne and others (1999) and Trevena and others (1999). This paper describes the results of investigations into another outbreak of VTEC O157 on an open farm and includes a follow-up longitudinal bacteriological study of the carriage of VTEC O157 in the various species of animal kept on the farm. Replacement animals entering the farm were also tested for VTEC O157 while they were being kept in isolation.

MATERIALS AND METHODS

Outbreak of VTEC O157

In August 1997, surveillance by the local health authority revealed 12 cases of VTEC O157 infection in nine households. Subsequent investigations linked four clinical cases and one asymptomatic excreter in two separate family groups (X and Y) to visits to an open farm in East Anglia. A 10-year-old boy in family X became ill the day after a visit at which he had handled goats. He was admitted to hospital with gastroenteric symptoms and VTEC O157 was isolated from his faeces. He was later discharged without adverse consequences; other family members were unaffected. A two-year-old boy in family Y developed fever, abdominal pain, vomiting and bloody diarrhoea six days after a visit at which he also had touched the goats and a cow. He subsequently developed haemolytic uraemic syndrome and had serum antibodies to *E coli* O157 lipopolysaccharide but a stool culture did not yield VTEC O157. His 18-month-old sister became ill 10 days later with bloody diarrhoea; VTEC O157 was isolated and she also developed haemolytic uraemic syndrome. The late onset of this infection suggested secondary person to person spread within the household. The parents were culture-positive for VTEC O157 but only the father was unwell.

Farm details

The farm was open to the public throughout the year and maintained a variety of animals on a few acres in a rural location. A cafeteria and public conveniences were located on the same site but separate from the farm. It had 60,000 to 70,000

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visitors annually (up to 1000 per day), mainly in family groups but including about 3000 children in prearranged school parties. The farm operated to good standards of hygienic practice; mains drinking water was used for the livestock. Before the outbreak, knee-operated washbasins for visitors had been installed near the exit. Notices were prominently displayed advising the need for good personal hygiene, and educational and advisory leaflets were also provided. Additional health and safety talks were given to all the visitors attending the closely supervised bottle feeding sessions for lambs.

The numbers, ages, species and groupings of livestock varied with the time of year and the availability of suitable young animals from local farms for short-term rearing to maintain adequate farm attractions. Resident stock usually comprised a minimum of about 12 sheep, four goats, two cows, two calves, three pigs, two donkeys, two ponies, 12 rabbits, 15 guinea pigs, six chipmunks, 80 laying hens, 10 bantams, 20 to 30 ducks, four geese and a variety of wild waterfowl. At the time of the outbreak the farm was unusually overstocked, with 30 goats and eight calves. Each year between February and April there was an influx of about 100 to 150 orphan lambs from several local sources specifically for bottle-feeding by the visiting public. A domestic dog was introduced during the latter part of the longitudinal study described below. Most of the animals were housed in pens between November and March and then went out to small grass paddocks or concrete-floored open yards. When lambs, calves and pigs became too old to handle they were either fattened elsewhere or sent for slaughter.

Initial field investigations

The farm was visited on September 9 and 18, 1997, to investigate the possible source of the human infection. The visits followed enquiries by environmental health officers from the local authority and were initially undertaken 21 days after family X and 16 days after family Y visited the farm. Individual rectal swabs were taken into Amies gel transport media, or freshly voided individual or pooled faecal samples were collected in new 50 ml plastic pots with separate sterile wooden spatulas or new disposable plastic gloves from groups of animals, particularly those handled by the affected families. Microbiological investigations were undertaken to test for the presence of VTEC O157 and any isolates were compared with those from the human cases. Hygiene and husbandry practices were also assessed as possible risk factors.

Longitudinal study

Before the 12-month longitudinal microbiological study of VTEC O157 infection commenced in November 1997, most of the goats and calves present at the time of the outbreak, including all those identified as excreters of VTEC O157, were removed from the farm and the buildings were thoroughly cleansed and disinfected. A small grass paddock used by the infected goats was left empty for two months in an attempt to prevent a carry over of infection. Subsequently, 10 soil scrapings, each of about 50 g and to a depth of about 1 cm, were taken with sterile wooden spatulas from an area of about 20 m^2 around a shelter in the above paddock, and cultured for VTEC O157.

Study design Samples were taken monthly to monitor the prevalence and persistence of VTEC O157 in the different species and groups of resident stock and in the environment. Brought-in animals were also sampled while they were kept in isolation for two weeks to determine whether pre-entry bacteriological testing might have a role in preventing the introduction of infection. After leaving isolation, these animals were included in the monthly sampling of resident stock. Goats, sheep (excluding orphan lambs) and calves were isolated in fibreglass calf hutches (JFC Manufacturing) on a concrete platform located at least 20 m from resident stock. The hutches were thoroughly washed out, steam cleaned and disinfected before and after use. Smaller animals, such as rabbits, were isolated in separate pens or enclosures elsewhere on the farm. Because of the large numbers involved, orphan lambs were kept in isolation on completely separate premises before joining the main farm at three or four weeks of age.

Sample collection from animals and the environment Slightly different collection methods were used for different groups and species of animals for reasons of practicality but they were consistent throughout the study and were similar to those used for the investigation of the outbreak. Individual rectal swabs, or swabs taken from freshly voided individual samples of faeces, were used for calves, goats and cows. With animals closely housed in small groups, composite samples were taken from at least six randomly selected freshly voided individual faeces. Brought-in animals were all individually swabbed rectally or, in the case of small mammals, samples of freshly voided faeces were collected immediately they arrived in isolation and seven days later. Environmental contamination (including that from wild birds), was monitored on a 3 m length of wooden fencing between two adjoining paddocks used by sheep and goats, and, on an area of about 1 m^2 on the front of an animal shelter. These sites, to which the public did not have access, were swabbed with cotton wool pads soaked in buffered peptone water (BPW) in sterile 250 ml honey jars.

Action following isolation of VTEC O157 At the outset, contingency plans were drawn up with the owners, the Consultant in Communicable Disease Control (CCDC) and the local authority (which had statutory responsibility under the Health and Safety at Work Act 1974), to ensure that public safety was not compromised in the event of VTEC O157 being isolated. Culture-positive animals, and those sharing the same pen, were segregated from public access as soon as VTEC O157 was suspected. They were not allowed back into contact with visitors until faecal samples had tested negative for VTEC O157 at least twice, 24 hours apart, seven days after the initial isolation. The pens used by infected animals were thoroughly washed out, steam cleaned and disinfected.

Surveillance of human VTEC O157 infection All human incidents of VTEC O157 infection encountered during the study period, including sporadic cases and general outbreaks (cases in two or more households), were notified to the CCDC, and environmental health officers then followed up the cases by using a standard questionnaire to investigate possible links with livestock and visits to farms.

Detection and isolation of VTEC O157

For the isolation of putative VTEC O157 from animals, 1 g of homogenised faeces, or one faecal swab, was mixed with 9 ml or 5 ml of BPW, respectively, supplemented with 0·05 mg/litre cefixime, 10 mg/litre cefsulodin, and 8 mg/litre vancomycin (BPW-VCC). For the environmental samples, 10 g of homogenised soil were mixed with 100 ml BPW; the honey jars containing cotton wool pads were made up to 200 ml with BPW. Enrichment cultures were incubated for six to seven hours at 37°C and *E coli* O157 separated with immunomagnetic particles (Dynabead anti-*E coli* O157; Dynal) (Chapman and others 1997). The beads were inoculated on to sorbitol MacConkey's agar supplemented with cefixime and potassium tellurite (CT-SMAC). Sorbitol non-fermenting colonies were examined by an *E coli* O157 latex agglutination test (Oxoid DR620G). A positive control sample was included with each day's cultures for internal quality control. Human faecal samples were plated directly on to CT-SMAC agar and sorbitol non-fermenting colonies were identified as described

TABLE 1: Properties of verocytotoxin-producing *Escherichia coli* O157 strains isolated from human and animal sources

Sample date	Source	Phage type (PT)	VT type	VT2 subtype	PFGE profiles*
Human cases					
22/8/97	10 y male, family X	2	2	VT2	PT2-12
4/9/97	18 m female, family Y‡	2	2	VT2	PT2-12**
6/9/97	Adult female, family Y	2	2	VT2	PT2-12**
10/9/97	Adult male, family Y	2	2	VT2	PT2-12**
Farm outbreak					
9/9/97	14 wk calf R	2	2	VT2	PT2-12
9/9/97	14 wk calf S	2	2	VT2	PT2-12**
9/9/97	Adult female goat	2	2	VT2	PT2-12**
18/9/97	8 wk calf T	2	2	VT2	ND
18/9/97	8 wk goat kid	2	2	VT2	PT2-12**
Longitudinal study					
2/12/97	4 wk calf A	21/28	2	VT2+VT2c	†
27/4, 2/5 and 4/5/98	13 wk calf B	21/28	2	VT2+VT2c	†
3/5/98	13 wk calf C	21/28	2	VT2+VT2c	†
Other infections of farm visitors					
19/3/98	Adult female D	2§	2	VT2+VT2c	PT2-3

* PFGE profiles were designated according to the nomenclature of Willshaw and others (1997) PT2-12 patterns designated ** showed minor variations from the index case affecting the mobility of two fragments of about 50 kb (Fig 1)
† PT21/28 strains with identical undesignated profiles
‡ Family Y also included a two-year-old male with haemolytic uraemic syndrome who was culture-negative but had serum antibodies to O157 lipopolysaccharide
§ Resistant to streptomycin, sulphonamide and tetracycline; all other isolates sensitive to 15 antimicrobial agents
PT2-3 defined by Willshaw and others (1997) differed from PT2-12 by at least eight fragment positions
ND Not done, PFGE Pulsed-field gel electrophoresis, y Year, m Month, wk Week, VT Verocytotoxin

above. The isolates were identified biochemically as presumptive *E coli* and sent to the Laboratory of Enteric Pathogens (LEP) Colindale, for confirmation and typing.

Confirmation and typing of VTEC O157 isolates

Animal and environmental isolates were examined for the presence of VT gene types 1 (VT1) and 2 (VT2) by PCR. All presumptive *E coli* O157 were characterised by the LEP on the basis of their antibiotic sensitivity, serotyping, phage typing and hybridisation with DNA probes for VT1 and VT2 genes (Frost 1994, Thomas and others 1996). All the isolates were compared by DNA-based tests (Willshaw and others 1997) including VT2 gene subtype and pulsed-field gel electrophoresis (PFGE). Some strains were also compared by the hybridisation of genomic DNA with a probe comprising a VT2-encoding phage.

RESULTS

Outbreak investigation

A total of 64 faecal samples were collected during the investigations on the farm. At the second visit the calves were all resampled and samples were also collected from the goats that had not been sampled previously. Thirty samples were taken from adult goats and kids, 16 from calves, eight from adult sheep and lambs, two from cows, three from donkeys, four from adult pigs and piglets, and one from a guinea pig.

VTEC O157 PT2 was isolated from five (7·8 per cent) of the 64 samples. They came from three of eight calves being reared for local farms, from one adult goat and from a two-month-old goat kid (from a total of 30 goats on the farm). Two of the calves (R and S), both aged 14 weeks, came from the same farm and one (T), aged eight weeks, was from an unrelated source. Calves R and S were identified as VTEC-positive from the samples collected on September 9, but were both negative by the second visit nine days later. Calf T was negative at the first visit but positive at the second.

The five animal isolates were typed as VTEC O157 PT2, VT2 and were fully sensitive to the range of antibiotics tested; VT2 gene subtyping showed they all carried the sequence for VT2 but not VT2c. They were indistinguishable in these properties from four VTEC O157 strains infecting the human cases (Table 1). Genomic DNA fragments of four human and three animal strains were hybridised with a VT2-encoding bacteriophage: identical patterns of hybridising fragments were obtained. Fingerprinting of all the PT2 isolates by PFGE showed that the strains gave at least 23 DNA fragments after digestion with *Xba*I (Fig 1). The profiles of the index case (family X) and calf R were identical and classified as PT-12 (Table 1).

All the other profiles were very closely similar to PT2-12 with only minor differences involving two fragments of about 50 kb (Fig 1). These differences were not considered to be significant in the light of the epidemiological and other typing data. Phenotypic typing and genotypic analysis by three independent methods therefore implicated the farm animals as the initial source of the human infection and supported the epidemiological links between the human cases and the farm.

Longitudinal study

Sample collection from animals and the environment

A total of 474 faecal samples were cultured from 15 species of resident stock of different ages. Twenty-three samples were taken from adult sheep, 59 from lambs, 40 from adult goats, four from goat kids, 24 from cows, 29 from calves, 20 from donkeys, 13 from ponies, 73 from adult pigs, 24 from young pigs, five from the dog, 44 from rabbits, 22 from guinea pigs, 12 from chipmunks, 60 from chickens and bantams, 19 from ducks, two from geese, and one from a pheasant. The 204 brought-in animals were each sampled twice while they were in isolation; they consisted of 138 orphan lambs, 30 assorted rabbits and guinea pigs, 11 other sheep (including adults), 10 calves, nine pigs, four goats, one cow and a dog. Twenty-four environmental samples were collected (in addition to the 10 taken before the longitudinal study was started).

Isolation of VTEC O157 VTEC O157 was isolated from two (0·4 per cent) of the 474 samples from the resident stock. The isolations were made from samples collected on December 2, 1997, from a calf (A) aged four weeks, and on April 27, 1998, from a calf (B) aged 13 weeks. Both calves had been introduced at about one week of age from unrelated local farms, were housed in different buildings and were negative for VTEC O157 when they were tested in isolation. Calf A was from the same source as the infected calves R and S associated with the human outbreak. No VTEC O157 was isolated from any of the other animal species on the farm (either resident or when in isolation) or from any of the environmental samples.

Further sampling As soon as the preliminary bacteriological results were known (about three days after the samples were collected) the infected calves, and other animals sharing the same pen, were removed from public access and individually resampled during the next two weeks. No VTEC O157 was isolated again from calf A or another calf, cow and donkey in the same pen. Calf B was positive on the fifth and seventh days after the first positive sample was collected and another calf (C), which shared its pen, was positive on day 6 only. All three calves subsequently returned to public display after their pens had been cleaned and disinfected. There were no further isolations of VTEC O157 from any of the calves up to the time of their departure in April (A) and July 1998 (B and C).

Typing of VTEC O157 isolates from animals All three infected calves carried antibiotic-sensitive VTEC O157 PT21/28, VT2. These strains were indistinguishable from each other by VT2 subtyping and PFGE (Table 1). This suggested a common source of infection and its apparent persistence on the farm

for at least 146 days between December 2, 1997, and April 27, 1998.

Surveillance of human VTEC O157 infection Sixteen human cases of VTEC O157 infection were notified to the CCDC during the study period but only one of them (adult female D) had any apparent association with the open farm. She was diagnosed with VTEC O157 infection in March 1998, two days after visiting the farm. The strain isolated was PT2 and had both VT2 and VT2c gene sequences (Table 1). It was resistant to streptomycin, sulphonamide and tetracycline. Its PFGE profile differed at more than eight fragment positions (out of 25) from the PT2 strain associated with the outbreak of human infection the previous year. No strain of VTEC O157 with these properties was isolated from animals on the farm during the study period.

DISCUSSION

During the past 10 to 15 years there has been a remarkable growth in the numbers of open farms, farm open days and rural attractions. This growth has unfortunately been associated with a very small proportion of visitors who have acquired illnesses associated with VTEC O157 infection or other zoonotic conditions such as cryptosporidiosis (Pritchard and Fleetwood 1995). The serious public health issues associated with VTEC O157 infection are the life-threatening complications (Dundas and others 1999) of haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura which can develop mainly among children under four and adults over 65 years of age. Young children, especially those from urban areas, are a particularly vulnerable group by virtue of their age and immunological naivety to common endemic infections present in the farm environment.

Between 1994 and 1999, eight (9 per cent) of 89 general outbreaks of VTEC O157 infection in England and Wales were linked to visits to open farms (LEP, unpublished data). These outbreaks, like that described here and that described by Milne and others (1999), each generally involved only a few cases (51 in total, including secondary spread within households) although there were at least 20 primary cases in the widely publicised outbreak in North Wales in 1999. The authors are not aware of any outbreaks comparable in scale to those linked to foodborne infection, like those in the USA or central Scotland (The Pennington Group 1997) or to the large scale outbreaks of cryptosporidiosis that have occurred among farm visitors, particularly during lambing (CDSC 1994, Pritchard and Fleetwood 1995). The small number of cases of VTEC O157 infection compared with the large number of visitors to farms – estimated by the National Association of Farms for Schools as almost 13 million in England and Wales in 1998 alone – suggests that the risk of acquiring the infection is very small. However, most human VTEC O157 infection is sporadic (Trevena and others 1999) and recent evidence indicates that such cases may be more closely linked to direct or indirect zoonotic contact with farm livestock than previously suspected. This evidence is based on an evaluation of risk factors (Coia and others 1998, Parry and others 1998, Chalmers and others 2000) and case-control studies (Adak and others 2000, Locking 2000). Trevena and others (1999) found microbiological evidence of zoonotic transmission of VTEC O157 from farm animals in seven cases, and links with farm animal contact in another 16 of 69 index cases in Cornwall and west Devon between 1994 and 1997.

There is little published information concerning the prevalence and persistence of VTEC O157 on open farms. Unpublished epidemiological investigations, including the typing of isolates by LEP, undertaken by VLA regional laboratories (formerly Veterinary Investigation Centres) into outbreaks on open farms in England and Wales since 1994 have implicated goats and cattle more frequently than other species. However, it is not possible to reach any definite conclusions about its relative prevalence in different species because not all species were present or sampled in each incident. Nevertheless, it is apparent from this study and previous accounts of outbreaks of VTEC O157 on open farms (Parry and others 1995, Shukla and others 1995, Trevena and others 1996, Milne and others 1999) that cattle (mainly calves) and goats appear to present particular risks. Unpasteurised goats milk has also been implicated in human infections (Bielaszewska and others 1997). Synge (1999) linked human cases of VTEC O157 infection in Scotland to sheep, goats, horses and domestic geese, in addition to cattle. The absence of isolates from small mammals, such as guinea pigs, may be associated with the different bacterial flora in their hind gut (Rasmussen and others 1999).

During the longitudinal study, VTEC O157 was isolated from three of the 10 calves that were present on the farm but not from any of the other 14 species sampled. The very low overall prevalence (0·4 per cent) in the faecal samples was similar to that reported by Rahn and others (1997) in a multispecies longitudinal study on known infected dairy farms in Canada. They also found VTEC O157 only in cattle and not in the other species

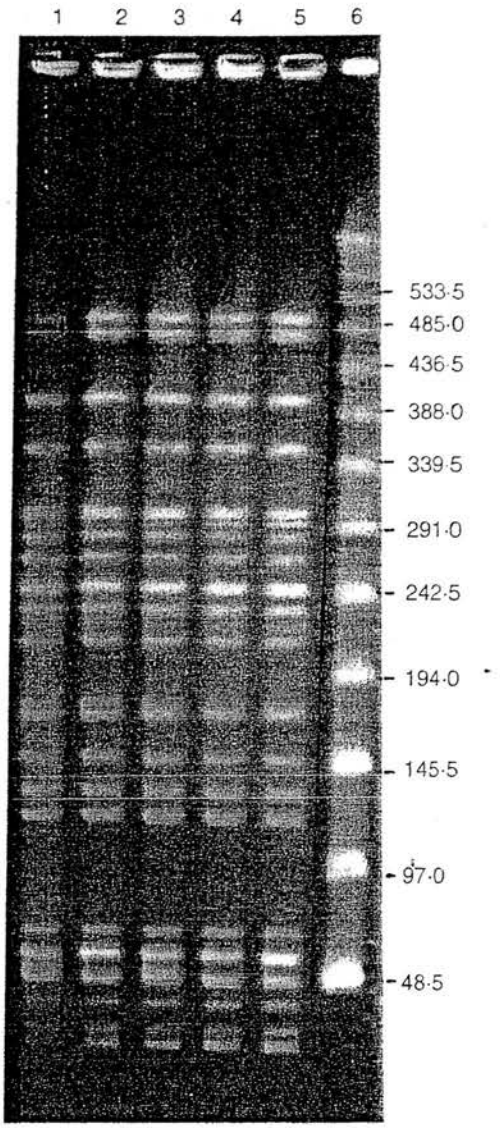

FIG 1: Pulsed-field gel electrophoresis of genomic DNA from strains of verocytotoxin-producing *Escherichia coli* isolated from studies associated with the farm. DNA was digested with restriction enzyme *Xba*I and separated as described by Willshaw and others (1997). Lane 1 10-year-old, family X (index case), Lane 2 Calf S (also adult female, family Y), Lane 3 Calf R, Lane 4 Adult goat, Lane 5 Adult male, family Y (also 18-month-old child, family Y), Lane 6 Molecular size marker, 50 kb to over 500 kb

sampled. The higher proportion (7·8 per cent) of VTEC O157-positive samples obtained from the farm outbreak, including three of the eight calves and two of the 30 goats, was probably associated with the larger number of these species on the farm at that time. Bacteriological investigations during the outbreak, and the additional sampling during the longitudinal study confirmed the transient pattern of shedding of the organism, the maximum period of excretion detected being seven days (calf B). In field studies by Mechie and others (1997) and Rahn and others (1997), the longest periods of excretion detected in cattle were four weeks and two months, respectively, although these periods could have included reinfections. Naturally infected animals may shed the organism for shorter periods than those infected experimentally (Wallace 1999).

A different strain of VTEC O157 was isolated during the longitudinal study (PT21/28) from that isolated during the outbreak (PT2). Unlike the outbreak strain, PT 21/28 appeared to persist on the farm for at least 146 days, despite the absence of direct contact between the two sets of infected calves and the adoption of disinfection procedures similar to those applied after the outbreak. There was no evidence that the strain spread to other species. Studies made under simulated environmental conditions (Maule 1997, Randall and others 1999) have shown that VTEC O157 can survive in soil for up to 150 days, in cattle faeces for up to 90 days, and in straw and tap water for up to 38 weeks. These results suggest that once pasture, buildings, water supplies or other non-animal habitats on the farm become contaminated, the organism can remain viable for several months. In the present study, in common with that of Rahn and others (1997), no VTEC O157 was isolated from the environmental samples, suggesting that if the organism persisted it was below detectable levels. Unfortunately, the environmental sampling could not be extended into the buildings because it would have necessitated stopping the routine cleaning and disinfecting in these areas and might have compromised public safety.

No link was detected between the human case of VTEC O157 PT2 infection (D) and the animal isolates. This suggested that the farm was probably not the source of the infection. However, in view of the intermittent and short-lived excretion of the organism, the possibility that this, or other strains, were present but not detectable by the sampling protocol and isolation methods used, particularly the inclusion of antibiotics in the broth culture media (Duffy and others 1998), could not be ruled out completely. In 1998, PT2 was the most commonly identified VTEC O157 phage type from human infections in England and Wales (31 per cent) compared with PT8 (18 per cent), PT21/28 (16 per cent) and all other phage types (35 per cent) (CDSC 1999).

The effects of dietary changes and management factors on the proliferation and faecal shedding of VTEC O157 by animals are unclear. There is, however, accumulating evidence (Mechie and others 1997, Synge 1999) of a seasonal excretion pattern (early summer and autumn), partly coinciding with peaks in human infection (late summer). The negative culture results from the 204 samples from various species of brought-in animals (including 138 orphan lambs and 10 calves) which were sampled while they were kept in isolation provided no evidence to suggest that stress factors associated with recent transportation and introduction to a new farm environment led to an increase in the shedding of the organism. Although calves A, B and C had come on to the farm during the previous three to 12 weeks they were apparently not excreting VTEC O157 on arrival. Rahn and others (1997) observed that open herds have a higher risk of VTEC infection; preventing the introduction of infected cattle may therefore reduce the prevalence of VTEC O157. However, from the limited evidence provided here, there appears to be no obvious value in the bacteriological testing of brought-in replacements while they are kept in isolation, at least not within two weeks of their arrival. Routine bacteriological screening of resident stock for evidence of VTEC O157 infection is also likely to give misleading results, because of the intermittent and transient excretion of the organism. Moreover, positive screening results can present a moral dilemma for those managing open farms and could lead to the arbitrary culling of infected animals. However, as demonstrated here, faecal culture and phenotypic and DNA-based typing of isolates are clearly invaluable epidemiological tools in investigations of an outbreak.

The public is often unaware or dismissive of the zoonotic risks associated with farm visits, although attitudes are likely to change in the light of recent media attention. Concerns for public safety have led to the introduction of advisory guidelines for visits to open farms and the control of VTEC O157 infection (Health and Safety Executive 2000, Public Health Laboratory Service 2000). Although some observers may consider that the very small risk of acquiring serious illness is not worth taking, the risk needs to be carefully balanced against the undoubted educational and amenity value of farm visits. If visitors adopt a common sense approach and organisers provide good standards of hygiene, hand-washing facilities, supervision and advice, and eating and animal handling areas, are strictly separated, the risks can be minimised.

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Verocytotoxin-producing *Escherichia coli* infection and private farm visits

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DIRECT or indirect contact with animals and their faeces is an important transmission route for verocytotoxin-producing *Escherichia coli* O157 (VTEC O157). Several outbreaks and sporadic incidents of infection have involved open farms or contact with farmland (Willshaw and others 2001). Case-control studies in England and Scotland have demonstrated animal contact as a significant risk factor (Locking and others 2001, O'Brien and others 2001). Children are particularly vulnerable to infection by this route and the low infectious dose of VTEC O157 results in secondary spread in families. VTEC O157 survives well in the farm environment depending on the matrix in which it is present as summarised by McDowell and Sheridan (2001). This presents problems for the control of infection, particularly on farms that are open to the public and which attract large numbers of young visitors. This short communication describes two separate incidents of infection separated over 30 months in visitors to the same commercial farm that were caused by very closely related VTEC O157 strains.

Infection with presumptive *E coli* O157 was diagnosed in a four-year-old boy who developed bloody diarrhoea after a visit to a family dairy farm in south-east England in November 1998. He visited cattle sheds, stroked a dog and a horse and drank unpasteurised milk. His six-month-old brother was asymptomatic but culture-positive. Presumptive *E coli* O157 was isolated from 10 of 28 rectal swabs collected from cattle of varying ages on the farm in January 1999. The organism was not isolated from the unpasteurised milk, the dog or the horse. All isolates from the children and cattle were confirmed as *E coli* O157 phage type (PT) 8 and possessed genes for both VT1 and VT2 toxins. All were sensitive to antimicrobial agents. Microbiological investigation of animal speci-

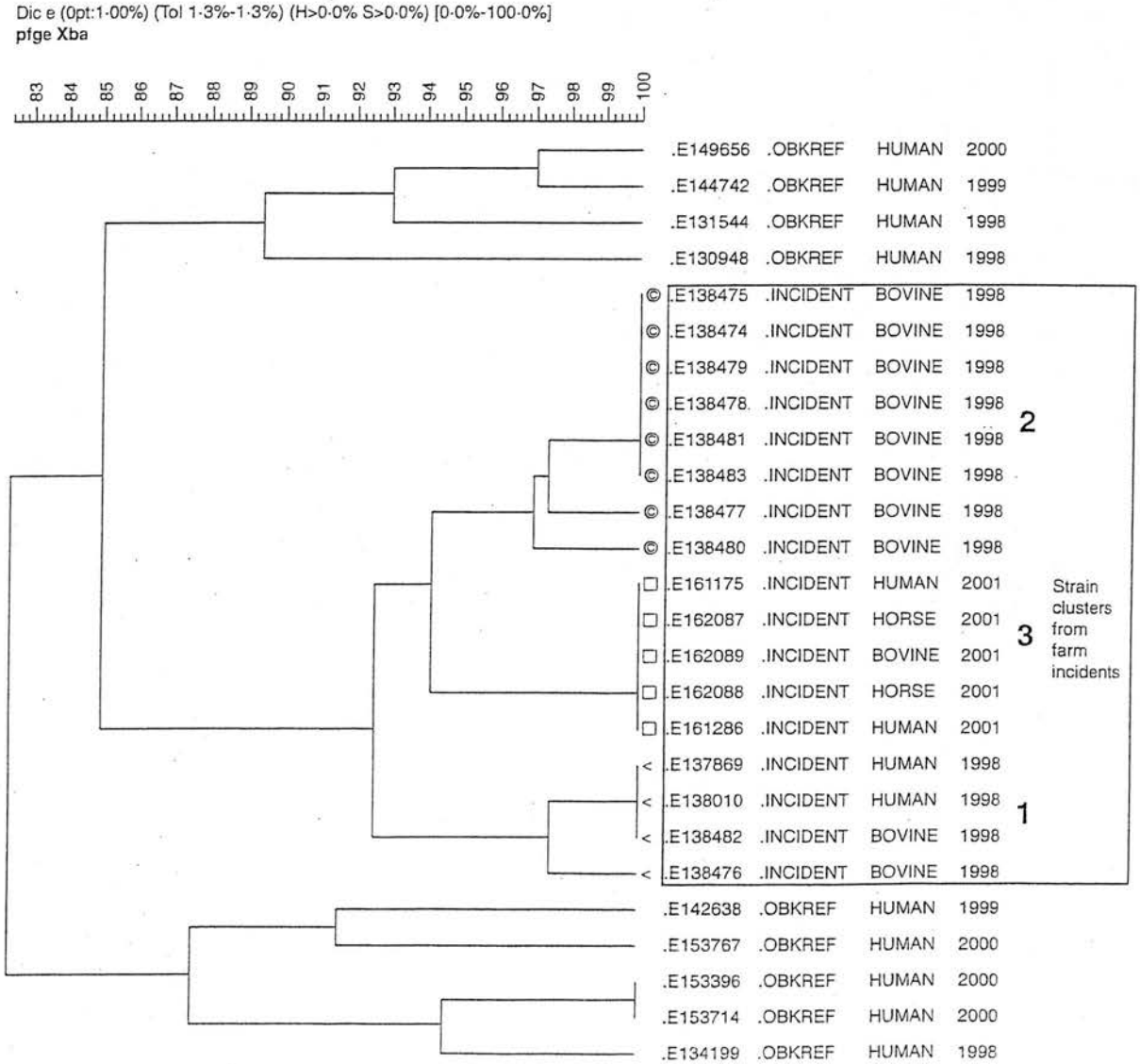

FIG 1: Cluster analysis of pulsed field gel electrophoresis profiles of verocytotoxin-producing *Escherichia coli* O157 PT8 strains with Bionumerics software by the unweighted pair group method using arithmetic means. Strains within the box were from the incidents at the farm in 1998 and 2001. Clusters identified in the text are indicated (1 <, 2 ©, 3 □). The other strains caused general outbreaks of infection between 1998 and 2001 and were not epidemiologically linked to each other or to the farm

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mens as described by Pritchard and others (2000) was carried out, isolation and typing of the human strains were summarised by Willshaw and others (1997, 2001).

In August 2001, a different family picnicked at the same farm in a field contaminated with faeces from 20 dairy calves and four horses. A one-year-old child, who had contact with faecal material became ill with bloody diarrhoea. Presumptive *E coli* O157 was isolated from her stool and from that of her three-year-old asymptomatic brother. He had also visited areas of the farm where young calves and horses were kept. Neither child consumed unpasteurised milk. Three of 18 calf faeces yielded presumptive *E coli* O157, one from the picnic field and two from the calf house and so did two of four equine samples. Rabbit droppings from the pasture were culture-negative. All the human and animal isolates were confirmed as drug-sensitive *E coli* O157 PT8, VT1 and VT2.

Images from pulsed field gel electrophoresis (PFGE) of *XbaI* fragments of genomic DNA of the isolates (Willshaw and others 1997) were analysed with Bionumerics software (Applied Maths). Profile comparisons were performed with a 1·3 per cent tolerance, and a similarity matrix was generated using the Dice coefficient. Clustering was by the unweighted pair group method using arithmetic means (Fig 1). Pairwise similarity values of strains from the 1998 incident ranged from 90 to 100 per cent, and they formed two clusters. Cluster 1 included the human isolates and a bovine strain indistinguishable from them (Fig 1) and cluster 2 was comprised of a further eight bovine isolates. The human, bovine and equine PT8 isolates from 2001 were indistinguishable (cluster 3) and had between 92 and 95 per cent similarity with the 1998 isolates. Strains within clusters were thus more closely related than those from different clusters but the similarities for all strains from both incidents was 90 to 100 per cent. Profiles of VTEC O157 PT8 from nine outbreaks of infection between 1998 and 2000 did not cluster with the farm incident isolates (Fig 1) and their similarity to the human isolates from 1998 and 2001 ranged from 61 to 91 per cent.

In both these incidents, isolation and typing of VTEC O157 from human and animal sources supported the epidemiological conclusion that infection resulted from visiting the farm. Separate incidents of VTEC O157 infection have rarely occurred at the same premises. General outbreaks in 1997 and 1999 involved the same open farm, but the strains were of different phage types (PT2 and PT43) and were distinct by PFGE (G. A. Willshaw, T. Cheasty, unpublished observations). PFGE results on the incidents reported here suggest that either highly related PT8 strains were independently present on the farm on the two occasions or that genotypic variation of a single strain was occurring. The PFGE profiles among the bovine isolates in 1998 indicated that a population of strains was present that could have given rise to the strain detected in 2001, but without isolates from the intervening period this is impossible to determine. However, the lack of clustering of the farm strains with PT8 strains from other sources does suggest a group of strains more closely related to each other than to those from other sources.

The incidents arose from private visits to commercial premises by urban residents. Guidelines exist to reduce the risk of infection with VTEC O157 on open farms, particularly among parties of children (Health and Safety Executive 2000). It is necessary to emphasise that any farm livestock premises may pose a potential hazard to visitors and that the very young may be vulnerable even without direct contact with animals. Although not implicated here, unusual vectors such as wild rabbits should be considered when investigating farms as sources of human infection (Pritchard and others 2001).

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2.6

Wild rabbits – a novel vector for verocytotoxigenic *Escherichia coli* O157

SIR, – An outbreak of verocytotoxigenic *Escherichia coli* (VTEC) O157 infection was identified between mid-July and early September 2001, in 10 children aged nine years and under, and two adults, in unrelated families from different parts of Britain who visited an animal collection open to the public. Visitor attendance during this period averaged about 500 daily. Symptoms varied from mild to acute haemorrhagic diarrhoea; one child developed haemolytic uraemic syndrome. Four patients were hospitalised, and all cases recovered. The causative strains belonged to phage type (PT) 2 and had genes for verocytotoxin (VT) 2; isolates examined by pulsed-field gel electrophoresis (PFGE) were shown to be indistinguishable (Willshaw and others 1997).

An outbreak control team (OCT), as previously described by Milne and others (1999), convened to investigate the source of infection, its likely method of transmission and to advise on control measures. Food and drink were eliminated as potential sources of infection at an early stage of the inquiries and an animal-associated outbreak seemed likely. Direct contact (touching) was only possible with two exotic pigs and two goats in pens, although visitors had potential contact with faeces from a few captive muntjac deer kept on part of the site. The enterprise was well managed and operated to reasonably high standards.

Microbiological investigations, as detailed by Pritchard and others (2000), yielded *E coli* O157 PT8, VT1 and VT2 from one of 21 pooled faecal samples from the deer; *E coli* O157 PT34, VT2 was isolated from one of the goats. Since neither phage type was implicated in the human infections, bacteriological screening was extended to include water, soil, herbage and wild bird droppings, and faeces from animal species to which the public had no direct access: VTEC O157 was not isolated.

In spite of the absence of an identified source for the outbreak strain of VTEC O157, the OCT recommended strict adherence to the advisory guidelines provided by the Health and Safety Executive (HSE 2000) to prevent further cases occurring. Visitors were physically separated from the deer, goats and pigs by installing double fencing; areas potentially contaminated by faeces from these animals were cleaned and disinfected. Clearer signs and public information sheets were provided to increase awareness of good personal hygiene. Despite these stringent control measures, new cases of human infection continued to occur and the premises were closed to the public, pending further investigations.

The search for the source of the outbreak strain was extended beyond the boundary fence to include an adjacent pasture used by a small beef suckler herd, in different ownership, with which the public had no contact. *E coli* O157 PT2, VT2, indistinguishable from the human outbreak strain, was subsequently isolated from 17 (46 per cent) of 37 cattle environmental faecal samples collected randomly at three sampling visits to the farm from pastures and an adjoining covered yard. No other VTEC O157 strains were detected.

The same organism was also isolated from three of 12 pooled environmental samples of wild rabbit faecal pellets collected from the pasture used by the cattle. Further sampling of rabbit faeces was carried out in non-animal (play and picnic) parkland areas within the perimeter fence of the animal collection. *E coli* O157 PT2, VT2, also indistinguishable by PFGE from the outbreak strain, was isolated from 17 (59 per cent) of 29 pooled samples collected at four separate visits during the latter part of September. Appropriate precautions were taken during sample collection to avoid cross contamination.

Our findings suggest that contact with wild rabbit faeces can be a risk factor for acquiring human infection. In this outbreak, rabbits appeared to have carried the infection from the cattle farm to recreational areas on the adjacent animal park by consumption of contaminated herbage. At present it is not clear whether the rabbits were maintenance hosts or merely vectors. Attempts to isolate the organism from culled rabbits are in progress. Human infection appeared to follow visits made during or soon after heavy rainfall, which could have resulted in rabbit faecal matter adhering to visitors' shoes and clothing. The topography of the land is such that contaminated surface water from the cattle pastures could not have drained onto the premises of the animal collection. Efforts are currently underway to reduce the rabbit population at the site by culling and fencing; the public has also been prevented from gaining access to contaminated areas of grassland. Although the outbreak strain was not isolated from the animals in the collection, the presence of two other strains of VTEC O157 in deer and goats emphasised the potential risk of human infection.

Cattle are considered to be the main reservoir for VTEC O157, but various other species, particularly goats, have been implicated in outbreaks on open farms (Pritchard and others 2000). Rabbits have been used as experimental models for VTEC O157 infection but we are not aware of any reports of the isolation of this organism from wild rabbits or its association with human cases.

The circumstances here, with a nearby heavily infected cattle herd, a prolific animal vector (the local rabbit population had increased significantly in 2001) and a susceptible population of visitors, provided ideal circumstances for transmission of VTEC O157. Investigations of human VTEC O157 infections with potential links to animals or agricultural land may need to include testing of rabbit faeces. Parks, play areas and gardens many also become contaminated and contact with faeces from wild rabbits needs to be considered as a risk factor when investigating outbreaks and sporadic cases of VTEC O157 infection.

We greatly appreciate the help and cooperation of the owner of the animal collection and the next-door farmer in this investigation.

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2.7a

VTEC O157 AND WILD RABBITS

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Wildlife gardens open to the public. Mainly exotic animals in enclosed buildings. Up to 500 - 1000 visitors daily. Picnic and adventure playground areas. Located on rural village outskirts adjacent to a small beef suckler herd (no direct contact) in predominantly arable area.

Bug spread by bunnies

Cattle are considered to be the main reservoir for VTEC O157 infection but it has also been isolated from various other species including goats and deer. An outbreak in 10 children and 2 adults who visited a wildlife gardens in 2001 was linked to contact with wild rabbits. This was the first report of its association with this species.

Pritchard and others (2000) Wild rabbits - a novel vector for verocytotoxigenic *Escherichia coli* O157. Veterinary Record **149**, 567

HISTORY

- VTEC O157 PT2 VT2 in 12 visitors July - Sept 2001
- Well managed premises with good animal husbandry
- Reasonable hygiene measures in place
- Enquiries excluded food and drink as source
- Direct/indirect animal contact possible source
- Potential contacts: 2 pigs, 8 deer and 2 goats only..

INVESTIGATION

- Identify risk factors
- Loczate likely source(s) of infection,
- Collect appropriate samples
- VTEC O157 culture -IMS/CT-SMAC
- Phagetype/ PFGE profile VTEC O157 isolates
- Compare isolates with outbreak strain
- Advise on control measures

ISOLATION OF VTEC O157

- Goats → VTEC O157 PT34, VT2*
- Deer → VTEC O157 PT8, VT1+2*
- Adjacent beef suckler cattle → VTEC O157 PT2, VT2*
- Rabbits → O157 PT2, VT2* (farm, gardens+rectum)
- Grass play area → VTEC O157 PT2, VT2*

* Incidental isolate
* OUTBREAK STRAIN

Rabbit faeces in wildlife gardens

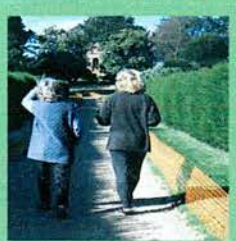
Sample collection July-Sept 2001

Sample	Site	Tested	Positive		VTEC
Goats	W	11	1		O157 PT34 VT2
Pig	W	6	0		
Deer	W	21	1		O157 PT8 VT1+2
Birds	W/F	27	0		
Horse	F	3	0		
Exotics	W	9	0		
Dogs	W	3	0		
Water	W/F	16	0		
Misc.	W/F	23	2	(grass W)	O157 PT2 VT2*
Cattle	F	34	15		O157 PT2 VT2*
Rabbit ●	W/F	31	20	(3F, 17W)	O157 PT2 VT2*
Rabbit ☞	W/F	23	1	(rectum)	O157 PT2 VT2*

W Wildlife gardens F farm ● faeces ☞ carcase * Outbreak strain
Note: some sites sampled several times

Control measures

Improve general hygiene procedures
Observe Health and Safety guidelines
Remove rabbit faeces and cull rabbits



Temporary fencing

Keep off grass

FURTHER STUDIES:

Despite the high isolation rate from rabbit faeces, the outbreak strain of VTEC O157 was isolated from the rectum of only 1 of 23 rabbit carcases each cultured at 10 sites, including different levels of alimentary tract. This suggested very localised colonisation.

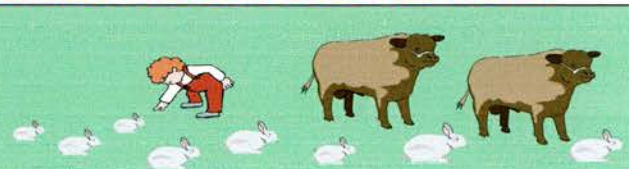
Repeated resampling of partly covered infected rabbit faeces, enclosed on an outdoor experimental plot, demonstrated persistence of outbreak strain of VTEC O157 for 9 months.

Experimental plot

IMPORTANT QUESTIONS

How prevalent is VTEC O157 infection in wild rabbits ? - survey needed

Are wild rabbits involved in sporadic VTEC O157 cases elsewhere ?



2.7b

ORAL PRESENTATIONS

O-14

VTEC O157 Infection from Wild Rabbits

Pritchard G, VLA.
1) Williamson S; Carson T, 2) Bailey,J; Warner, L, 3) WillshawG, Cheasty T. 1) VLA, Bury St Edmunds, Suffolk, 2) Norfolk Health Authority, Norwich, Norfolk, 3) Public Health Laboratory Service, London.

During summer 2001, VTEC O157 PT2 VT2 infection was identified in 10 children and two adults who visited an exotic animal wildlife gardens. Food and drink were eliminated as potential sources. Animal and environmental samples were subsequently examined for VTEC O157 using immunomagnetic separation and inoculation onto CT-SMAC.

Contact between visitors and animals was limited to two exotic pigs, two goats and several muntjac deer. VTEC 0157 was isolated from the deer and goats but the phagetypes (8 and 34 respectively) differed from the outbreak strain. However, VTEC 0157 PT2 VT2, indistinguishable by pulsed-field gel electrophoresis from the outbreak strain, was isolated from 20 of 30 composite samples of wild rabbit faeces and from two herbage samples from childrens' play and picnic areas. It was also found in faecal pellets from the rectum of one of 23 rabbits necropsied.

Further investigations yielded the outbreak strain from three of 12 composite samples of rabbit faeces on an adjacent pasture used by a small beef suckler herd in different ownership. This farm shared a boundary with the wildlife gardens but was inaccessible to the public. More significantly, this strain was also isolated from 15 of 34 faeces samples (freshly voided or aged) from the cattle grazing the same pasture (but not from those grazing elsewhere). It was concluded that the rabbits probably acquired infection from the cattle during co-grazing.

No further human cases were reported after September 2001. This followed adoption of control measures, including temporary closure, improved handwashing facilities, fencing grass areas, removal of rabbit faeces and a culling programme. Follow-up sampling of rabbit and cattle faeces continued until November 2002 but there were no further VTEC O157 isolates after October 2001. In a related study, VTEC O157 persisted for nine months in rabbit faeces kept under environmental conditions.

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PAPERS

Verocytotoxigenic *Escherichia coli* O157 in animals on public amenity premises in England and Wales, 1997 to 2007

G. C. Pritchard, R. Smith, J. Ellis-Iversen, T. Cheasty, G. A. Willshaw

At the request of the public health authorities, 31 public amenity premises in England and Wales containing animals of various species were investigated for the presence of verocytotoxigenic *Escherichia coli* (VTEC) O157 between 1997 and 2007, because of putative associations with human cases. VTEC O157 was confirmed in one or more species on 19 (61·3 per cent) of the premises. There were significant associations between the presence of VTEC O157 and the number of species sampled, the size of the enterprise, the presence of young cattle and the presence of adult pigs. *E coli* O157 was isolated from 305 (17·8 per cent) of 1715 samples taken from all the premises, and verocytotoxin genes were detected by PCR in 184 (98·4 per cent) of 187 representative isolates. On positive premises, the highest mean proportion of positive samples (29·0 per cent) was in cattle, followed by sheep (24·4 per cent), donkeys (14·6 per cent), pigs (14·3 per cent), horses (12·3 per cent) and goats (9·9 per cent). A high proportion of positive samples was obtained from camelid species sampled on three of the premises. The main phage types (PT) were 2 and 21/28, which were those most commonly isolated from human cases during the same period. A single PT was detected on 14 of the 19 positive premises, with up to six different species having the same PT.

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VEROCYTOTOXIGENIC *Escherichia coli* (VTEC) O157 has emerged as an important human pathogen during the past 25 years. It causes a broad range of human illnesses, including diarrhoea, haemorrhagic colitis and severe systemic complications, particularly haemolytic uraemic syndrome (Lynn and others 2005). Most cases are sporadic and unattributable, although case-control studies have indicated that contact with animals is a major risk factor (Locking and others 2001, O'Brien and others 2001). Infection may be acquired on commercial livestock farms through occupational or residential exposure or the consumption of contaminated unpasteurised milk; cases have also been associated with private farm visits by children (Willshaw and others 2003). Of greater significance are the clearly defined outbreaks that have occurred in England (Milne and others 1999, Pritchard and others 2000, 2001) and elsewhere, including the USA and Canada (Lejeune and Davis 2004, Durso and others 2005), which have been linked to visits by the public to farms or other animal amenity premises. In Great Britain, the major VTEC O157 outbreaks have invariably occurred as a result of indirect contacts with animals via contaminated food or water, but the potential for large outbreaks to occur from direct contact with animals on premises visited by the public has been demonstrated in the USA and Canada (Lejeune and Davis 2004).

Open farms and similar premises often provide the only significant opportunity for the general public to have close contact with animals, and they fulfil an important educational role and provide major contributions to the tourism and leisure industries. The potential risk is the exposure of people to zoonotic hazards such as VTEC O157 (Pritchard and others 2000) and *Cryptosporidium parvum* (Pritchard and others 2007). The prevalence of VTEC O157 in farmed livestock, especially cattle, has been extensively researched, including comprehensive abattoir surveys (Milnes and others 2008) and intensive farm studies (Ellis-Iversen and others 2008), but relatively little is known about its prevalence among the wide range of species found on animal amenity premises. Since the mid-1990s, the Veterinary Laboratories Agency (VLA) has assisted public health authorities in England and Wales with the investigation of human VTEC O157 infections linked to animal contact on such premises, and this paper presents the findings from 1997 to 2007.

Materials and methods

The premises investigated were all essentially 'open farms' – a generic category of animal establishments, specifically operating as an attrac-

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tion for visits by the general public; they included petting zoos, children's farms, city farms, wildlife parks and other premises with animals. The investigations were undertaken at the request of the public health authorities when epidemiological inquiries suggested that animal contact was the likely source of human VTEC O157 infection. They usually concerned outbreaks affecting more than one household, but occasionally followed single sporadic cases or family outbreaks. Open farm premises investigated between 1997 and 2007 were included in the study; conventional commercial farms inaccessible to the public were excluded.

Field investigations and data collection

Visits were undertaken by veterinary investigation officers (VIOs) of the VLA as soon as possible after the request to investigate. They were often accompanied by members of the public health authorities, such as environmental health officers or consultants in communicable disease control. In addition to collecting samples, veterinary advice was provided on animal husbandry and hygiene aspects potentially relevant to the risk of human infection. General health and safety precautions, such as the supervision of visitors, advisory notices and hand-washing facilities, were assessed but not analysed further. A standardised questionnaire was used by VIOs to collect epidemiological data, including the type and size of the premises, the animal species (type, number, age and grouping), the number of days open, and the numbers of staff and visitors. The data were entered into a Microsoft Access database and checked for errors, and validated by a visual check linked to a random selection of 10 per cent of the unique farm identifiers and a search of the database for outliers and impossible values.

Sample collection

The primary aim of collecting samples was to detect VTEC O157 for characterisation and comparison with strains from human cases by phage type (PT) and pulsed-field gel electrophoresis (PFGE), as described by Pritchard and others (2000). A standardised sampling method was used to detect at least one positive sample in each epidemiological group with 95 per cent confidence, assuming a prevalence of at least 10 per cent (Cannon and Roe 1982); in some investigations more samples were collected. An epidemiological group was defined as a group of animals, usually of the same species, which were housed together or had other close contacts through management and husbandry practices. Each sample consisted of a minimum of 1 g of fresh-looking faeces from the animal enclosure or environment, or occasionally from the rectum (depending on availability, safety and practicality), either from single animals or pooled from several animals in the same group (Arnold and others 2008). Pooled samples were usually collected from species producing small faecal pellets, such as sheep and rabbits.

Detection and isolation of VTEC O157

The methods for culture and isolation were as described by Pritchard and others (2000) and Paiba and others (2003); pre-enrichment in buffered peptone water was followed by immunomagnetic separation (Chapman and Ackroyd 1997) and the inoculation of beads (Dynabead anti-*E coli* O157; Dynal) on to sorbitol MacConkey's agar supplemented with cefixime and potassium tellurite (CT-SMAC). Non-sorbitol-fermenting colonies were examined by an *E coli* O157 latex agglutination test (DR260G; Oxoid). Biochemically confirmed *E coli* O157 were examined for verocytotoxin (VT) genes 1 and 2 by PCR (Willshaw and others 2001) and phage typed (Khakhria and others 1990). PTs were presented as the PT number, but if strains reacted with phages but failed to conform to a recognised type, they were classified as RDNC (reacts, does not conform). Because of the cost, only a representative selection (approximately 10 per cent) of *E coli* O157 isolates from epidemiological groups or species on each premises were examined for VT genes. If VTEC O157 was confirmed in these, it was inferred that other *E coli* O157 isolates were also VT strains, on the basis of the findings of Paiba and others (2003) and Synge and others (2003), and a recent study by Ellis-Iversen and others (2009) in which 91·4 per cent of *E coli* O157 were VT-positive. The term VTEC O157 has therefore been used synonymously for all non-sorbitol-fermenting strains of *E coli* O157.

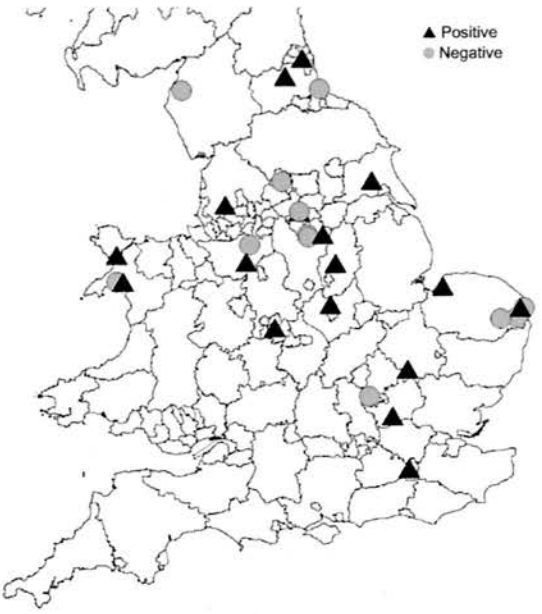

FIG 1: Geographical location and verocytotoxigenic *Escherichia coli* status of open farm premises investigated in England and Wales between 1997 and 2007 as potential sources of human cases

Statistical analysis

The numbers of staff and the annual numbers of visitors, and the numbers and types of animal species, were used to subdivide the premises subjectively into small, medium and large, for simple comparative purposes. Quantifying the premises in a more precise way was not feasible because of the diversity of the enterprises. The species were classified as cattle, sheep, goats, pigs, horses, donkeys, llamas, alpacas, deer, poultry (chickens and turkeys), waterfowl (geese and ducks), other birds (parrots, quails, gamebirds and pigeons), dogs, cats, small pet mammals (guinea pigs, pet rabbits, rats, ferrets, chipmunks, and so on), wild rabbits, other free-living wild mammalian species (badgers, bats, foxes and otters) and reptiles and amphibians.

A farm was defined as positive if VTEC O157 was isolated from one or more samples (at least one *E coli* O157 isolate per positive premises was invariably confirmed as a VT by PCR). This was analysed at premises, group and species level. The within-group proportion of positive samples (an approximate guide to prevalence) was defined as the proportion of VTEC O157-positive samples from the total number of samples analysed at the level of interest. The location and VTEC O157 status of the premises was plotted using ARCGIS v9.1 (ESRI) to show their geographical distribution. Group ages (adult only or young/mixed ages) were compared for the major species, defining adult as 24 months or older for cattle, five months or more for pigs and 12 months or more for sheep and goats. Sample groups with missing ages were excluded. Individual characteristics were evaluated against status by Yates-corrected Chi-squared test, Fisher's exact P values and tests for linear trends as appropriate, using Epi Info 6 software (Dean and others 1995). Crude odds ratios (ORs) were used to measure the strength and direction of significant associations. Logistic regression was used to compare group ages, using STATA 9 (Stata Corp), including the cluster function to account for the clustering of samples at the premises level.

Results

Investigation and premises

Thirty-one investigations were undertaken between 1997 and 2007 (between one and six premises a year); all of the premises (apart from

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TABLE 1: Numbers and proportions of VTEC O157-positive samples from different animal species sampled on 31 open farm premises

Species	Number of premises sampled	Total number of samples collected on all premises*	Number (%) of positive samples on all premises	Number (%) of positive premises	Mean within-group proportion (%) of positive samples on positive premises	Median within-group proportion (%) of positive samples on positive premises
Cattle	21	365	87 (23·8)	14 (67)	29·0	20
Sheep	19	427	120 (28·1)	11 (58)	24·4	12·5
Goats	27	281	25 (8·9)	12 (44)	9·9	0
Pigs	22	168	30 (17·9)	10 (46)	14·3	0
Horses	19	85	10 (11·8)	7 (37)	12·3	0
Donkeys	8	26	6 (23·1)	2 (25)	14·6	0
Alpacas	1	4	4 (100)	1 (100)	100	100
Llamas	3	13	9 (69·2)	2 (67)	40·7	33·3
Deer	4	27	1 (3·7)	1 (25)	1·5	0
Poultry	15	45	1 (2·2)†	1 (7)	6·7	0
Waterfowl	11	20	0	0	0	0
Other birds	4	44	0	0	0	0
Dogs	6	12	0	0	0	0
Cats	3	7	0	0	0	0
Small pet mammals	19	129	0	0	0	0
Wild rabbits	3	32	12 (37·5)	1 (33)	15·8	0
Other wild mammals	3	28	0	0	0	0
Lizards and amphibians	1	2	0	0	0	0

* Includes individual and pooled samples
† Chicken

TABLE 2: Numbers and percentages of VTEC O157-positive samples taken from farm animal species kept on open farms, by age, adjusted for clustering on premises

Species	Age	Number of samples	Number (%) of positives	P
Cattle	Adult	125	13 (10·4)	
	Young or mixed age	227	69 (30·4)	0·054
Goats	Adult	113	13 (11·5)	
	Young or mixed age	165	12 (7·3)	0·379
Pigs	Adult	60	18 (30·0)	
	Young or mixed age	91	12 (13·2)	0·013
Sheep	Adult	83	20 (24·1)	
	Young or mixed age	317	89 (28·1)	0·731

one in January) were sampled between April and October, with most (12 premises) in July and August. Three premises were investigated twice, several years apart, and for the purposes of the analyses these investigations were classed as being on different premises because the livestock populations and other farm characteristics had changed significantly during the intervening periods. There was an average delay of 24 days (range five to 70 days) between the identification of the primary human case and the request for a field investigation. Twenty-six of the investigations were on typical open farms, of which 14 had a shop or cafeteria, two operated a campsite and three had associated commercial farms. The other five premises were essentially livery stables or farm campsites containing a number of animal species to which the public had general access. On average, there were seven different species on each premises, with a median of eight and a range from two to 12. The average area of the premises was 87·8 ha with a median of 10·1 ha and a range from 0·4 to 1012 ha, which often included land to which the public had little or no access, such as that used for commercial agriculture. The farms had an average number of 156,000 visitors annually (median 68,000, range 175 to 900,000), were open for an average of 344 days each year (median 365) and had an average of 14 full-time staff members (median six). Seventeen of the investigations were undertaken on small premises, 11 were on medium-sized premises and three were on large premises.

VTEC O157 status of the premises

VTEC O157 was identified on 19 (61·3 per cent) of the 31 premises. The time interval between the identification of the primary case and the investigation had no significant effect on the status of the premises. Large premises were more likely to be positive than smaller premises (P=0·051). The geographical distribution and VTEC O157 status of the 28 different locations investigated (including the three premises that were investigated twice) are shown in Fig 1; they covered most of England and Wales, but there were none in south Wales or south-west England.

An average of 55 samples (range two to 189) from six different species was collected per investigation, with wide variations depending on putative contact as perceived by the investigators of the outbreak on the basis of the case history. The probability of detecting VTEC O157 on a premises was greater when more than three species were sampled (linear P=0·045). When four to six species were sampled on eight premises, five (62·5 per cent) premises were positive (OR 4·2, P=0·315), and when seven or more species were sampled on 16 premises, 12 (75·0 per cent) premises were positive (OR 7·5, P=0·066).

VTEC O157 status by species

Although the samples were collected from epidemiological groups, for the purpose of this study the analyses were completed at sample level due to difficulties in consistently assigning the samples to groups. *E coli* O157 was isolated from 305 (17·8 per cent) of the 1715 samples collected from all premises; 187 (10·9 per cent) were examined for VT genes and 184 (98·4 per cent) were confirmed as VTEC O157. All but two of these contained the VT2 gene; the exceptions were classified as VT1+2, a sheep isolate that was RDNC and a deer isolate that was PT8. VTEC O157 was found in more than one species on 15 of the 19 positive farms. The three VTEC O157-negative isolates were from two premises, on both of which VTEC O157 was definitively confirmed by PCR in other species.

Table 1 shows the proportions of VTEC O157-positive samples by species, both overall and on positive premises only. Multiple isolates of VTEC O157 were obtained from cattle, sheep, goats, pigs, horses, donkeys and llamas on more than one premises. Of the traditional domesticated animal species, the highest mean proportion of positive samples on positive premises (29·0 per cent) was detected for cattle, followed by sheep (24·4 per cent), donkeys (14·6 per cent), pigs (14·3 per cent), horses (12·3 per cent) and goats (9·9 per cent); the ranking of the top two species was the same with the median results. Samples from camelids had a high proportion of positives; however, samples were collected on only three premises, of which two premises were positive, including one where *E coli* O157 was isolated from 12 of 13 samples from llamas and alpacas. There were multiple positive samples from wild rab-

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bits on one of the three premises on which wild rabbits were sampled. Single positive samples, each from single premises, were obtained from a deer and a chicken. No VTEC O157 was isolated from dogs, cats, small pet mammals, waterfowl, other avian species, lizards and amphibians, or wildlife other than wild rabbits.

Age and VTEC O157

Samples taken from cattle in a group containing young cattle were more likely to be VTEC O157-positive than samples from groups containing only adult cattle. Conversely, samples from pigs were less likely to be positive if they were in a group containing young pigs rather than in a group of adults only (Table 2).

VTEC O157 phage types

A total of 174 VTEC O157 isolates (between one and 22 from each positive investigation) were phage-typed and 10 different PTs and RDNC types were identified. Fourteen (73·7 per cent) of the 19 positive premises had only a single PT or RDNC strain, despite the isolates originating from up to six different animal species. One premises had two different PTs in cattle and four premises had two or more PTs or RDNC strains in up to six different species. The most common was PT 2 (six premises), followed by PT 21/28 (five premises) and PT 32 (three premises). PT 8 and PT 4 were each detected on two premises, and PTs 14, 33, 34, 38 and 43 were all detected on single premises. Three premises had RDNC strains. Because of the small sample sizes it was not possible to assess any statistical associations between PT and animal species, but no obvious links were observed.

Discussion

The 31 premises were all sampled because of perceived links with human cases of VTEC O157 infection and were not part of a structured survey. It cannot therefore be assumed that any associations detected were representative of all open farms. Furthermore, it is likely that there was a significant cluster effect, whereby animals within groups and within farms were more alike. The influence of clustering on the effect of age was accounted for in the analyses but the data set was not suitable for multivariable analysis owing to the diversity of the premises. As a result, it cannot be concluded that any associations, such as with the number of species sampled, were causal or not confounded by other factors. Nonetheless, the study was one of the largest investigations into VTEC O157 among the wide range of species in mixed-species environments and has generated hypotheses that merit further investigation. The lack of premises in south Wales and south-west England did not appear to be associated with fewer human cases (Health Protection Agency [HPA] 2006, 2007) or fewer open farms in these areas (National Farm Attractions Network 2008). VT genes were detected in 98·4 per cent of 187 *E coli* O157 isolates examined by PCR, a slightly higher proportion than reported by other researchers in Great Britain (Paiba and others 2003, Synge and others 2003, Ellis-Iversen and others 2008).

Over 60 per cent of the premises were infected, a much higher proportion than the between-herd prevalence of 40 per cent or less in cattle herds surveyed in England and Wales by Paiba and others (2003) and in Scotland by Synge and others (2003). This result suggested either that the initial screening of cases carried out by public health authorities for possible association with animal contact was successful in identifying premises with a higher risk of being VTEC O157-positive, or that the open farms had an intrinsically higher prevalence of infection. However, causal associations with human cases, assessed by PT and PFGE profiles, were not confirmed in all the positive premises, and therefore some of the VTEC O157 infection detected was incidental. Sampling at least four different species significantly increased the farm-level sensitivity of detection, but this association may have been confounded by the number of species present, the size of the farm and the number of samples tested. A larger sample size would have made a multivariable analysis possible to separate the effect of each variable and achieve a more accurate conclusion.

The high proportion of VTEC O157-positive samples (a crude estimate of prevalence) on the positive farms in this study could have been associated with different husbandry and management practices on open farms compared with the typical commercial farms surveyed previously. Open farms are more likely to have higher stocking rates, more movements of staff and equipment, less stable animal group structures (Ellis-Iversen and others 2008) and may comply less carefully with biosecurity precautions (Pritchard and others 2005). Furthermore, open farms often introduce lambs, calves or other species to the resident animal population, depending on the season and their availability, which can increase the risk of introducing infection. The larger premises may have been more likely to be infected than the smaller premises because they maintained larger animal groups (Ellis-Iversen and others 2008), kept more staff and had more visitors, leading to an increased risk of introducing the organism via fomites and a greater risk of within-farm transmission. There was no evidence that the period from the identification of a human case to sampling the farm influenced the detection of VTEC O157. However, Pritchard and others (2000) detected a much lower prevalence of infection during the 12 months after an associated VTEC O157 outbreak on an open farm than during the initial investigation, and it is possible that the prevalence of VTEC O157 fluctuates on open farms and that periods of higher prevalence may contribute to outbreaks. However, further longitudinal studies are needed to investigate this hypothesis.

The findings suggest that young cattle were a significant risk factor, as is the case on commercial cattle farms (Paiba and others 2003). However, there was less supporting evidence for the association with older pigs housed without younger pigs. This could have been associated either with the fact that many of the farms housed pigs of different ages together, to increase visitor interest, or with the fact that the adult pigs were often tame Vietnamese pot-bellied pet pigs, which may have had more contact with all the other animal groups. The data were inconclusive.

The highest proportion of positive samples in farm species on positive premises was detected in cattle, followed by sheep, donkeys, pigs, horses and goats, although the greatest proportion of positive samples from all premises (28·1 per cent) was in sheep. VTEC O157 infection has been described in all these species (Trevena and others 1996, Chalmers and others 1997, Milne and others 1999, Chapman 2000). An unexpectedly high proportion of positive samples was detected in camelids, although they were sampled on only three premises. This appears to be the first report of VTEC O157 infection in these species and further studies are needed to determine whether they constitute a particular zoonotic hazard on open farms or elsewhere. VTEC O157 was isolated from a deer on one of the farms, and has also been described in deer by Chapman and Ackroyd (1997). VTEC O157 was detected in one chicken and the organism has been reported in laying hens in Italy (Dipineto and others 2006). However, the single isolate from birds suggested that they constitute only a minimal risk to visitors, despite reports from elsewhere of infection in various avian species (Wallace and others 1997, Synge 1999, Ejidokun and others 2006). The extensive infection in wild rabbits on one premises was described in detail by Pritchard and others (2001) and followed up by Scaife and others (2006). Domesticated rabbits can be infected experimentally with VTEC O157 (Ashkenazi and others 1992), but there was no evidence of the infection in pet rabbits or other small pet mammals in the many samples taken on 19 premises, which suggests that there is no significant risk of acquiring the infection from these species. The infection was not detected in any of the dogs or cats, but VTEC O157 infection has been described in farm dogs by Trevena and others (1996) and Hogg and others (2009).

A single PT or RDNC strain was found on almost 75 per cent of the positive premises and was often isolated from several different species. Where multiple PTs were present, they usually occurred in different species. The prevalent PTs (2 and 21/28) were the same as those most commonly isolated from human infections in England and Wales over the same period (HPA 2002, 2003, 2004, 2005, 2006, 2007). There was no apparent link between the animal species and the PT, and the PTs appeared to be associated with individual farms rather than species, suggesting that within-farm transmission was more common than the introduction of new strains.

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McMillan and others (2007) and Weese and others (2007) described high-risk behaviour and poor hygienic practices by visitors to animal amenity premises. However, despite the high prevalence of VTEC O157 detected, the risk of acquiring the infection on open farms and other animal premises is very small in relation to the large numbers of visitors each year. It is also necessary to balance this small risk against the undoubted benefits of allowing the public to interact with farm animals (Pritchard and others 2000). The risk of people acquiring an infection from animals depends more on the degree of contact and the precautions adopted than the prevalence of infection in a particular species. Procedures to prevent the faecal-oral transmission of VTEC O157 and other zoonotic infections to people visiting open farms have been described (Health and Safety Executive [HSE] 2002). Strain typing can be used to confirm or refute a causal association between a human infection and an animal source, as has been described by Milne and others (1999) and Pritchard and others (2001).

Acknowledgements

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2.9a

OUTBREAKS OF VTEC O157 INFECTION LINKED TO ANIMAL CONTACT IN ENGLAND AND WALES BETWEEN 1996 AND 2005

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INTRODUCTION

- Direct contact with animals or indirect exposure via faecally contaminated environment recognized as one of the main routes of transmission of VTEC O157 to man
- Case-control studies of sporadic cases identified contact with animals (other than pets) and rural environment as major risk factors[1]
- Persistence of VTEC in agricultural environment shown experimentally and in sampling of cattle herds[2,3]
- Carriage of VTEC by vector species (birds, flies, wild animals) permits transfer between sites[4,5]
- General and household outbreaks linked to farms in England and Wales; first open farm outbreak in 1994
- Infections linked to agricultural premises trigger veterinary and environmental investigation; role of reference laboratory to compare human and non-human isolates for epidemiological purposes

METHODS

Clinical and epidemiological

- Isolation of presumptive VTEC O157 from human infections
- Epidemiological investigation of cases; assessment of risk factors; identification of possible link to agricultural premises

Veterinary

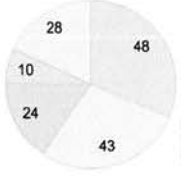
- On-farm sampling; isolation of presumptive VTEC O157

Reference laboratory

- Biochemical confirmation and serotyping of human and animal isolates
- Phage typing (>80 types recognised)
- Identification of VT1 and VT2 genes by PCR
- Pulsed field gel electrophoresis (PFGE) of *Xba*I fragments
- Profile comparison by Dice coefficient; cluster analysis by unweighted pair group method using arithmetic means (UPGMA)

RESULTS

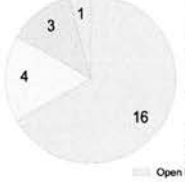
Data on 153 general outbreaks between 1996 and 2005 showed that in 24 (16%) the principal transmission route was direct or indirect contact with animals



The number of animal-related general outbreaks per year ranged from 1 to 6

In 8 waterborne outbreaks, ultimate source probably animal faeces but isolates not obtained from animals

Settings of 24 general outbreaks linked to farms



Open farms included wildlife parks and sanctuaries

Camp sites on private farms; music festival

Commercial farms included those at open prisons

Other: index case handled animals in his domestic farm setting

Premises in all regions of England and Wales except London

ACKNOWLEDGEMENTS

Thanks to all involved in the investigations summarized here and to the staff of the Laboratory of Enteric Pathogens and Veterinary Laboratories Agency for work on identification and typing of isolates.

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General outbreaks linked to farms: human infections

- Cases (158) comprised 2% of the total human VTEC O157 between 1996 and 2005 (8314)
- Children <5years of age accounted for 43 cases (27%). This age group has the highest incidence per 100,000 of VTEC O157 and HUS[6,7]
- 39 of the cases in <5y group were from open farm outbreaks
- Annual visits to open farms estimated at 13 million[8]. Risk of infection is very low but likely to include most vulnerable group
- In half the outbreaks, person-to-person transmission spread infection to family members and other contacts
- Identification of cases often difficult; might not reside in location of premises

Animals implicated in general outbreaks

In 18/24 outbreaks animal isolates of VTEC O157 were obtained that had the same PT, VT type and PFGE profile as the strain infecting human cases. A wide range of animals was identified and the number of positive species in each outbreak ranged from 1 to 5.

Goats	61	1	4
Cattle	61	2	7
Pigs	44	3	2
Sheep	39	>3	5
Equines	28		
Other*	17		

* Wild rabbits, llama, cockerel (1 outbreak each)

All identifications of goats and pigs carrying VTEC O157 were at open farms: in 16 outbreaks, 11 had +ve goats and 8 had +ve pigs

Examples of family outbreaks linked to animal contact

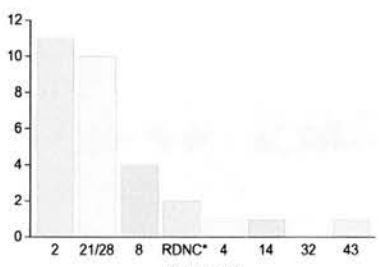
In 7 household outbreaks, sampling of implicated animals resulted in isolation of strains indistinguishable by PT, VT type and PFGE profile from those infecting humans. Further spread by person-to-person transfer in some incidents

Family of farm worker	2000	4 (3)	Cattle	PT2,VT2
Private farm: two incidents at same premises	1998 2001	2 (2) 2 (2)	Cattle, horse Cattle	PT8, VT1+2 PT8, VT1+2
Private farm	2003	2 (1)	Cattle	PT21/28, VT
Open farm	2004	3 (2)	Indirect: cattle on adjacent farm	PT2, VT2
Domestic garden	2004	9 (4)	Orphan lamb	PT4, VT2
Open farm	2005	2 (1)	Cattle	PT2, VT2

Transmission increasingly recognized as potentially complex

- Role of vector animals: transfer of outbreak strain from commercial farm to adjacent open farm by wild rabbits
- Some activities may pose additional risk – eg. bottle feeding of lambs
- Risk of transfer of VTEC O157 to family members from clothing and footware of farm workers
- Hazards of farm campsites due to direct faecal exposure or contamination of untreated water sources
- Microbiologically indistinguishable VTEC O157 in humans and putative animal sources but direct evidence for method of transfer unclear; problem for implementation of control measures

Phage types of VTEC O157 from general and household outbreaks linked to animal contact



*RDNC: reacts with typing phage but does not conform to a recognised type
Two different RDNC strains found
PT8 strains were VT1+2; other strains were VT2

- Types reflected the general prevalence of PT2, PT21/28 and PT8 in human infections between 1996 and 2005 (see poster P14)

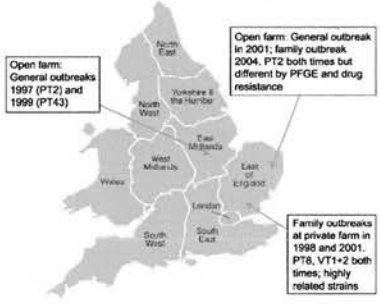
PFGE of isolates from general and family outbreaks showed that only 2 outbreak strains (PT2) shared the same *Xba*I profile

In 4 of the general outbreaks, VTEC O157 in addition to the human outbreak strain were recovered from animals

PT8 (sheep, cattle)	PT32 (sheep)
PT2 (sheep, goat, pony, pig)	PT4 (goat)
PT21/28 (goat)	PT4 (cattle, sheep, goat)
PT2 (wild rabbits, cattle on adjacent farm)	PT34 (goat); PT8 (deer)

These other strains could have caused human infection but did not as far as we were aware

Three farm premises were the settings for more than one outbreak



DISCUSSION AND CONCLUSIONS

- Animal contact common outbreak route; true number of family outbreaks and sporadic cases per year attributable to this route unknown; better information gathering needed
- Investigations now more complex; require collaboration and sharing of data
- Number of cases linked to open farms is small in context of annual farm visits and total VTEC O157 per year
- Involvement of young children who are most vulnerable group
- Increased awareness of risk attached to farm visits led to publication of Health and Safety Guidelines for teachers[9] and to Farms for Schools organisation
- Improvements in hand-washing facilities; separation of eating and playground areas from animals; better signage and information for visitors
- But outbreaks still occur at well-run open farms with good hygiene
- Range of species implicated on open farms; cattle may be ultimate source leading to acquisition by other animals and establishment in environment
- Goats may pose a particular risk due to gregarious habits
- PTs of VTEC O157 reflect those found generally; use of PFGE to identify specific animals or environments and provide evidence for control measures
- Impossible to eliminate risk but aim to manage it successfully

2.96

VTEC O157 outbreaks linked to animal contact in England and Wales between 1996 and 2005

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Transmission of VTEC O157 by direct or indirect contact with animals was identified in primary cases from 24 general outbreaks of infection between 1996 and 2005 occurring in all health regions of England and Wales. Sixteen outbreaks involved agricultural premises open to the public, 3 were at commercial farms, including those at prisons and 4 at on-farm campsites. Investigations were carried out jointly by public health professionals and veterinary staff. Human isolates (156), including those acquired by secondary person-to-person spread, were typed and compared with those obtained from putative sources. VTEC O157 with the same phage type (PT), Vero cytotoxin (VT) type and PFGE profile were obtained from humans and animals in 18 outbreaks. Species harbouring outbreak strains included cattle, sheep, goats, pigs, equines and wild rabbits. On some open farms, up to 5 animal types carried the outbreak strain. VTEC O157 of other PTs were recovered from animals in some investigations in addition to the strain causing human infection. In 7 family outbreaks (23 isolates) microbiologically linked to animals, infection was acquired at private farms, open farms or followed contact on domestic premises. Predominant PT and VT types of VTEC O157 in the 31 incidents described were PT2, VT2 (11), PT21/28, VT2 (10) and PT8, VT1+2 (4) with 6 strains of other PTs. PT21/28 has been the most common type from human infections since 1999 while PTs 2 and 8 are the next most common types. Three premises had episodes of infection in two different years; persistence of related strains appeared likely on one farm but at the other two, strains with different properties were recovered. Cases from all these incidents represented 2.3% of human VTEC O157 in the time period but included cases in children <5 years of age, the most vulnerable group for infection and serious illness.

2.10

E.COLI 0157 INFECTION IN GOATS

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INTRODUCTION

Verocytotoxin-producing *Escherichia coli* (VTEC) have only emerged as a cause of serious human illness during the last few years. Food poisoning caused by ingestion of VTEC 0157 came to the forefront of public attention with the large scale outbreak in Central Scotland in 1996. This led to the Pennington Report and recommendations for improvements in hygiene of slaughterhouses, meat production premises and butchers' shops. Pennington (1997) also devoted a chapter to farms and *E.coli* 0157 in livestock, since it is generally considered that cattle and other livestock species constitute the main reservoir of infection. Although this has stimulated much further work into various aspects of *E.coli* 0157 infection, its precise epidemiology and natural history is still not fully understood. *E.coli* 0157 is the commonest VT-producing *E.coli* isolated from cases of human illness but it is important not to forget that human illness can also be produced by non-0157 VT-producing *E.coli,* biochemically atypical *E.coli* 0157 and VT-producing organisms other than *E.coli.* Human infection with 0157 can be asymptomatic but usually presents as diarrhoea, accompanied by severe abdominal cramps. Up to 50 percent of such cases progress to haemorrhagic colitis requiring hospitalisation. More seriously, a small percentage of cases develop the haemolytic uraemic syndrome (HUS) which can be fatal. Most cases occur in children under five years or in the elderly but infection can occur at all ages.

E.COLI 0157 AND ANIMALS

VTEC 0157 is primarily a foodborne infection although secondary person-to-person and faecal-oral spread are also important features of outbreaks. Products of bovine origin, including burgers and minced products, are commonly implicated in human disease. However, a small number of cases are not attributed to foodborne infection but are instead associated with direct contact with infected animals and their faeces. There are varying reports of the prevalence of 0157 excretion in different species although most work has been done on cattle. There is a very low animal specific prevalence of infection within herds and excretion tends to be intermittent and seasonal, with most excretion (and human cases) occurring during the summer months. Factors such as changes in intestinal flora following dietary changes or periods of starvation have been suggested as influencing the excretion pattern. In addition to healthy cattle, the organism has also been isolated from other species including, sheep, goats, deer, pigs, horses, dogs, cats and birds. The accepted view is that *E.coli* 0157 infection does not cause illness in carrier animals.

GOATS AND *E.COLI* 0157

As mentioned above, goats are one of those species from which *E.coli* 0157 has been isolated in association with human illness. One such case (Sukla and others 1995) was associated with a farm visitor centre where VTEC 0157, indistinguishable from human isolates, was isolated from cattle and goats on the farm. There is also a report of a cluster of cases of HUS in children in the Czech Republic, attributed to drinking unpasteurised goats' milk (Bielaszewsca and others 1997). In 1997 in Britain, four small outbreaks of human 0157 infection were linked to visits to open farms. In each case goats were one of those species from which 0157, indistinguishable by pulsed field gel electrophoresis from the human isolates, were detected.

INVESTIGATIONS AND OBSERVATIONS ON OPEN FARMS

Whilst there are considerable potential concerns regarding unpasteurised goats' milk, recent investigations concerning goats have concentrated on risks to the general public on open farms. In the case of one outbreak investigated by the author, and which received much media attention, 0157 PT 21 (one of the more common phage types) was isolated from five (5.6%) of 89 faecal samples taken over a period of two months from a variety of species and their environmental niches, including goats, horses, pigs, rabbits, chickens, sheep and guinea pigs. Three of the isolates were from goat faeces obtained from one particular paddock and the other two isolates were from dairy cows. The author also investigated another open farm under similar circumstances.

In this case *E.coli* 0157 PT2 was isolated from five (7.8%) animals (an adult goat, a kid goat and three calves) from a total of 64 faecal samples from a similar range of species. A follow-up study currently underway on this farm has, however, only detected 0157 in three calves, despite regular monthly testing of all resident stock, including goats.

CONCLUSIONS

There is no doubt that goats, like many other species, can excrete VTEC 0157 and that this can result in human disease, either through direct contact with infected faeces or through raw milk. Since the organism appears to be so widespread and is excreted only intermittently, there is probably little point in testing all goats for the presence of infection. In view of this, and the relative difficulty with which the organism is isolated, it may be more prudent to assume that all farm livestock, including goats, are potentially infected and to adopt appropriate hygiene procedures at all times. Nevertheless, there is some circumstantial evidence to suggest that groups of animals may become infected through recently added stock and the value of quarantining and testing replacements before mixing with resident animals is still open to debate. Recently, the Health and Safety Executive (HSE 1998) have produced guidelines for the prevention of *E.coli* 0157 infection in visitors to open farms, including precautions such as providing adequate numbers of washbasins, supervision of visitors and separation of eating and animal handling areas.

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POTENTIAL ZOONOTIC PROBLEMS ON GOAT FARMS WITH PUBLIC ACCESS

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INRODUCTION

This paper updates a presentation given at a previous meeting of the Society (Pritchard 1988). The main development since then has been the emergence of verocytotoxin-producing *Escherichia coli* (VTEC) O157, the significance of which in goats was also discussed recently by Pritchard (1999a). Cryptosporidiosis has also assumed increased importance, particularly in relation to farm visits (Pritchard and Fleetwood 1995). The arrival during the 1990s, of caseous lymphadenitis (CLA) in sheep and goats, caused by infection with *Corynebacterium pseudotuberculosis*, added yet another, albeit very minor, potential skin zoonoses to the list (Pritchard 1999b). There has been continuing debate about the possible association between *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) and Crohn's disease in man. Although the case is far from proven, the fact that Johne's disease is not uncommon in goats inevitably raises potential concerns regarding the sale of unpasteurised milk.

POTENTIAL ZOONOTIC INFECTIONS FROM GOATS IN BRITAIN

The following tables, derived partly from Smith and Sherman (1994) and Palmer and others (1998), provide an updated list of the main potential zoonotic diseases and infections that may be acquired from goats in Britain, including some of unlikely significance. For further details also see Pritchard (1988).

Direct/Indirect Transmission

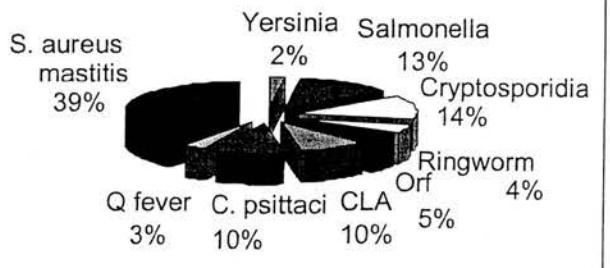
VTEC O157
Cryptosporidium parvum
Salmonella sp
Orf
Ringworm
Coryne. pseudotuberculosis
Chlamydia psittaci
Mycobacterium bovis
Q fever
Campylobacter sp
(Brucella abortus)
Toxoplasma gondii
Yersinia sp

Milk-Borne/milk contaminant

VTEC O157
Staphylococcus aureus
Salmonella sp
Campylobacter sp
Q fever
Listeria monocytogenes
Streptococcus zooepidemicus
Toxoplasma gondii
Yersinia sp
(Brucella abortus)
Mycobacterium bovis
Louping ill
C. pseudotuberculosis

The relative frequency with which diagnoses of potential zoonotic significance in goats have been made from submissions to Regional Veterinary Laboratories during the last five years (Veterinary Investigation Diagnosis Analysis III) is shown below. VTEC O157 infection does not cause clinical disease in animals and is not represented. Although the total number of relevant diagnoses was small (178) and the data inevitably highly biased, the findings still provide a useful overview. In the light of comments made above, it is noteworthy that 157 diagnoses of Johne's disease were made during the period reviewed.

Potential goat zoonoses from VIDA 1995-99



ENTERIC ZOONOSES

As shown above, *Staphylococcus aureus* mastitis is frequently diagnosed and, as discussed by Pritchard (1988), unpasteurised goats milk

presents a potential risk of staphylococcal enterotoxicosis food poisoning although cases appear to be rarely reported. Cryptosporidiosis is probably the enteric zoonosis most frequently acquired from ruminants. Both diarrhoeic and clinically normal animals (mainly up to about four weeks of age) can excrete cryptosporidial oocysts. These are very resistant and can persist in the environment for several months. Strict attention to hygiene, including steam cleaning of buildings and the use of specific anticoccidial disinfectants such as "Oocide" (Antec) prevent a build up of infection. Other potential zoonotic enteric conditions from goats include *Salmonella sp* and *Yersinia sp* infections.

VTEC O157 infection in goats was discussed at a recent meeting (Pritchard 1999b). The serious public health issues associated with VTEC O157 infection are the life threatening complications of haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura, which can develop mainly among children under four and adults over 65 years of age. Human infection can occur from the consumption of contaminated unpasteurised goats' milk or cheese or from faecal contamination acquired after pasteurisation. Goats have also frequently been implicated as a possible source of infection for visitors to open farms (Pritchard 2000) through direct or indirect contact with infected faeces. However, cattle are the main reservoir of infection and recent surveys from England, Scotland and Wales, by the Veterinary Laboratories Agency and the Scottish Agricultural colleges have indicated about 5 – 9% of faecal samples and 25 – 44% of herds are infected. No comparable prevalence data are currently available from goats. Their apparent involvement in open farm outbreaks is likely, in part at least, to be associated with their frequent presence on open farms and their increased contact with the public by virtue of their friendly and inquisitive nature.

Between 1994 and 1999 eight (9%) of 89 general outbreaks of VTEC O157 infection in England and Wales were linked to visits to open farms (Pritchard and others 2000). These included a total of just 51 human cases from an estimated 13 million visitors annually. However, most human VTEC O157 infection is sporadic and the source is very difficult to determine. As reviewed by Pritchard (2000), recent case control studies suggest that many of these sporadic cases could be linked to direct or indirect contact with farm animals, including goats, rather than being derived from contaminated food sources. As with other enteric infections, it is essential that goat owners are aware of the potential zoonotic risks both to themselves and to members of the public, from direct or indirect contact with faeces of infected (although clinically normal) animals, and the need for good hygienic practices.

Some goat owners have expressed interest in screening their herds for VTEC O157. It is important to be aware of the significant difficulties with this approach, mainly because of the intermittent excretion pattern – probably only a few days in most cases. Testing is also expensive and a single negative result is meaningless. Furthermore, there is the vexed question of what action to take if an animal proves to be positive. Goat owners may not wish to wrestle with their conscience if, for example, infection is identified in an animal they are about to sell, perhaps to a family with young children. It is far preferable to assume that all animals, including goats, are potentially infected and routinely adopt appropriate hygiene precautions. Detailed guidelines on avoiding ill health at open farms from VTEC O157 and similar organisms is given in the Agriculture Information Sheet 23 (revised) available from the Health and Safety Executive.

SKIN INFECTIONS

The three main diseases in this category – orf, ringworm and CLA – are all characterised by their potentially long persistence in the environment. Human cases can occur amongst the general public where there is contact with infected livestock. Orf is more likely to be a problem on mixed farms where sheep are also present. Similarly, ringworm can occur in goats from contact with infected calves or calf houses contaminated with ringworm spores. If any of these infections are present in goat herds in contact with the public, it is important to ensure that thorough cleaning and disinfection of the environment is undertaken because of the risk of indirect, as well as direct transmission. Remove any animals showing clinical signs from contact with the public.

SUMMARY AND MAIN CONCLUSIONS

In Britain, zoonotic infections acquired from goats appear to be uncommon. However, it is important that goat owners and veterinary surgeons working with goats are aware of the main zoonoses and their relative prevalence.

- Be aware of the main goat zoonoses
- Regularly inspect animals and seek veterinary advice when appropriate
- Keep diarrhoeic and other sick animals (including skin infections) away from the public

- Observe strict hygiene of farm premises and milk production
- Provide hand washing facilities for visitors where appropriate
- Ensure eating and animal handling areas are strictly separated
- Provide good supervision and advice for members of the public
- Keep pregnant women away from kiddings and particularly abortions
- Consider milk pasteurisation.

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Goats and Zoonoses

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Introduction

Zoonoses are infections naturally transmissible between vertebrate animal hosts and man. Transmission can occur in a number of settings, ranging from indirect contact through food or drinking water to direct occupational exposure on farms, during leisure pursuits or from pets. Some farm animal zoonoses may be clinically unapparent and economically insignificant which can sometimes make it difficult to encourage farmers to take them seriously. The Veterinary laboratories Agency (VLA) is funded by Defra to provide surveillance information on zoonotic diseases (mainly in farm animals) in England and Wales; some current issues and findings from recent investigations are outlined here, with particular reference to goats where possible.

The role of the VLA in Zoonotic diseases

Zoonoses surveillance within the VLA falls within the Food and Environmental Safety programme, although it relies heavily on information from the Emerging Diseases and Welfare programme. It is subdivided into several projects including antimicrobial resistance, potential food safety incidents, salmonella and non-statutory zoonoses. The latter category includes a wide variety of non-notifiable zoonoses such as Q fever, cryptosporidiosis and leptospirosis and is discussed here.

Surveillance data on non-statutory zoonoses, and infections shared between animals and man, are collected by VLA from various sources. These include diagnostic and disease monitoring submissions to VLA's regional laboratories, information from farm visits, surveys or projects and general communications with farmers, private veterinary surgeons, human health professionals and the general public. Clinical disease diagnoses conforming to strict criteria are recorded on the VIDA database which is an integral part of the scanning surveillance system used to detect trends. More targeted surveillance data is also collated for some of the main infections. The information gathered is used in intelligence reports to Defra and other stakeholders. Much of the information is available on Defra and HPA websites. One of the main published outputs is the UK Zoonoses report which is published annually.

A strong multi-disciplinary approach is essential for effective zoonoses surveillance and there are good on-going communications at all levels between veterinary and medical professionals. A cornerstone of this is provided by the Human Animal Infections and Risk Surveillance group (HAIRS) which is a multi-agency horizon scanning group which meets monthly to discuss and assess recent infectious disease issues with possible zoonotic potential (Walsh and Morgan 2005). VLA also assists the HPA (usually through the local

Consultant in Communicable Disease Control) with the investigation of zoonotic incidents linked to animal contact (see below).

Some examples

VTEC O157

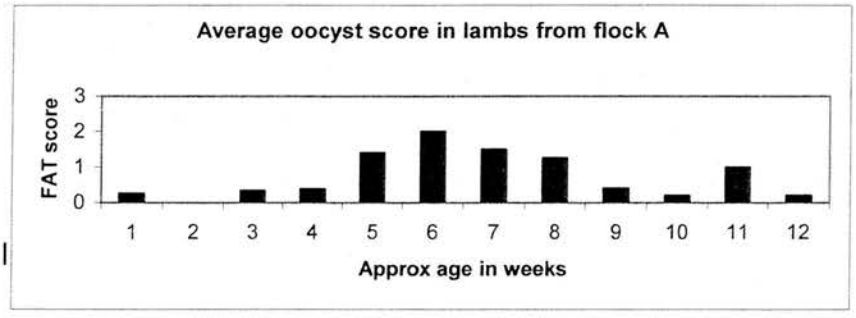
Verocytotoxigenic *Escherichia coli* (VTEC) O157 is a particular serogroup of *E. coli* that is zoonotic and can be responsible for human outbreaks which command significant media attention. VTEC O157 are common commensal bacteria found in the alimentary tract of many animal species (especially cattle, sheep and goats) without causing them any illness. In people, however, infection can lead to potentially very serious illness (notably haemolytic uraemic syndrome), especially in the main susceptible age groups (<5 yrs >65 yrs). Large outbreaks are usually related to consumption of contaminated food but most cases overall occur sporadically and animal contact is thought to be a major risk factor for these. Where appropriate, the VLA assists the HPA with investigation of VTEC O157 outbreaks linked to farms (especially those open to the general public). In addition to providing veterinary advice on husbandry and hygiene aspects, extensive faecal sampling of animals is undertaken and animal strains of VTEC O157 are compared with isolates from human cases mainly by phage-typing and pulsed field gel electrophoresis (PFGE) profiling.

Open farms are important components of the tourism and leisure industries, as well as fulfilling an important educational role. The downside is that they represent a zoonotic interface for members of the public who might otherwise have little contact with animals. An analysis of VTEC O157 outbreak investigations carried out by VLA on 31 open farms between 1997 and 2007 (Pritchard and others in preparation) identified the infection on 19 (61%) of premises. On these, VTEC O157 was found in 29% of samples from cattle, with isolation from the other main species as follows: sheep 24%, donkeys 15%, pigs 14%, horses 12% and goats 10%. A high prevalence of infection was also was detected in llamas and alpacas on the few premises on which they were sampled. Despite the lower prevalence of infection detected in goats than the other main domesticated farmed species, goats were actually implicated (by phagetyping and PFGE profiles) in slightly more incidents of zoonotic transmission than other species (Willshaw and others in preparation). This was probably because goats are a very common species on open farms and their friendly and inquisitive behaviour leads to increased contact with visitors.

One of the most interesting and unusual incidents investigated involved a series of visitors to a wildlife gardens acquiring infection from a picnic area through contact with faecal pellets from wild rabbits which had been co-grazing with infected cattle on the adjacent farm (Pritchard and others 2001). Elsewhere, farm dogs on a cattle farm were another unusual source of human VTEC O157 infection. It should be emphasised that the risk of acquiring VTEC O157 infection on open farms are very small and can be reduced further by increased public awareness and compliance with common-sense hygiene and supervisory precautions such as those detailed in a leaflet (HSE 2002) available from the Health and Safety Executive (www.HSE.gov.uk).

Cryptosporidium

Cryptosporidiosis (caused by *Cryptosporidium parvum* protozoa) is a common cause of diarrhoea in young ruminants, including goat kids, and often occurs alongside other infections such as rotavirus. It is also a common zoonotic infection and can be acquired from direct contact with the faeces of infected animals. It has long been known that healthy animals can shed Cryptosporidium oocysts, the extent of which was quantified by recent work by the VLA using a sensitive fluorescent antibody test (FAT). This clearly demonstrated the presence of very high oocyst counts – much higher than the minimal infectious dose for people (less than 1000 oocysts) in between about 10 and 50% of clinically normal lambs used for bottle feeding on open farms (Pritchard and others 2007). Peak oocyst output was at around 6 weeks of age (Fig 1).



FAT scores (oocysts/gram): 1 = >1000, 2 = 10,000 - 150,000, 3 = 150,000-700,000

Fig 1: Relationship between *C. parvum* oocyst shedding and age in orphan lambs (Adapted from Pritchard and others 2007)

In a further recent VLA study, *Cryptosporidium hominis*, which is associated with non-zoonotic cryptosporidiosis in people, was detected in a sheep and a goat (Giles and others in press), which adds a further dimension to transmission risks. Methods for the control and prevention of zoonotic transmission of Cryptosporidium from direct animal contact are essentially the same as for VTEC O157.

Q fever

Q fever is an important although relatively uncommon zoonosis, which is ubiquitous and occurs worldwide. It came to the forefront recently with large human outbreaks in Stirlingshire (2006) and Cheltenham (2007). The organism (*Coxiella burnetii*) has recently been reclassified by molecular sequencing as being a legionella-like bacterium and is not a rickettsia as formerly thought. There are two circulation cycles – one in ticks/wildlife and one in domesticated animals, especially cattle, sheep and goats. It occurs as either a spore-like small cell variant (SCV) form which is resistant to heat, pressure, desiccation, and disinfectants or a large cell variant (LCV) which multiplies and persists in host

monocytes and macrophages and localizes in the mammary gland and placenta. Common sources of infection are the placenta, birth fluids, urine, faeces and contaminated bedding, particularly from lambing, calving and kidding pens. The prevalence of animal infection in Britain is not known but it is thought to be fairly common – for example, a survey in 1995 using bulk milk antibodies detected it in 21% of dairy herds in England and Wales.

Most infection in animals is subclinical, although occasionally there can be large abortion outbreaks in farmed ruminants and particularly in goat herds. In man, Q fever usually causes just a mild flu-like illness but sometimes it can be more severe with persistent fever and occasionally chronic sequelae, such as in those with damaged heart valves. Pregnant women and immunocompromised people are at greatest risk. It is a common infection in occupationally exposed groups – in a recent survey in Northern Ireland about 54% of farmers and 59% of veterinary surgeons were found to have serological evidence of exposure. The source of large and otherwise unexplained human outbreaks is thought to be windborne aerosol transmission from farm animal sources although it can also (rarely) be acquired from tick bites or drinking unpasteurised milk. It is important to be aware of the latter since Q fever has been diagnosed by the VLA in a number of milking goat herds. Guidelines for farmers to reduce the risks from Q fever infection, particularly by using appropriate husbandry and hygiene measures, including careful handling and disposal of high risk bedding material, were recently published on the HPA and Defra websites (www.hpa.org.uk www.defra.gov.uk) where there is other useful information about Q fever and the other topics covered here.

Toxoplasmosis

Toxoplasmosis is zoonosis which has come to increased prominence recently following a major report in 2007 by the European Food Safety Authority (EFSA) which highlighted it's importance as a foodborne zoonosis. Toxoplasmosis may have been somewhat underestimated in the past, at least in Britain, where we tend to think of the main risk being contact with cat faeces, particularly pregnant women handling litter trays. In the USA it is considered to be a very important disease because of the severity of the chronic human illness it causes. Medical and veterinary initiatives are currently underway in Europe to improve monitoring, surveillance, diagnosis and control of toxoplasmosis, the main driving force being the total cost of the associated human disease burden.

Toxoplasmosis is caused by *Toxoplasm gondii,* which is a protozoan parasite that affects all warm blooded animals and is a major cause of abortion in goats and sheep. Asexual multiplication of the parasite occurs in intermediate hosts and sexual multiplication occurs in the cat, which is the definitive host. Human infection is very common but mainly asymptomatic, although congenital infection can result in abortion, stillbirth, hydrocephalus and chronic ocular disease. Lymphadenopathy, retinitis and encephalitis may follow post-natal infection in immunocompromised people, such as those infected with HIV. Human infection can be acquired from sporozoites or oocysts from environmental sources or tachyzoites and bradyzoites in tissue cysts. Potential sources of human infection include raw or undercooked meat (particularly pork), unpasteurised milk (goats milk is often highlighted in this context), unwashed vegetables and cat faeces.

Some other recent topics

VLA has been involved in the investigation of a number of minor incidents involving what are often termed "orphan zoonoses". One such incident involved a human case of *Leptospira* Icterohaemorrhagiae infection (Weil's disease) acquired from contact with pet rats. The source was traced back to a pet shop where infection was detected by PCR in several further rats (Gaudie and others in press). Another area of recent interest is human cases of diphtheria caused by *Corynebacterium ulcerans,* possibly acquired by zoonotic transmission from the pharynx of dogs and cats. The usual agent of human diphtheria is *C. diptheriae* but there are close similarities to *C. ulcerans.* Improved guidelines for the medical/veterinary reporting and investigation of miscellaneous zoonotic conditions in England and Wales are currently being drawn up by a multidisciplinary group led by HPA.

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Goats and Public Health

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Summary

The structure of the goat industry and the present legal situation regarding food production from goats in the UK are described. The main potential zoonotic diseases from goats are discussed with emphasis on possible risks from milk-borne infection and parturition. It is concluded that, whilst there is no evidence of any significant zoonotic problem at present, it is important that goat owners and veterinary surgeons are aware of the potential risks so that sensible precautions may be taken. In addition, there is a need for stricter control over the hygiene and production of goats' milk and goats' milk products in the UK. Despite this, the desirability of compulsory pasteurisation is debatable at the present time because of consumer interest in an unadulterated product.

Introduction

The increasing popularity of goat keeping, both as a hobby and a small commercial enterprise, epitomises the current health food and self sufficiency revolution and the desire of many people in the western world to 'return to the land'. Furthermore, goats not only supply food products but they also serve as companion animals.

Numerous articles in the farming and national press have proclaimed the resurgence of interest in goat keeping and the profits to be made. The manufacturing industry offers a wealth of equipment to cater for all aspects of goat keeping, with particular emphasis on the cottage industry and small-holding aspects. Reliable techniques for controlled breeding, embryo transfer, artificial insemination and pregnancy diagnosis of goats have emerged in recent years. Despite the folklore and traditional remedies that surround the goat, the veterinary profession stated its commitment with the formation of the Goat Veterinary Society in 1979. Breed societies and goat clubs throughout the UK are very active and flourishing.

Structure of the Goat Industry in the UK

Until very recently, there was little clear information about the structure of the goat industry in the UK. Since most goats are kept in very small numbers on unregistered holdings, much information has failed to reach the Government's statisticians and estimates of goat numbers have varied widely. In August 1985, in response to these problems, the Islay and Jura Goat Society (IJGS) carried out a statistical survey of goat

husbandry in the UK and its findings (IJGS, 1986) represent the most accurate information now available. The Society estimated that there were nearly 8 000 herds and a total UK goat population of over 75 000. The survey revealed that 53 per cent of goat keepers had herds of five goats or less; only 0.6 per cent of owners had large herds (100-500). Thirty per cent of all goats were kept on properties of under an acre. British Saanen (21 per cent); British Toggenburg (14 per cent) and Anglo Nubian (12 per cent) were the most common breeds. However, as might be expected, 21 per cent of all goats were of unclassified varieties. According to the survey, 88 per cent of owners kept goats for private milk supply, 38 per cent for meat, 22 per cent for commercial reasons and 3 per cent for their fibre. Pleasure (74 per cent) and showing (37 per cent) also featured prominently.

Since there is no requirement for registration, owners can sell surplus milk direct to the public — either fresh or frozen — usually in plastic bags from the farm gate. There is a growing home and export market for dried goats' milk and other milk products such as cheese or yoghurt. Several co-operatives — including the recently established Goat Producers Association of Great Britain — have been formed for the sale and distribution of these products. Retail outlets are predominantly health food shops but supermarkets are showing increasing interest.

In contrast to Mediterranean countries such as Greece, goat meat consumption in the UK is minimal. Although some finds its way to ethnic groups, there is no established goat meat market and most surplus fattened kids are consumed by goat keepers or their friends and neighbours.

There is a small but potentially lucrative market for goat fibre, particlarly high quality cashmere, and recently the Angora goat has been imported into Britain. There is also a small market for goat skins.

Legal Aspects of Food Production from Goats in Great Britain

The slaughter of goats is covered by the Slaughterhouses Hygiene Regulations (1977) in England and Wales and by the Slaughterhouse Hygiene (Scotland) Regulations (1978) in Scotland. However, it is in the rapidly growing field of goats' milk and goats' milk products that legislation is lacking. There is little organised marketing and there are no legal standards for goats' milk in the UK. Furthermore, goats' milk is not considered to be 'milk' under the Milk and Dairies (General) Regulations (1959) or the Milk and Dairies (Scotland) Act (1914) which control the hygienic production of cows' milk. It is, however, classed as 'food' under the food legislation.

Lewis (1985) reviewed the present legal situation regarding goats' milk, with particular reference to the retail aspect. In Britain, legislation is available to local authorities under the Food Act (1984) for England and Wales and the Food and Drugs Act (1956) for Scotland and it is an offence to offer for sale any food commodity which is not of the nature, substance or quality demanded by the purchaser. Although the initial stages of goats' milk production are exempt from the Food Hygiene (General) Regulations (1970), processing, packaging and distribution are included. Enforcement of the legislation is primarily the responsibility of the environmental health departments of the local authorities; labelling is the brief of the local trading standards officers. However, according to the IJGS survey, even these limited regulations are not widely enforced. There are no legal requirements or specifications for testing of milk other than cows' milk.

Diseases Transmissible from Goat to Man

Man can acquire disease directly from goats or indirectly via vectors such as milk or other animal hosts. The main potential goat zoonoses in the UK are shown in Table 1.

Table 1 Potential zoonotic diseases and infections from goats in the UK

Direct transmission	Indirect transmission	Organisms associated with a milk-borne infection
Anthrax	Fascioliasis	*Campylobacter* spp.
Brucellosis	Hydatidosis	*Coxiella burnetti*
Campylobacteriosis	Toxoplasmosis	*Escherichia coli*
Chlamydiosis		*Listeria monocytogenes*
Cryptosporidiosis		Louping ill
Dermatomycosis		*Salmonella* spp.
Leptospirosis		*Staphylococcus aureus*
Listeriosis		*Streptococcus* spp.
Orf		*Toxoplasma gondii*
Pseudotuberculosis		*Yersinia* spp.
Q fever		
Salmonellosis		
Tuberculosis		

Adapted from Forbes 1985.

Direct zoonoses

Goat keepers themselves are clearly at the greatest risk from direct zoonoses, particularly in the hobby farm environment of goat keeping in which there is likely to be close contact between goats and man.

Parturition — either at full term or prematurely due to abortion — presents several potential zoonotic risks to the goat keeper and it is important that strict hygiene is observed with the handling and disposal of abortion material. There are also possible risks to the veterinary surgeon attending the kidding or performing caesarian operations.

Chlamydia psittaci is a major cause of sheep abortion and can similarly affect goats. An outbreak in Scotland was described by Appleyard and others (1983). Although mammalian strains of chlamydia are generally regarded as having a low virulence for humans compared with avian isolates, there have been several reports of abortion in women assisting with lambings in infected flocks (Buxton, 1986). Although there does not appear to be any documented case of human infection acquired from goats, this remains a distinct possibility for the future.

Placentitis and abortion in the goat can result from Q fever *(Coxiella burnetii)* infection (Robinson, 1983); latent infection appears to be activated during late pregnancy and the organisms are excreted in the urine, faeces, milk and foetal fluids. The organism is very resistant in the environment and aerosol infection can be signficant. Although the prevalence of *C. burnetii* infection in goats in Britain is not known, Little (1983) commented that several people in Britain have developed Q fever after attending kiddings. The infection appears to be fairly prevalent in cattle in Britain. For example, in a recent survey, Pritchard and Pritchard (unpublished data) found serological evidence of infection in 22 (8.5 per cent) of 260 herds in East Anglia. Q fever in sheep and goats poses quite serious problems in other parts of the world, particularly Mediterranean countries and it may be a somewhat under-diagnosed zoonosis in the UK.

Listeria monocytogenes can be a significant cause of abortion in goat herds and large numbers of organisms are excreted in the vaginal discharges of affected females (D.G. Harwood, personal communication). This organism appears to pose a potential risk to man although it is debatable whether it is a true zoonosis because it is so widely disseminated in the environment. However, there appear to be particular risks for pregnant women and immuno-suppressed patients. Veterinary surgeons have acquired papular cutaneous listeriosis whilst delivering infected calves (Robinson, 1983) and it would be reasonable to assume that similar risks exist with the delivery of infected kids.

Infection with *Campylobacter* spp. is an important cause of sheep abortion and the disease has also been reported in goats. Although the main zoonotic risk relates to the consumption of infected milk, Duffell and Skirrow (1978) described a case of acute campylobacter enteritis in a farmer who gave oral resuscitation to a moribund lamb infected with campylobacter. There must therefore be a similar possibility of transmission of infection to man from goats, particularly since people with little or no knowledge of animal husbandry are perhaps more likely to be involved with goats than sheep.

Toxoplasma gondii is an important cause of abortion and perinatal mortality in goats in Britain (Lloyd, 1982). Direct zoonotic infection from abortion material seems very unlikely. The risk from infected milk or meat will be considered later.

Leptospira interrogans serovar *hardjo* is now a major cause of abortion and milk drop in cattle in the UK. It also presents a significant zoonotic hazard via infected urine, aborted fetuses, foetal fluid and placentae. According to Ellis (1985), *L.hardjo* is also the main serovar infecting sheep in Ireland and reproductive failures have been described. Although the leptospira situation in goats in the UK is not known, it seems likely that it is similar to sheep (D.G. Pritchard, personal communication).

Brucella melitensis infection in goats is a significant problem in Mediterranean goat herds but it has never been reported in the UK. The few cases of human infection recorded in Britain have either been associated with imported goats' milk cheese or the infection was acquired abroad (Gayford, 1985; Barrett, 1986). *Brucella abortus*

infection has occasionally been reported in goats but has not been recorded in Britain since 1965 (Lewis, 1985).

Enteric disease and healthy intestinal carriage of disease organisms in the goat constitute another area of potential zoonotic risk. *Cryptosporidium*, a protozoan parasite of several animal species including calves, lambs and kids, offers a particular risk to young children. Salmonella infection — *S.typhimurium*, *S.dublin* and *S.abortus ovis* have all been described in goats (Buxton, 1959) and could similarly be transmitted to man. Although Jackson (1985) found no association between consistency of faeces of young kids and isolation of campylobacter organisms, campylobacter enteritis has been described in lambs (Firehammer and Myers, 1981) and it seems likely that a similar disease could occur in goats.

A further potentially important aspect of zoonotic disease in the goat relates to skin infection. Orf (contagious ecthyma) is not uncommon in goats. Kids may show lesions on the lips, gums, muzzles, and nostrils; the teats and udders of adult females can also be affected. The risk of transmission to man is high in artificially reared goats and there is a need for particular care when children are bottle feeding kids. Care is similarly needed when handling goats suspected of being infected with ringworm, particularly *Trichophyton verrucosum* which occurs occasionally in goats (Robinson, 1983).

Indirect zoonoses

Indirect zoonotic disease from the goat appears to be of minimal significance. However, hydatidosis has been described in the goat (Jackson and others, 1984) and human infection could occur via dogs that have eaten raw infected goats' meat. Similarly, man could become infected with *Toxoplasma gondii* following the consumption of inadequately cooked meat infected with toxoplasma tissue cysts. There is also a very small risk of man indirectly acquiring *Fasciola hepatica* infection from goats via metacercariae encysted on wild watercress used for human consumption.

Milk borne disease

Extravagant claims have frequently been made for the nutritive and therapeutic value of goats' milk despite lack of conclusive scientific evidence and possible dangers of its use as an infant feed (Taitz and Armitage, 1984; Archer and MacDonald, 1984; Thompson, 1984; Taitz and Wardley, 1985). As mentioned earlier, there is limited statutory control over the production and sale of goats' milk and very little is pasteurised for liquid sales. It is therefore not surprising that concern has recently been voiced from many quarters concerning the need for more legislation and for accepted standards of hygiene and quality similar to those applicable to cows' milk. Questions are also currently being raised concerning the possible need for routine testing of goats for brucellosis and tuberculosis as for dairy cattle.

Although over 95 per cent of cows' milk in England and Wales is now heat treated, milk-borne disease due to the consumption of raw or defectively pasteurised milk has still been a significant problem in recent years (Galbraith and others, 1982). Salmonellosis, campylobacter enteritis, staphylococcal enterotoxicosis and infection with *Yersinia enterocolitica*, *Streptococcus zooepidemicus* and *Corynebacterium ulcerans* all featured in the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS/CDSC) reports (Barrett, 1986), associated with cows' milk and dairy products, during 1983-1984. In marked contrast, there has been little evidence of human infection due to the consumption of raw goats' milk or milk

products. A small outbreak of *Salmonella typhimurium* gastro-enteritis associated with the consumption of Turkish goats' cheese was recorded in 1980 (Galbraith and others, 1982) and *Y.enterocolitica* infection was reported in 1981 in an 18-month-old child following the consumption of raw goats' milk (PHLS/CDSC, unpublished data). More recently, an outbreak of campylobacter enteritis associated with the consumption of raw goats' milk was described by Hutchinson and others (1985).

Regarding the concern over tuberculosis and brucellosis, *Brucella abortus* has not been confirmed in goats in the UK for at least 20 years and tuberculosis has not been confirmed for over 35 years (Gayford, 1985). Accordingly, it is the current policy of the State Veterinary Service only to test goats for these diseases when there is close contact with known infected cattle. However, owners can arrange for tests to be carried out privately through their own veterinary surgeons.

In addition to the infections mentioned already, there are several other potential zoonoses from raw goats' milk. Lloyd (1982) considered that toxoplasmosis in goats presented a significant public health hazard since the tachyzoites have been detected in the milk of experimentally infected goats (Dubey and others, 1980). Furthermore, Sacks and others (1982) reported an outbreak of human toxoplasmosis in which the evidence strongly implicated the consumption of raw goats' milk. Jones and others (1982) described caprine mastitis associated with *Yersinia pseudotuberculosis* infection: the organism was isolated in profuse pure culture from the mastitic milk. The goat was being kept to provide milk for a five-year-old boy with asthma and eczema but fortunately he had not consumed any of the milk from this animal. In a recent study, Reid and others (1984) found high levels of louping ill virus in the milk of experimentally infected goats. They commented that the virus was likely to be stable in unpasteurised milk and could be transmitted to man via raw milk or milk products, causing a severe meningoencephalitis. Louping ill may therefore present a significant risk to man from goats grazing tick infested upland areas of Britain.

Bacteriological and Hygienic Quality of Goats' Milk

Numerous surveys have been carried out in recent years to assess the bacteriological and hygienic quality of goats' milk — usually at farm gate or retail level. Lewis (1981) found a poor standard of hygiene in many herds in a small survey in East Anglia in 1980. Jackson (1985) found that 154 (11 per cent) of 1 404 individual goats' milk samples failed the methylene blue test (MBT) and gave a high plate count at 22°C and 37°C: *Staphylococcus aureus* was found in 66 (5.3 per cent) of the 1 250 samples that passed the MBT. Roberts (1985) reported on a similar large survey undertaken by the PHLS involving the examination of 2 493 samples of fresh and frozen goats' milk samples collected from a variety of retail outlets in England and Wales. Surface colony counts at 22°C and 37°C of $<10^5$ per ml of raw milk were found respectively in 76 and 79 per cent of samples examined: 86.7 per cent of samples passed the MBT. *Staph. aureus* was found in countable numbers in 4 per cent of samples examined but whereas Jackson (1985) failed to isolate any other significant pathogens, Roberts (1985) also recovered *Bacillus cereus* from three samples, *Yersinia enterocolitica* from two and *Campylobacter jejuni* from one sample. Roberts (1985) concluded that goats' milk was still a reasonably safe product although poor hygiene during production was a major cause of bacterial contamination. Surprisingly, neither Jackson (1985) nor Roberts (1985) isolated any salmonellas from the samples examined.

Stubbs and Hacking (1985) recently monitored the bacteriological quality of goats' milk from two large commercial enterprises in Wales — one a milk drying plant and the other engaged in the production of goat curd cheese. They found that the overall hygienic quality of the products was good but not up to current dairy cow standards. Poor bacteriological results from individual farms were primarily due to poor milk cooling. They related the total bacterial counts in goats' milk samples to the Milk Marketing Board's quality payments schemes and found a higher percentage of goats' milk fell into the penalty band than cows' milk.

Udder Infection

The picture is rather confusing regarding subclinical mastitis in the goat and the identification of the main pathogens. It is the general experience of the Veterinary Investigation Service that bacteriological examination of goats' milk samples is often unrewarding and that major pathogens are isolated with less frequency than from samples of cows' milk. From a total of nearly 200 samples of mastitic goats' milk examined at Norwich Veterinary Investigation Centre between 1980 and 1985, approximately 50 per cent failed to yield any significant growths of organisms or yielded only coagulase negative staphylococci. The main pathogens isolated were: *Escherichia coli* (18 per cent), *Staph. aureus* (15 per cent), *Bacillus* spp. (6 per cent), Streptococci (3 per cent), *Klebsiella* spp. (3 per cent) and *Pasteurella haemolytica* (2 per cent) (G. C. Pritchard and others, unpublished data).

The greatest problem with the bacteriology of goats' milk samples relates to the significance of coagulase negative staphylococci. This organism is frequently isolated both from apparently normal and abnormal caprine udders. Poutrel (1984) isolated it from 218 (15.7 per cent) of 1 385 samples and Hunter (1984) isolated it from 101 (20.9 per cent) of 483 samples. Manser (1985) suggested that coagulase negative staphylococci were common commensals similar to *Corynebacterium bovis* in cattle and this appears to be the most likely situation.

Although coagulase negative staphylococci are of no significant public health importance the apparently high prevalence of *Staph. aureus* mastitis is of concern because isolates from chronically infected goats have sometimes proved to be potentially enterotoxogenic (Pritchard and Wieneke, unpublished data). Whereas it is unlikely that clinically mastitic milk would be offered for sale, milk from subclinically infected animals could reach the consumer. Although the risk of staphylococcal enterotoxicosis from the consumption of infected milk or milk products from goats is probably minimal, it would be advisable for such milk to be pasteurised before consumption. A further problem with chronic staphylococcal mastitis in the goat is that, unlike the dairy cow, owners would be unlikely to cull affected animals because of sentimental attachment.

Numerous studies have been carried out to examine the somatic cell count of normal and mastitic goats' milk samples in an attempt to define acceptable standards. Apparently normal goats' milk often has a high somatic cell count and gives a positive reaction with the California Mastitis Test (Lloyd, 1982). Poutrel and Lerondelle (1983) suggested that a cell count of 10^6 per ml with the Coulter Counter represented the most satisfactory threshold for identifying infection with major pathogens. However, although somatic cell counts are usually higher in infected halves, apparently uninfected samples can also have cell counts of several millions. In a survey involving 62 predominantly small herds in the north of Scotland, Hunter (1984) found an extremely

variable cell count with the results usually much higher than cattle. A third of the samples had cell counts of $>2\times10^6$ ml. There is still no universally agreed standard for cell counts in goats but, as pointed out by Hunter (1984), tests for cattle milk may not be appropriate for goats.

Pasteurisation

Goats' milk, in common with milk from other species, constitutes an ideal medium for microbial perpetuation regardless of whether infection emanates from the udder or from the environment. In consequence, the potential for milk-borne disease from goats is considerable, particularly since most of the milk is sold and drunk untreated. Furthermore, it is likely that some goats' milk will be consumed by the most susceptible section of the general population — particularly young children.

On the face of it, there appears to be a strong case for compulsory pasteurisation. In Scotland, since August 1983, it has been compulsory to pasteurise all retail cows' milk but sales of raw goats' milk are currently unafffected by the legislation. There is a small but active lobby for the retention of green top cows' milk in Britain because of its purported wholesome and unadulterated properties. Similarly, there is possibly an even greater lobby that demands the availability of unpasteurised goats' milk. Compulsory pasteurisation of goats' milk would probably be very unpopular because this would go directly against the very reason for which the product is usually consumed. Significantly, at a joint meeting of the Goat Veterinary Society and the Veterinary Public Health Association held in Norwich in October 1984, delegates voted unanimously for the retention of raw goats' milk despite the potential hazards.

Although compulsory pasteurisation of goats' milk may have limited appeal, there appears to be universal agreement that stricter control over the hygienic production of goats' milk is essential. The initiative has already been taken by the Aylesbury Vale District Council (Lewis, 1985) and by the Scottish Home and Health Department (Forbes, 1985) to produce a voluntary code of practice for the hygienic control of goats' milk. The Scottish code (DAFS, 1984) is based on the general legal provisions for cows' milk production; it is anticipated that a similar code will be introduced shortly in England and Wales.

Conclusions

Despite the concern, there is little present evidence that goats and goats' milk have caused any significant degree of human illness or disease. However, the sale of a poor quality product or one that causes serious human infection could ruin the reputation and credibility of goats' milk and goats' milk products for the future.

The existing legislation for the protection of the public from milk-borne disease from goats is clearly inadequate. A voluntary code of practice is the first step towards rectifying the situation but in the longer term legislation is essential. This is particularly important as production and marketing methods for goats' milk and milk products are becoming more sophisticated and the products are becoming more widely available to the general public.

Regarding direct infections from goats, it is important that owners are aware of specific zoonotic diseases and veterinary surgeons should advise their clients accordingly. However, goats should not pose a greater risk to man than any other

species of livestock provided common sense precautions are taken to minimise the risks.

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2.14

VTEC O157

Verocytotoxigenic *Escherichia coli* O157 in camelids

WE would like to report the results of a survey of verocytotoxigenic *Escherichia coli* (VTEC) serogroup O157 in camelids carried out by the Veterinary Laboratories Agency (VLA). VTEC O157 has become an important human pathogen in recent years and causes a broad spectrum of illness. This includes diarrhoea, haemorrhagic colitis and occasionally severe systemic complications such as haemolytic-uraemic syndrome, which can be fatal, especially in young children and the elderly. Cattle and sheep are thought to be the main reservoirs for human infection, although a wide range of other domesticated species have been shown to carry VTEC O157 and may transmit the infection to people.

This survey was initiated following concerns over the detection of VTEC O157 in a significant number of camelids examined during investigations of human outbreaks, where the source of infection was thought to be contact with animals on premises open to the public during 1997 to 2007 (Pritchard and others 2009), and in three subsequent similar human outbreak investigations. The issue of the risk of acquiring VTEC O157 from contact with animals on open farms has assumed greater significance in the light of the large open farm-associated outbreak linked to Godstone Farm, Surrey, in 2009, although camelid species were not implicated in that outbreak (Griffin 2010). It was considered important to clarify whether alpacas and llamas present any significantly greater hazard from VTEC O157 than other animal species, particularly since the keeping of camelids has grown in popularity in recent years, especially on smallholdings and open farms where close contact between animals and people may facilitate the spread of zoonotic disease.

The 12-month opportunistic survey began in May 2009 using carcases submitted to VLA regional laboratories for diagnostic postmortem examination, and faecal samples submitted for routine monitoring or diagnostic purposes. Cultures for VTEC O157 are not normally routinely undertaken by VLA laboratories because the infection does not cause disease in animals. The British Veterinary Camelid Society was informed of the survey, and camelid owners could choose to opt out of testing for VTEC O157 if they wished. Microbiological examination of faeces was undertaken as described by Pritchard and others (2009). Biochemically confirmed *E coli* O157 were examined for the presence of verocytotoxin (VT) genes 1 and 2, and the attaching and effacing (*eae*) intimin gene (an additional virulence factor) using PCR (Willshaw and others 1997).

A total of 188 samples (from 96 separate premises) were examined during the survey; 168 (89 per cent) samples were from alpacas, 18 (10 per cent) were from llamas and two (1 per cent) were from camels. Three (1.6 per cent) samples tested positive for VTEC O157, with VT2 and *eae* genes detected. The three positive samples were all obtained from adult alpacas from the same premises. Non-verocytoxigenic *E coli* serogroup O157 was isolated from a further two samples from alpacas on different premises; in these isolates, only the *eae* gene was present. VTEC O157 was not isolated from any of the remaining alpaca, llama or camel samples.

Although this was not a structured randomised survey and was subject to significant selection bias, on the basis of the sample population examined, these results suggest that the prevalence of VTEC O157 infection in camelids is low, at around 2 per cent. This is reassuring and similar to that of other farmed species, where individual prevalences of around 4 per cent in cattle (Paiba and others 2003) and 2 per cent in sheep (Paiba and others 2002) have been reported. However, the prevalence in the close multi-species environment on most open farms may well be somewhat higher, as described by Pritchard and others (2009).

VTEC O157 is carried asymptomatically by animals, and owners of camelids should be aware of the potential for zoonotic transmission of VTEC O157 even from healthy animals. Avoiding contact with animal faeces and adopting principles of good hygiene following animal contact is crucial in reducing the risk of zoonotic infections. The tendency of alpacas and llamas to spit occasionally is another potential risk factor to consider, given that VTEC O157 has been isolated from the saliva of other animals (Keen and Elder 2002). Open farm owners should ensure that there is full compliance with the Health and Safety Executive Agriculture Information Sheet 23 (currently under revision). Further advice on reducing the risk of human VTEC O157 infection is provided on the Health Protection Agency website (www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1270122184581).

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2.15

SHORT COMMUNICATIONS

Probable zoonotic transmission of verocytotoxigenic *Escherichia coli* O157 by dogs

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VEROCYTOTOXIGENIC *Escherichia coli* (VTEC) of serogroup O157 is an important pathogen of human beings, with potentially serious consequences, including the development of haemolytic uraemic syndrome (Mainil and Daube 2005). It has been isolated from many animal species, but ruminants, especially cattle, are considered to be the main reservoirs of infection (Caprioli and others 2005). Contact with the farming environment, and direct and indirect contact with animals other than domestic pets are risk factors that were identified in case-control studies of sporadic VTEC O157 infections (Locking and others 2001, O'Brien and others 2001). Companion animals can sometimes be implicated, as reported by Trevena and others (1996) who recovered indistinguishable strains of VTEC O157 from a child, pony and dog in one incident. This short communication reports a case of apparent zoonotic transmission of VTEC O157 from dogs, which was identified in 2007 during the course of a joint medical and veterinary investigation into an outbreak of VTEC infection in a group of young children.

Three children in the group and two household contacts (adults) developed gastrointestinal symptoms. Non-sorbitol fermenting *E coli* O157 were isolated from stool samples of the five symptomatic cases and from an asymptomatic sibling contact. Methods summarised by Willshaw and others (1997) confirmed that all six isolates were VTEC O157, phage type (PT) 21/28, with genes for verocytotoxin 2. Pulsed field gel electrophoresis (PFGE) of *Xba*1 fragments showed that all six isolates had indistinguishable profiles.

The Outbreak Control Team, which was led by the Consultant in Communicable Disease Control of the Health Protection Agency (HPA), identified a number of potential sources for the outbreak. These included a small private farm visited by the index case (a child) five days before the onset of symptoms. Staff from the Veterinary Laboratories Agency (VLA) visited the farm at the request of the HPA and collected faecal samples and rectal swabs (Table 1) for *E coli* O157 culture using the standardised approach adopted within the VLA for VTEC O157 outbreak investigations (Pritchard and others 2000).

Non-sorbitol fermenting *E coli* O157 were isolated from seven (24·1 per cent) of the 29 samples collected from the farm. These were all subsequently confirmed as VTEC O157, PT 21/28, verocytotoxin 2; PFGE profiles of all seven isolates were indistinguishable from each other and from the six human isolates. VTEC O157, PT 21/28 was not isolated from the other potential sources investigated during the outbreak, such as a pet rabbit, other potential farm sources, other human contacts or environmental samples from food preparation areas, and it was concluded that the small private farm was the most likely initial source of the outbreak strain.

The presence of VTEC O157 in cattle and their farm environment was not unexpected since VTEC O157 carriage in farm animals, especially cattle and sheep, is relatively common (Paiba and others 2003, Milnes and others 2008). However, inquiries indicated that the index case did not have direct contact with cattle, but confirmed a history of close association during the visit to the farm with the two healthy dogs that were later found to be shedding VTEC O157 in their faeces. At the time of the VLA visit to the farm to collect samples, the dogs had access to the manure heap, the gates at the front of the calf pens and the calves through the gaps in the gates. This suggested that the dogs were likely to have been the immediate source of infection for the index case, while the cattle were probably the reservoir host on the farm.

There is little published information on the role of dogs in transmitting infection to people, or relevant control measures to minimise zoonotic risks from this source of infection. It is possible that the fur or hair of companion animals may become contaminated with VTEC O157 on livestock farms, and zoonotic transmission could occur when they are petted. Advice given to the owners of the dogs in this incident included the need for strict personal and domestic hygiene measures, with a strong emphasis on hand washing after contact, and separation and isolation of the dogs from visitors. Segregation of the dogs from cattle and cattle faeces on the farm (the main potential source of infection for the dogs) was advised.

Beutin (1999) reviewed the carriage of diarrhoeagenic *E coli* in dogs and showed that between 3·2 and 12·3 per cent of healthy dogs, and 8·8 per cent of diarrhoeic dogs carried VTEC, but VTEC O157 was very rare. Higher rates of carriage in diarrhoeic dogs have been identified in other studies; 10 of 58 (17·2 per cent) of dogs in Canada were positive for VTEC but serotypes were not reported (Hammermueller and others 1995), and in the UK, 23 of 82 (28·1 per cent) dogs carried VTEC but not strains of serogroup O157 (Sancak and others 2004). Faeces from dogs on cattle dairy farms have been shown to contain either non-O157 VTEC (Roopnarine and others 2007) or VTEC O157 (Hancock and others 1998).

The duration of faecal excretion by dogs naturally infected with VTEC does not appear to have been investigated. During follow-up studies in this outbreak investigation, faeces were collected from the two infected farm dogs approximately four and six weeks after initial testing. VTEC O157 strains were not isolated but one dog was shed-

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TABLE 1: Samples collected from a farm to investigate possible zoonotic transmission of *Escherichia coli*, and *E coli* O157 culture results

Source of samples (number of animals present)	Number of samples collected (type)	Number of *E coli* O157 isolates (type of sample)
Calves in pen (3)	2 (rectal swabs)	2 (rectal swabs)
	3 (freshly voided faeces)	
Calves in pen (3)	1 (rectal swab)	1 (rectal swab)
	3 (freshly voided faeces)	
Horse (1)	1 (freshly voided faeces)	0
Sheep (4)	5 (freshly voided faeces)	0
Dogs (2)	2 (freshly voided faeces)	2
Chickens (18) and duck (1)	1 (freshly voided faeces)	0
Calves at grass (5)	4 (freshly voided faeces)	0
Manure heap by calf house	1 (manure)	1
Trough in calf pen	1 (swab)	0
Gate in calf pen	1 (swab)	1
Wild rabbit faeces	2 (environmental faeces)	0

ding sorbitol fermenting *E coli* O157 on both occasions. These isolates were not VTEC since they did not produce verocytotoxin in the Vero cell cytotoxicity assay and did not have verocytotoxin genes detectable by PCR. They possessed gene characteristics of some strains of enteropathogenic *E coli*. These included genes for intimin (*eae*), which is associated with attachment and effacement of the intestinal epithelium, the bundle-forming pilus (*bfpA*), plasmid encoded regulatory (*perA*) genes and the increased serum survival (*iss*) gene (Anjum and others 2007). There are reports of recovery of non-verocytotoxigenic attaching and effacing *E coli* from healthy dogs (Holland and others 1999) and enteropathogenic *E coli* can cause enteric disease in dogs (Beutin 1999). Sancak and others (2004) recovered significantly more *eae*-positive *E coli* (both VTEC and non-VTEC) from diarrhoeic dogs than from healthy ones, and suggested that enteropathogenic *E coli* may play a role in the development of acute and chronic diarrhoea in dogs. The serotypes of enteropathogenic *E coli* associated with disease in dogs are not those that have caused human illness, even though virulence factors may be shared. There is no evidence that the carriage of an enteropathogenic-like *E coli* O157 resulted in human infection, although the role of dogs as vectors of potentially pathogenic organisms must be recognised.

In addition to the incident described by Trevena and others (1996), Parry and others (1995) reported VTEC O157 infection in a three-year-old girl who developed haemorrhagic colitis three days after visiting a farm visitor centre with a variety of animals, including dogs and cattle. She had extensively petted the farm dog but not the other animals. Synge and others (1993) similarly reported circumstantial evidence that VTEC O157 infection of a child was linked to dogs that had contact with cattle on an adjacent farm. In both these incidents, cattle were found to be excreting VTEC O157 strains indistinguishable from the human isolates, but isolates were not obtained from the dogs. It appears uncommon for dogs to be tested in connection with human illness. The cultures held by the Laboratory of Enteric Pathogens include only two other canine isolates resulting from such investigations. Both involved diarrhoeic or incontinent dogs that had been in contact with children with diarrhoea.

The present findings provide further evidence that contact with dogs may, on occasion, result in the transmission of VTEC O157 to human beings. However, the fact that case-control studies have not identified contact with dogs as a significant risk factor, and the paucity of reports in the literature, suggest that this happens infrequently and may be confined largely to circumstances where dogs have contact with cattle faeces, particularly on farms. In the present case, the absence of VTEC O157 from the dogs up to six weeks later (after contact with cattle was minimised) suggested that they were not true maintenance hosts but were short-term vectors that acquired VTEC O157 after contact with cattle faeces while exercising on the farm. Kusunoki and others (2004) infected four dogs experimentally by mouth with enterohaemorrhagic *E coli* O157:H7; the organism was detected in the faeces of all four dogs a day after infection, and in three at day 3 after infection, but it was not detected in the faeces of any dog by day 6. The ecology of VTEC O157 in naturally infected dogs does not appear to have been investigated. The risk of zoonotic transmission of VTEC O157 from dogs to human beings appears to be mainly confined to agricultural settings where dogs have intimate contact with cattle that are a main reservoir of infection.

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Investigation into the effectiveness of pooled fecal samples for detection of verocytotoxin-producing *Escherichia coli* O157 in cattle

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Abstract. Verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) may cause severe illness in people. Cattle are regarded as an important source of VTEC O157, and in an outbreak investigation, there is a necessity to establish whether or not the putative contact herd shares infection with the human case. The effectiveness of a herd investigation is impacted by the number of samples required, which will influence the time taken to collect samples and then process these in the laboratory. The objective of this study was to investigate the effectiveness of pooled sampling for detecting VTEC O157 in cattle herds in the United Kingdom. On farm 1, 150 individual fecal samples were collected during the course of a VTEC O157 outbreak investigation. One-gram and 10-g subsamples were tested from each individual sample. Once the culture results of the individual sample were known, pools comprising 5 and 10 individual samples were formed, with each pool containing a known number of positive samples. This data showed that the sensitivity of pooled sampling depended upon the proportion of positive samples in the pool. Further samples were collected from 2 more infected farms (2 and 3). Each individual sample was tested in duplicate. Pools of 5 feces were formed on-farm, and half the number of pooled feces were tested as individual feces. There was no significant difference between the number of cultures required for pooled sampling, as was the same for individual sampling, and therefore pooling did not improve the effectiveness of detection of VTEC O157.

Key words: *Escherichia coli*; modeling; pooled samples; surveillance.

Introduction

Verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) cause human disease outbreaks in the United Kingdom every year (http://www.hpa.org.uk/infections/topics_az/ecoli/O157/data.htm). Symptoms range from mild diarrhea to hemolytic uremic syndrome, which can be fatal especially for very young children. Cattle are the main reservoir for VTEC O157; they are asymptomatic carriers and shed the bacteria in feces.[7] In England and Wales, the Health Protection Agency (HPA) can request assistance from the Veterinary Laboratory Agency (VLA) to identify possible animal sources of human outbreaks of VTEC O157 using comparative molecular typing (mainly phagetype and pulsed-field gel electrophoresis).[16] The standard procedures adopted by VLA investigators use epidemiological groups rather than individual animals, because VTEC O157 excretion in individual cattle is intermittent, whereas group-level status remains stable for longer (http://www.defra.gov.uk/science/project_data/DocumentLibrary/OZ0138C/OZ0138C_3750_FRP.doc). Verocytotoxin-producing *E. coli* O157 is usually widespread in fecally contaminated environments of infected animals and tends to circulate between individual animals within close proximity of each other or indirectly via other epidemiological routes.[4]

Currently, during outbreak investigations, a large number (often 100 to 200 or more) of fecal samples are examined for VTEC O157 based on a 95% confidence of detecting at least 1 positive at a minimum estimated within-group prevalence of 10% within each distinct epidemiological group. Adopting a pooled approach for sample collection from epidemiological groups is potentially much more practical, cost-effective, and safer for the sampler as it does not require handling of individual cattle, provided it offers adequate statistical confidence and test sensitivity. Use of efficient sampling methods to minimize the cost and duration of an outbreak inquiry would facilitate an outbreak investigation. If costs are high, then this may discourage investigators from examining a potential farm source unless circumstantial evidence is compelling or other inquiries have failed to yield results. Thus, we consider

From the Centre for Epidemiology and Risk Analysis (Arnold, Ellis-Iversen, Cook), and the Department of Food and Environmental Safety (Davies, McLaren), Veterinary Laboratory Agency (VLA), New Haw, Addlestone, Surrey, UK; VLA Preston, Preston, Lancashire, UK (Kay); and VLA Bury St. Edmunds, Bury St. Edmonds, Suffolk, UK (Pritchard).

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that to be effective, an outbreak investigation should be epidemiologically robust, based on sample-size estimates being 95% confident of detecting infection if it were present at or above a threshold value in any epidemiological group (e.g., 10%). This demands that the sensitivity of the detection method be known. Furthermore, the minimum number of samples should be collected in the interests of not only economy, but also timeliness.

Pooling of fecal samples for the determination of the infection status of groups has been shown to be more efficient than the use of individual samples for *Salmonella* in pigs[1] and Johne's disease in cattle.[15] If validated for VTEC O157 in cattle, it offers considerable potential cost savings, and the approach could be adopted for surveys and intervention studies. One investigation into pooling fecal samples for detection of VTEC O157 in cattle has been performed;[12] however, the details of the test procedures in that study for the culture of VTEC O157 differed from those used routinely for outbreak investigations in the United Kingdom. Furthermore, in the previous study, pooling was restricted to samples pooled in the laboratory, whereas in this study samples were pooled on-farm for 2 of the 3 farms from which samples were taken, as would be carried out in practice. The objective of this study was to determine the sensitivity of pooled and individual-level sampling to determine whether pooled sampling offered reductions in the number of samples required to detect VTEC O157 on cattle farms in Great Britain.

Materials and methods

Testing of pools containing known number of positives

Samples were collected during the course of a VTEC O157 outbreak investigation undertaken by VLA at the request of the HPA. One hundred and fifty individual fecal samples were collected. On arrival at the laboratory, the samples were well-mixed and 1-g and 10-g subsamples were inoculated into 9-ml and 90-ml volumes of buffered peptone water (BPW) and incubated at 37°C for 6 to 8 hours. After incubation, the volumes of BPW were subject to automated immunomagnetic separation (AIMS) using Dynal O157 Dynabeads.[a] The Dynabeads were plated out onto cefixime tellurite sorbitol MacConkey's (CTSMAC) agar plates and incubated at 37°C for 18 hours. After incubation, the plates were examined for typical nonsorbitol fermenting colonies. Up to 10 typical colonies were tested by latex agglutination test[b] for the presence of O157. Once the individual sample results were known, a series of 1-g pools was created from the individual samples as follows:

- Pools of 5 individual feces with 0, 1, 2, 3, 4, and 5 positives per pool—10 replicates per pool for the pools of 0, 1, 2, and 3 positives; 5 replicates of 4 and 5 positives. This results in a total of 50 pooled samples.
- Pools of 10 individual feces with 0, 1, 2, 4, 6, 8, and 10 positives per pool—10 replicates per pool for the pools of 0, 1, 2, 4, and 6 positives; 5 replicates of 8 and 10 positives. This results in a total of 60 pooled samples.

Positive and negative samples were all selected at random from the set of test-positive samples (i.e., positive for at least 1 of the 1-g and 10-g cultures by AIMS) and test-negative samples, respectively, from the 150 samples from the outbreak farm, according to predetermined tables created using random numbers generated by Microsoft Excel.[c] While awaiting the result of the VTEC O157 culture, the samples were stored at 4°C, and all the pooled samples were formed and tested within a week of collection. All VTEC-positive individual samples were spiral plated[11] at the same time as the pooled samples were formed to determine the number of VTEC O157 organisms in the individual (and pooled) samples. A previous study on the survival of *E. coli* in naturally occurring cattle feces[11] had shown that *E. coli* counts start to die off in some of the pats after about 7 days, which would suggest that there would be no die-off in the interval between collection of the samples and the formation of the pools in the present study, since the pooled samples were all formed within a week of collection. The farm from which the samples were taken will be referred to as farm 1.

Testing of pools formed in the field

In order to provide data on the sensitivity of pooled samples as would be carried out in the field, samples were collected from 2 more infected farms (2 and 3). From each farm, the number of individual samples required to detect a positive at 10% prevalence with 95% confidence from each of the 3 groups of cattle was taken in accordance with the established VLA outbreak investigation protocol based on published tables for sample-size requirements.[2] Each group was a distinct epidemiological unit on the farm; that is, each group was housed separately, and all animals sampled were between 6 and 18 months of age. For each group sampled, $n/2$ pooled samples were collected in addition to the individual samples, where n was the number of individual samples collected. In total there were 3 sets of 22 individual samples and 3 sets of 11 pooled samples from farm 2, and 3 sets of 16 individual samples and 3 sets of 8 pooled samples from farm 3. Each pooled sample comprised approximately equal quantities (approximately 3 to 5 g) of 5 randomly selected freshly voided feces. These were collected into the same 150-ml plastic pot with a wooden spatula (the same for all samples comprising the same pool). In terms of practicality, some repeat sampling of feces from the same animals within a pen or group was considered acceptable. The pen from which the pooled samples were taken was recorded.

At the laboratory, each pooled sample and 2 1-g subsamples from each individual sample were well-mixed and 1-g removed for culture and examined for *E. coli O157* using the same method for the individual samples as described above and previously.[9] Representative O157

latex-positive isolates were sent to the Laboratory of Enteric Pathogens (HPA Colindale, Colindale, UK) and the Department of Food and Environmental Safety (VLA Weybridge, UK) to determine whether they were verocytotoxigenic strains. Spiral plating quantification of VTEC O157 in 1 set of individual samples from each farm was performed at the same time as the IMS to provide data that could potentially explain any farm-level differences in sensitivity that might be found.

Statistical analyses

Testing of pools with known number of positives. Logistic regression models were fitted to the data on the pools formed in the laboratory to determine whether the best-fitting model was one that depended on 1) the proportion of positives in the pool or 2) the colony-forming unit (CFU)/g of VTEC O157 in the pool (estimated by summing up the counts from the individual samples comprising the pool). For this analysis it was assumed that all positive samples were detected by at least 1 of the 1-g and 10-g samples tested by AIMS. For the proportion of positive samples in the pool as the explanatory variable, this leads to the probability of a pool testing positive, η_{pool}, being equal to:

$$\eta_{pool} = [\exp(\alpha + \beta\pi_{pool})]/[1 + \exp(\alpha + \beta\pi_{pool})], \quad (1)$$

where π_{pool} was the proportion of samples in the pool that are true positives, and α and β were parameters determined by maximum likelihood, with a similar formula for the CFU/g model. The adequacy of the fit of each model to the data was assessed by a Hosmer-Lemeshow chi-square test[3] (implemented in Limdep 7.0[d]). A likelihood ratio test was carried out to determine whether there was a significant difference between the parameters α and β for the pools of 5 and 10, respectively, and thus determine whether the data could be pooled for a combined estimate depending only on the proportion of pools positive, rather than taking into account the number of samples in the pool (i.e., a pool of 5 with 1 positive has the same sensitivity as a pool of 10 with 2 positives).

Testing of pools formed in the field. The results from the pools formed on-farm provided data on the sensitivity of pooled samples as would be carried out in the field, relative to individual samples. In this case the number of positive samples in each pool was not known but was assumed to follow a binomial distribution dependent on the number of samples in the pool and the prevalence of VTEC O157 infection in the group of cattle from which the pooled sample was formed. The best-fitting model from the previous section was used to describe the sensitivity of the pool according to the proportion or count of VTEC O157 in the pool. It was assumed that the tests had 100% specificity.

It was assumed that the individual-level test sensitivity was equivalent to a pooled sample containing all positive samples. The log-likelihood of the data from the 2 outbreak farms was maximized with the group-level prevalence on each farm and the parameters α and β in Equation (1) as unknowns, resulting in 8 unknown parameters. The optimization was then repeated with the parameters α and β, allowed to vary between the 2 farms, and a likelihood ratio test carried out to determine whether a significantly better fit was obtained. The motivation behind this is that data from a recent study,[8] in which multiple subsamples were tested from individual samples, suggested that the sensitivity of detection in individual-level samples could vary considerably between herds; central estimates of sensitivity of 24%, 55%, and 82% for prevalence equal to 15%, 46%, and 91%, respectively, were obtained.

The effectiveness of pooled versus individual sampling was compared by calculating the number of samples that would be required in order to detect VTEC O157 with 95% probability in each group of cattle on farms 2 and 3 for pooled and individual sampling. For individual sampling, the probability that a single test would be positive was given by the product of the group-level prevalence and the individual-level test sensitivity. For pooled sampling it was given by

$$\sum_{i=1}^{5} P(i \text{ positive samples in pool})\eta_{pool}\left(\frac{i}{5}\right), \quad (2)$$

where the summation is up to 5 since there are 5 samples per pool, and the probability of i positive samples in the pool follows a binomial distribution with parameters given by the pool level prevalence and the number of samples in the pool. Sample sizes were then calculated according to the formula[2]

$$n = \ln(1 - \tau)/\ln(1 - \eta), \quad (3)$$

where τ is the statistical power required and η the probability that each test would be positive. Equation (3) relies on the assumption that the number of fecal samples would be much larger than the number of samples required. When this is not true, one would be required to take multiple samples from some individual fecal pats. This would tend to result in slightly smaller sample sizes (for both individual and pooled sampling).

Results

Pools formed in the laboratory

Of the 150 individual samples collected from the outbreak investigation (which were to be used to create the pools), 137 were positive for at least 1 test, of which 121 were positive at 1 g and 133 were positive at 10 g. Assuming that all true positive samples were positive for at least 1 of the 2 tests, this gives 88% sensitivity for 1-g culture and 97% sensitivity for the 10-g culture.

As the number of positive samples in the pool increased, an increased proportion of the pools tested were positive (Table 1). The logistic regression provided an adequate fit to the data according to the Hosmer-Lemeshow chi-square test ($P = 0.40$), and

Table 1. The proportion of positive pools with various proportions of positive samples included in the pool.

No. of samples in pool	Proportion of positives in pool						
	0	0.1	0.2	0.4	0.6	0.8	1.0
5	0/10	—*	2/10	6/10	7/10	4/5	5/5
10	0/10	0/10	0/10	5/10	7/10	5/5	5/5

* — = no pools tested.

showed that the dependence on the proportion of positive samples in the pool and the probability that a pool would test positive for VTEC O157 was significant ($P < 0.0001$), thus showing that there was a dilution effect of mixing true-positive with negative samples on the test sensitivity. There was no significant difference between the estimated parameters for the pools of 5 and 10 ($P = 0.24$), respectively. This indicated that the model depended only on the proportion of positives in the pool and not the size of the pool. The parameter values from the logistic regression were $\alpha = -3.69$ (95% confidence interval [CI]: -5.03, -2.35) and $\beta = 8.20$ (95% CI: 5.30, 11.10).

The logistic regression model that used the count of VTEC CFU/g as the explanatory variable also provided an adequate fit to the data according to the Hosmer-Lemeshow chi-square test ($P = 0.57$), but showed a lower positive predictive value and negative predictive value than the model that used the proportion of positive pools as the explanatory variable, largely a result of some of the pools with very high counts testing negative (Fig. 1b). As a consequence, the proportion positive model was preferred.

Pools formed in the field

Table 2 gives the results for the individual-level and pool-level samples collected from 2 further known infected farms. Individual-level test sensitivity was estimated to be equal to 84% if assumed not to vary between farms 2 and 3. The values of α and β, taking into account the data in Table 2, were (with 95% confidence intervals) $\alpha = -3.2$ (-3.7, -2.8) and $\beta = 4.8$ (4.3, 5.4). However, a likelihood ratio test showed that α and β varied significantly between the farms ($P = 0.01$), and that the test sensitivity was lower in farm 3 than farms 1 and 2; the sensitivity of the individual-level test was estimated to be approximately 89% on farm 2 and 47% on farm 3, and the corresponding estimates for the pool-level test sensitivity are shown in Fig. 1.

The distribution of VTEC O157 CFU/g within the AIMS-positive fecal samples was compared for each of the farms in the study (Table 3). There was a wide

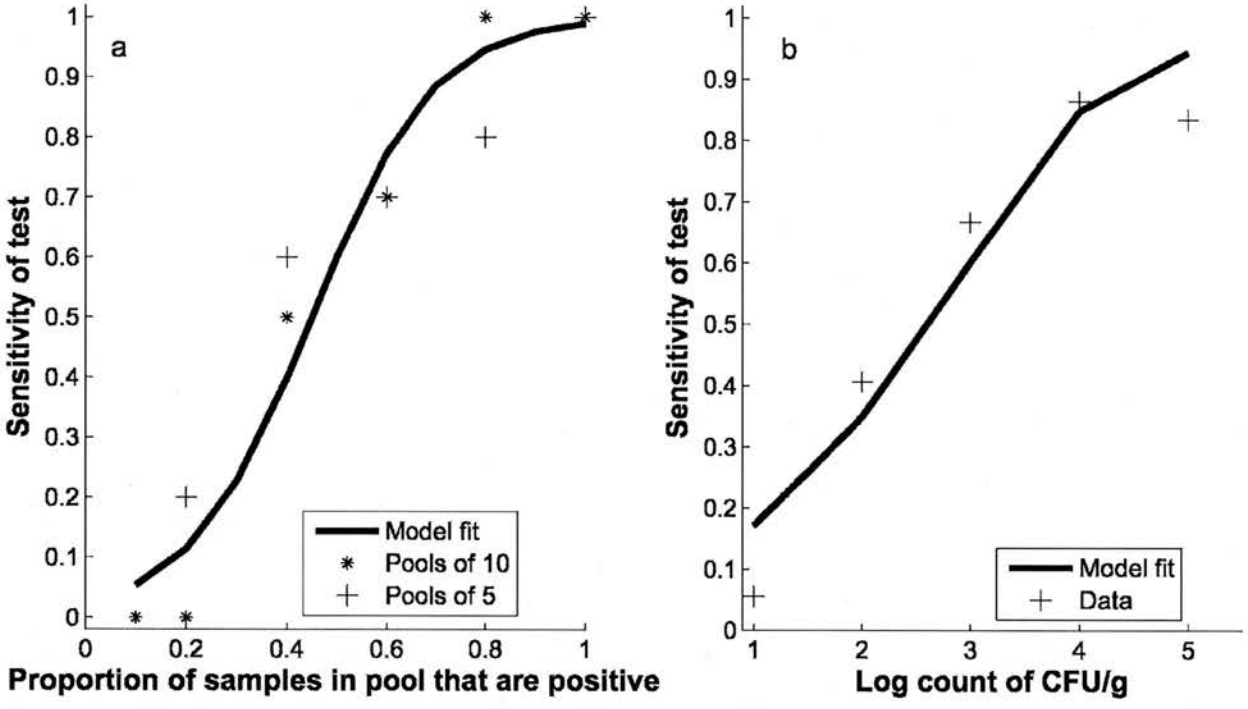

Figure 1. The fit of the logistic regression models to the sensitivity of laboratory-formed pooled samples when the dependent variable is **a**, the number of positive samples in the pool, or **b**, the log CFU/g of verocytotoxin-producing *Escherichia coli* O157.

Table 2. Summary of results for farm-level sampling of verocytotoxin-producing *Escherichia coli* O157 using individual and pooled samples.

Farm	Group	Individual samples: Positive for replicate 1	Individual samples: Positive for replicate 2	Individual samples: Positive for both replicates	Pooled samples
2	A	8/22	10/22	8	3/11
2	B	15/22	11/22	11	6/11
2	C	15/22	15/22	14	7/11
3	D	0/16	2/16	0	0/8
3	E	5/16	2/16	2	1/8
3	F	1/16	3/16	1	2/8

distribution of CFU/g obtained and a similar proportion of samples (approximately 75%) with <200 CFU/g in each of the farms.

There were several samples from both farms that tested positive for only 1 of the 2 duplicate samples taken from each individual-level sample (Table 2), suggesting a heterogeneous distribution of the organisms within the fecal sample. There was a far greater difference in prevalence between the 2 farms than between the groups on each farm (estimates of 45%, 69%, and 75% for groups A through C, respectively, from farm 2; 14%, 41%, and 33% for groups D through F, respectively, from farm 3).

The resulting relationship between the number of positives in a pool and the probability of a test-positive is shown in Fig. 2 for each of the 3 farms in the study. The reducing probability of detection as the proportion of positives in the pool decreases indicates a dilution effect of mixing positive and negative samples on the probability of detection.

Comparison of pooling and individual-level sampling

The number of samples required to obtain at least 1 positive test for each group on farm 2 was 9, 4, and 4 for pooled sampling for groups A through C, respectively, and 6, 4, and 3 for individual sampling. For farm 3, the sample sizes for groups D through F were 48, 17, and 21, respectively, for pooled sampling,

Table 3. Comparison of the verocytotoxin-producing *Escherichia coli* count distribution in individual samples identified as positive between the 3 farms in the study.

No. of CFU/g*	Frequency: Farm 1	Farm 2	Farm 3
<200	100	32	8
200–1,000	16	5	2
1,001–10,000	10	4	0
10,001–100,000	8	1	0
100,001–1,000,000	2	1	0
>1,000,000	1	0	0

* CFU = colony-forming unit.

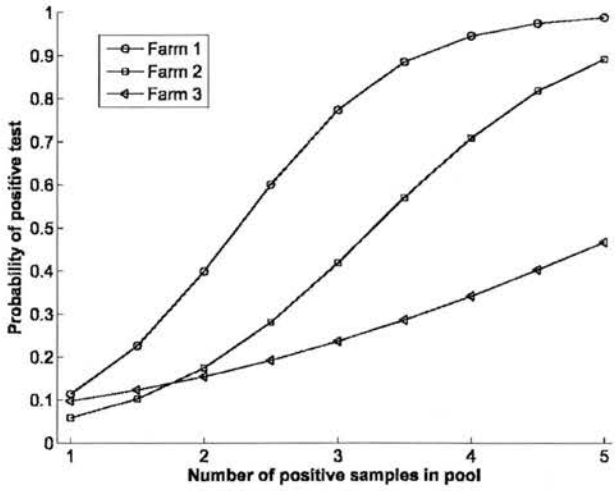

Figure 2. The estimated probability of obtaining a positive result from a pooled sample according to the number of true positives in the sample for each of the 3 farms.

and 46, 15, and 19 for individual level sampling. These results indicate that pooled sampling does not result in a reduction in the required number of cultures to detect VTEC O157. The individual sampling required the same or fewer samples in each case. A sign test[17] was employed to determine whether the observed differences between pooled and individual-level sampling (Table 2) were significant. Of the 12 individual-level sample results, there were 7 instances when the proportion of individual-level positives were greater, 3 when the pooled samples were greater, and 2 ties, which resulted in no significant difference between pooling and individual-level sampling ($P = 0.34$). This shows that the sample-size requirements for pooled sampling were either the same as for individual sampling, or too small a difference from individual sampling to be detected by the study.

Discussion

The results from this study suggest that pooling of 1-g fecal samples is no more efficient than testing individual 1-g samples during outbreak investigations. This is caused by the reduced probability of detecting VTEC O157 when true-positive samples are diluted with true-negative ones, which cancels out the increased probability of including VTEC O157 in the pooled sample compared with the individual sample. Reduced sensitivity for the pooled samples compared with individual samples caused by the dilution effect was also described in a previous study.[12] However, the expected proportion of pooled samples that tested positive with 1/5 or 2/5 positive samples in the pool was much higher for that study, at around 50% for

both cases, than was found in the present study, with 7% and 20% for 1/5 and 2/5, respectively. The differences between the present study and the previous one could have arisen as a result of differences in the experimental protocols for the bacterial culture. For example, in the previous study a stomacher was used prior to culture, whereas in the present study samples were mixed by hand, and it is possible that the difficulties of effectively homogenizing the pooled samples led to the small estimated difference between the sensitivity of pooled and individual samples observed in this study. This would be a key difference between pooling samples for VTEC O157 in cattle and *Salmonella* in pigs; in the latter case, pooling was shown to be more efficient than individual sampling without the use of a stomacher.[1] There were also other differences between this study and the previous study[12] that could cause differences in results. First, in the present study feces were pooled on-farm, as would be done in practice if pooling was routinely adopted for outbreak investigations, rather than mixing sets of individual fecal samples in a laboratory. Second, the distribution of VTEC O157 CFU/g in individual fecal samples in the present study was markedly lower than those obtained in the previous study, with only 10% of samples >5,000 CFU/g in the present study, compared with approximately 50%. The CFU/g is likely to affect the rate of positives in diluted pools as there is a correlation between the probability of successful culture and the number of CFUs/g. The large difference between the distributions of CFU/g of this study and the previous study could have been caused by differences in the sampling ages of the animals between the studies (as there is an age-association on shedding patterns[6]), the method of enumeration of the VTEC O157, the fact that the previous study used inoculated calves rather than naturally infected ones, or merely the considerable natural variability of shedding. A comparison of the shedding distribution of cattle in the present study showed no significant difference (as measured by a chi-square test) from 2 other studies in the United Kingdom,[5,8] and a similar range of CFU/g to a third study.[10] Overall, therefore, while the CFU/g distribution would have an impact on the performance of pooling, there is nothing to indicate that the farms sampled in the present study had atypical counts of CFU/g.

The estimated individual-level test sensitivity was highly variable between the farms, with a lower estimate of 52% in farm 3 and approximately 90% for farms 1 and 2. This is in accordance with the findings of a recent study[8] where the estimated test sensitivity was also higher where the estimated prevalence was higher. The most likely reason for the lower test sensitivity in farm 3 is lower VTEC shedding rates in the individual animals compared with the other 2 farms. While the proportion of positive samples with a count of less than 200 CFU/g was similar for each farm (Table 3) at approximately 75%, 2 factors should be borne in mind. First, the low prevalence for farm 3 results in a small number of positive samples and a large uncertainty in the VTEC CFU/g distribution. Second, taking into account the expected number of true-positive samples that were missed by the duplicate testing of individual-level samples, the proportion of samples with <200 CFU/g for farm 3 rises from 8 to 12, which then gives 85%. The authors cannot easily quantify the effect on the AIMS of such an increase in the proportion of samples with a low CFU/g since the relationship between CFU/g and the probability of detection of the AIMS for low CFU/g for naturally infected samples is not accurately known. The actual CFU/g in samples below the detection limit for the spiral plating technique is also not known in the present study. However a lower level of shedding on farm 3 remains a possible explanation for both the lower prevalence and the lower test sensitivity. External factors have been reported to have an effect on shedding rate or infectious burden of VTEC O157 in groups of cattle. Clean animals, dry bedding, avoiding movement between epidemiological groups, and clean water troughs have been reported to reduce VTEC O157 within groups, which supports the importance of environment in the transmission dynamics (http://www.defra.gov.uk/science/project_data/DocumentLibrary/OZ0138C/OZ0138C_3750_FRP.doc). Individual animals have been found to excrete different concentrations of CFU, and heterogeneity in floor pats was described and modeled recently.[4] However, little is known about the reason for "super-shedding" and factors that may reduce the CFU/g excretion, but various feeds and the presence of other animal species have been suggested.[13,14]

The superior fit of the proportion of positives dependent logistic regression model over one that depended on the VTEC CFU/g was unexpected; the VTEC CFU/g is likely to have a large influence over the probability of successful culture, although clearly there will be a correlation between the 2 models. There is, however, uncertainty in the measurement of the CFU/g by the spiral plating method,[11] which could have influenced the fit of the model to the data. It is also possible that background flora could have influenced the culture of VTEC 0157, leading to occasional test failures even for high VTEC loads.

The primary use of sampling for VTEC O157 in human outbreak investigations is to determine whether and where in the farm VTEC O157 identical to human cases is present, rather than to determine the actual

prevalence of infection. The results of this analysis are important to determine the number of samples required to show whether the prevalence is less than a design prevalence, currently set at 10% for outbreak investigations in the United Kingdom. The basic message from the on-farm sampling is that the pools of 5 did not reduce the sample-size requirements compared with individual-level sampling. However, the 10-g samples were found to be more sensitive than the 1-g samples in farm 1, and it may be that testing larger samples would be a better route to increase the efficiency of sampling schemes than pooling. Another factor to take into account is that, if the test sensitivity were influenced by the prevalence of VTEC O157, this would have an impact on the sample sizes required, especially for detection of VTEC O157 on low prevalence farms. Therefore it is vital that further investigation into the variation of test sensitivity between farms is carried out. This could be done by multiple testing of individual-level samples collected during outbreak investigations. If farm-level factors were also noted, then it may be possible to determine whether there are any farm-level factors that affect the test sensitivity in a predictable way.

The present study has shown that pooling of samples requires at least as many cultures as the testing of individual samples for outbreak investigation. This study has also found that the individual-level test sensitivity for VTEC O157 was highly variable between farms and possibly correlated with the on-farm prevalence. Further work would be useful to investigate the between-farm variability of test sensitivity for VTEC O157, as it is crucial for the appropriate design of sampling protocols. It would also be useful to explore whether more sensitive methods of VTEC detection (e.g., culturing 10 g rather than 1 g) for pooled samples or individual samples would be worthwhile.

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Sources and manufacturers

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b. Oxoid Ltd., Basingstoke, Hampshire, UK.
c. Microsoft Corporation, Redmond, WA.
d. Econometric Software, Plainview, NY.

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Identification of management risk factors for VTEC O157 in young-stock in England and Wales

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Abstract

We conducted a cross-sectional study on 255 cattle farms in England and Wales to identify risk factors for verocytotoxin-producing *E. coli* O157 (VTEC). Exposure variables were collected at the levels of the farm and of the group of young-stock within the farms. On each farm a group of young-stock (6–18 months of age) was sampled to establish VTEC status.

In our multiple logistic regression, farm VTEC status was associated with access to springs (OR: 0.31, $CI_{95\%}$: 0.12, 0.78) and assessing the wetness of the bedding material less frequently than daily (OR: 3.89 $CI_{95\%}$: 1.5, 10.2). At group-level we found no associated risk factors for animals housed outdoors in fields. Significant for groups housed in pens were wet bedding (wet OR: 3.43, $CI_{95\%}$: 1.3, 9.4; very wet OR: 4.24, $CI_{95\%}$: 1.2, 14.6), number of animals in the group (10–15 OR: 2.72, $CI_{95\%}$: 0.75, 9.9, 16–24, OR: 3.78, $CI_{95\%}$: 1.2, 12.3; >25 OR: 3.78, $CI_{95\%}$: 1.1, 12.7) and feeding straw (OR: 2.29, $CI_{95\%}$: 1.2, 5.5).
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Keywords: VTEC; STEC; Zoonoses; Cattle; Risk factor

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1. Introduction

Human exposure to verocytotoxin-producing *Escherichia coli* O157 (VTEC) is a public-health concern. In the UK, the main reservoir of VTEC is cattle, especially young-stock <2 years old, which shed the agent via faeces and saliva (**Paiba et al., 2003**). Exposure can occur through contaminated food or via direct contact with infected animals or contact with a contaminated environment or via person-to-person spread (**HPA, 2000**; **Locking et al., 2001**).

In 1997, legislation was introduced to prevent dirty cattle from entering slaughterhouses in England and Wales to reduce the risk of cross contamination from hides to meat and thus, reduce transmission of VTEC through meat (**MHS, 1997**). However, the prevalence of human outbreaks traced to surface water, direct contact with farm animals or contaminated fields is increasing and interventions need to be implemented at farm-level to reduce human cases infected from these sources (**Shukla et al., 1995**; **Licence et al., 2001**; **Locking et al., 2001**; **Ogden et al., 2002**).

Commercial young cattle are managed and kept in groups; most farm practices that might influence the epidemiology of VTEC are applied to groups rather than individual animals. Furthermore, VTEC shedding by individual animals is intermittent and the agent appears to recycle and survive within a group of animals rather than persist as a life-long infection of one individual (**Midgley and Desmarchelier, 2001**; **Robinson et al., 2004**; **Matthews et al., 2006a**). To date most studies have identified risk factors (such as biosecurity, housing, age, breed, season, feeds and other animals) for individual animals, but these studies might not provide powerful information on the group or farm-level, because the animals often originate from a small number of farms (**Rugbjerg et al., 2003**; **Synge et al., 2003**).

Our objective was to investigate management risk factors of VTEC status on cattle farms and on the group level of young-stock with the long-term aim of recommending farming practices that might reduce VTEC in cattle. The study was carried out as a screening survey to identify and enrol VTEC-positive farms in a future intervention study (**DEFRA, 2006**).

2. Materials and methods

2.1. Farm selection

Farms were selected through the Veterinary Laboratory Agency's network of Regional Laboratories (rl) representing most regions of England and Wales. A Veterinary Investigation Officer (VIO) at each rl was supplied with a list of the 10 private veterinary practices (pvp) that had submitted the greatest number of bovine samples to the rl within the previous 12 months. The pvp were approached by the VIO, who explained the proposed study and asked for permission to contact and seek to enrol their clients, who had submitted samples. Each pvp was also asked to suggest other clients whom the pvp anticipated might be willing to participate in the study. A telephone questionnaire was completed by a VIO to assess the farms eligibility and willingness to participate in the study. Farms were excluded if they had: (i) <60 cattle; (ii) <20 young-stock of <24 months old; (iii) main enterprise was production of cattle for sale to other farms; (iv) members of the public visiting (i.e. an open farm); (v) a bed-and-breakfast enterprise on the premises or (vi) if they sold unpasteurised milk. Exclusions (i) and (ii) were excluded, because positive farms would be invited to participate in an intervention study for which larger farms were needed. (iii–vi) were excluded to avoid economic and ethical consequences should VTEC be detected in these enterprises.

With 255 cattle farms enrolled, the study yielded a power of 96% to detect an odds ratio (OR) of 1.2 with a 95% confidence assuming a ratio of exposed/unexposed of 1:2. Because multiple exposures were examined a precise ratio of exposed and non-exposed was unattainable. However, the large power was selected to create a sample size that would allow adjustment for variation in the exposure ratio and for variables with multiple categories.

2.2. Sample and data collection

2.2.1. Samples

Farms were visited and sampled once by trained staff between August and December 2003. At each farm, a total of 20 fresh faecal samples were collected off the ground of enclosures containing groups of weaned young-stock (3–18 months of age). If the young-stock were housed in several pens or fields, the number of samples was divided amongst all enclosures according to the proportion of young-stock in each. The 20 samples from one farm were interpreted as an aggregate-testing scheme and the farm was classified as positive if one of the samples were positive. This approach was considered to have a herd-level sensitivity of 83% assuming a 77% sensitivity of individual floor-pat sampling (**Sanderson et al., 2005**).

2.2.2. Questionnaires

Two questionnaires were completed at the sampling visit in discussion with the farmer to obtain information on exposure levels to potential risk factors. One questionnaire was designed to obtain farm-level information on cattle purchase, quarantine procedures, grazing and housing, water sources and general hygiene procedures (**Tables 1–3**). The other questionnaire was designed to obtain information on the management practices and specific details (age, number of animals, etc.) for the group of young-stock that was sampled. Both questionnaires were a slightly revised version adopted from a previous study for which they had been trialled and used (**DEFRA, 2006**). Most of the questions were closed to avoid ambiguous or lengthy answers and both the farmer and the interviewer/sampler were unaware of the VTEC status of the farm at the time of the interview.

2.2.3. Assessment of physical parameters

Some parameters regarding the group of sampled young-stock were rated by the sampler whilst collecting the samples. The wetness of the bedding ('the squelch-score') was assessed on a scale from 1–5 for each enclosure sampled; a score of 1 meant very dry and a score of 5 meant very slippery (**Hughes, 2001**). The colour (1 = very pale, 5 = very dark) and consistency of the faeces (1 = very dry, 5 = liquid) collected was also scored for each faecal pat sampled (**Robinson et al., 2004**). The median hide cleanliness of the group of sampled calves was rated using the Meat Hygiene Service Hygiene Assessment Score, where only animals with cleanliness scores of 1 or 2 are allowed for slaughter in UK abattoirs (**MHS, 1997**). A score of 1 was very clean and 5 was heavily soiled. In the analysis the median score of five animals in each group was used as a group cleanliness score. The five animals were selected by location in the enclosure, where one animal near each corner and one in the middle was chosen for scoring by the sampler independently of cleanliness. The number 5/group was based on logistic consideration of available time rather than mathematical calculations of sensitivity.

Samplers were given a 1-day training course to ensure that the physical parameters were scored in a consistent way; samplers were supplied with a pictorial description of faecal colour and consistency.

Table 1
Description of farm-level management practices and VTEC O157 status for young-stock on 255 cattle farms in England and Wales

Variable	Exposure level	N[a]	% of row, VTEC-positive farms
Total cattle on farm	60–176	59	27
	177–260	62	32
	261–369	59	29
	370–1300	60	35
Type of cattle on farm	Dairy	216	31
	Suckler calves	57	32
	Fattener	95	34
	Breeding bulls	10	30
Source of cattle in the last 12 months	Market/auction	78	30
	Private farm	108	32
	Dealer	12	50
	Leasing	5	31
	Closed farms	109	32
Quarantine of all in brought cattle	No	102	35
	Yes	43	28
	Some	20	20
Minimum length of quarantine (days)	<7	16	19
	7–13	19	16
	14–28	20	20
	>28	9	56
Test new cattle for diseases other than VTEC	No	228	31
	Yes	27	33
Contact between farms stock and neighbouring farms in the last 2 months	Yes	79	28
	Unknown	10	40
	No	166	33
Horses[b]	Present	58	43
	Absent	22	28
Pigs	Present	6	83
	Absent	249	68
Sheep	Present	79	29
	Absent	176	32
Poultry	Present	37	35
	Absent	218	31

[a] Missing values are omitted.
[b] p-Value < 0.25 and included in the multivariable model.

2.2.4. Sample testing

The faecal samples were stored on ice at collection and transported to the nearest VLA rl. Culture and identification of *E. coli* O157 was undertaken using immuno-magnetic separation (IMS) and culture on sorbitol McConkey agar containing cefixime and tellurite as previously described (**Paiba et al., 2003**). Putative VTEC O157 colonies were identified by latex agglutination and confirmed by serum agglutination for O157 antigens, after which we used a

Table 2
Field management of young-stock at farm-level on 255 cattle farms in England and Wales and the VTEC O157 status

Variable	Exposure level	N^a	% of row, VTEC-positive farms
Spread manure/slurry/dirty water on cattle grazing land	No	39	28
	Yes	212	31
Time from spread of manure to grazing	<2 weeks	16	38
	2–8 weeks	67	33
	>8 weeks	85	31
Time from spread of slurry to grazing	<2 weeks	12	33
	2–8 weeks	80	31
	>8 weeks	66	26
Time from spread of dirty water to grazing	<2 weeks	59	36
	2–8 weeks	90	27
	>8 weeks	33	30
Water sources present in cattle grazing fields	Mains	201	33
	Borehole	87	26
	Well	37	32
	River	139	30
	Lake	27	33
	Drains	40	38
	Springs	59	19
Presence of water sources shared with other farms	No	162	34
	Yes	88	26
Presence of spring in cattle grazing field	No	196	35
	Yes	59	81
Grazing land used for other ruminants	No	109	30
	Yes	138	31
Sheep co-graze with cattle	No	82	32
	Yes	56	30

[a] Missing values are omitted.

polymerase chain reaction to detect the presence of verocytotoxin-producing genes *vt1* and *vt2* and attaching and effacing gene *eae* (Paiba et al., 2003). A farm or group was considered VTEC O157 positive, if at least one isolate of *E. coli* O157 contained *eae*-genes and either one or both *vt*-genes.

2.3. Data analysis

The results from the questionnaires and laboratory analyses were entered into a Microsoft Access 97® (Microsoft Corporation, Redmond, WA) database by a clerk and a visual quality check was performed on the entered data. The RAND function in Microsoft Excel was used to randomly give each form a reference number and then the 10% of forms with the lowest numbers were selected for a 100% visual check and the error risk was 0.27%. Outlying and non-plausible values were checked against the original questionnaires and if needed traced back to the farm for confirmation.

Table 3
Indoor hygiene-practices in young-stock group-management on 255 cattle farms in England and Wales and their VTEC O157 status

Variable	Exposure level	N[a]	% of row, VTEC-positive farms
Boot dips outside pens	No	242	31
	Yes	7	29
Protective clothing used on the farm	No	134	32
	Yes	110	27
Enclosure designated overcoats[b]	No	224	32
	Yes	16	13
Ringworm observed in the last year	No/unsure	106	26
	Yes	139	35
Frequency of assessing the wetness of bedding	Daily	192	28
	Less than daily	26	62
Frequency of bedding down young-stock[b]	Daily	131	25
	Every other day	59	34
	Twice a week or less	56	41
Straw type used for bedding[b]	Long	191	33
	Chopped	15	27
	Both	36	28
	Barley	74	41
	Wheat	58	23
	Mixed straw	110	31
Water source	Treated mains	183	33
	Borehole	77	27
	Well	31	32
	River	13	15
	Lake	2	0
	Drain	4	50
	Spring	22	32
Location of water trough in enclosure	Front	131	31
	Middle	37	27
	Back	120	34
Presence of raised water troughs	No	209	33
	Yes	22	23
Frequency of cleaning water troughs[b]	More than monthly	28	18
	1–6 Months	48	31
	>6 Months	152	35
Frequency of emptying water troughs	Weekly	14	14
	>Weekly	224	31
Feeding practices	Floor	45	29
	Buckets	34	32
	Built in troughs	202	32
Clear old food away before feeding	No	18	33
	Yes	205	31

[a] Missing values are omitted.
[b] p-Value < 0.25 and included in the multivariable model.

Separate analyses were carried out for farm-level exposures and for exposures relating to the specific groups tested. Univariable χ^2 analyses were performed to investigate the crude association between the presence of VTEC and farm and group-level exposures to identify variables to include in multivariable models. All variables with a $p < 0.25$ in the univariable analysis were tested for collinearity, before included in a multivariable logistic-regression model.

Initially, a full logistic regression model, containing all variables selected in the univariable analysis, was constructed. Backwards stepwise elimination of non-significant variables ($p < 0.05$ Wald's test), using likelihood-ratio tests for comparison of nested tables, was carried out to build the best-fit final model consisting only of significant variables. Possible confounders were included in the model if they were either (1) associated with both the explanatory variable and the outcome, (2) biologically meaningful as confounders or (3) if they caused a biologically important change in the OR of the risk factor when included in the model (Dohoo et al., 2003). Typically, changes in the OR of greater than 30% were considered to indicate confounding. Potential two-way interactions and collinearity between the variables in the final model were investigated. Collinearity among predictor variables was assessed by examining variance inflation factors (VIFs) following. VIFs > 10 were considered to be evidence of strong collinearity (Dohoo et al., 2003). Additionally any observed inflation of the standard errors of the coefficients during final model fitting was investigated. A multilevel approach was not used because only one or two groups were sampled on most (87%) of farms.

The group-level dataset was stratified into groups housed in pens and groups kept in fields, because many exposures were strongly associated with housing arrangements (e.g. bedding variables and stocking density). We built logistic-regression models as described above and the final variables in the final model were investigated for potential interaction and collinearity as described above.

All analyses were carried out using Intercooled Stata 8.0 (Stata Corporation, College Station, TX).

3. Results

3.1. Descriptive results

A total of 321 (81%) of the 411 farms contacted initially were eligible to participate and 255 farms were enrolled in the study. The remaining 76 (24%) farms were unwilling to

Table 4
Physical measures used to classify sample condition and enclosure hygiene of young-stock on 255 cattle farms in England and Wales and their VTEC O157 status

Scored factor	VTEC O157 status	Minimum score	Median score	Maximum score
Muck	Positive	1	2	3
	Negative	1	2	4
Faecal consistency	Positive	1	3	4
	Negative	1	3	5
Faecal colour[a]	Positive	2	4	5
	Negative	3	4	5
Bedding squelch[a]	Positive	1	2	5
	Negative	1	2	4

[a] Included in multivariable model for groups of young-stock housed indoors.

Table 5
Distribution and univariable associations of group-level exposures and VTEC O157 status in young-stock

Variable	Exposure level	Indoor		Outdoor	
		N^a	%VTEC-positive farms	N^a	%VTEC-positive farms
Age of cattle indoor (quartiles) (months)[b]	2–4	47	28	–	–
	5–6	37	38	–	–
	7–10	39	33	–	–
	>10	28	14	–	–
Age of cattle outdoors (quartiles) (months)	<8	–	–	49	16
	8–11	–	–	41	17
	12–14	–	–	47	19
	>14	–	–	43	14
Number of animals in group quartiles (indoor)[b]	<10	35	14	–	–
	10–15	28	29	–	–
	16–24	51	33	–	–
	>25	37	38	–	–
Number of animals in groups (quartiles) (outdoors)	<12	–	–	49	6
	12–20	–	–	44	18
	21–30	–	–	46	17
	>30	–	–	38	24
Animal density indoors (quartiles) (m^2)	<3	31	32	–	–
	3–6	39	26	–	–
	6.1–10	33	39	–	–
	>10	36	24	–	–
Animal density outdoors (quartiles) (m^2)[c]	<6	–	–	49	6
	6–61.9	–	–	44	8
	62–1950	–	–	46	17
	>1950	–	–	38	24
Presence of ringworm	No	121	28	129	18
	Yes	29	35	19	26
% of animals in enclosure with ringworm[b]	0	34	28	129	18
	1–50	6	29	15	20
	51–100	4	50	4	50
Silage used as feed[c]	No	81	33	152	13
	Yes	70	24	28	36
Straw used as feed[b]	No	59	20	164	16
	Yes	92	35	16	25
Presence of a hay/silage rack[c]	No	61	33	126	12
	Yes	90	27	54	28
Type of bedding	Other	6	33	–	–
	Straw	145	29	–	–
Use of chopped straw for bedding[b]	No	143	30	–	–
	Yes	8	13	–	–
Squelch score of bedding (median)	1	42	14	–	–
	2	85	33	–	–
	>2	24	42	–	–

Table 5 (*Continued*)

Variable	Exposure level	Indoor		Outdoor	
		N^a	%VTEC-positive farms	N^a	%VTEC-positive farms
The number of water troughs in the enclosure[c]	1	110	31	114	14
	2	24	26	30	20
	3	4	50	11	46
	>3	7	14		
Height from bedding to top of the water trough (cm)[b,c]	1–30	28	21	88	19
	31–100	113	30	76	12
	>100	10	50	16	25
Emptying of water troughs by hand[b]	No	27	15	73	18
	Yes	124	32	107	16
Access to surface water[c]	No	–	–	153	18
	Yes	–	–	27	7

[a] Missing values are omitted.
[b] Included in the multivariable model for indoor-housed groups.
[c] Included in the model for outdoor-housed animals.

participate due to lack of interest, time or staff availability. The median herdsize was 240 [range: 62–1300] and most sampling visits (91%) were in September and October 2003. A total of 42.7% of the farmers reported maintaining a closed herd (i.e. not introducing cattle from outside the farm).

At least one VTEC-positive sample was isolated from 87 (32%) of the 255 farms. The VTEC status was not associated with production type on farm ($p = 0.78$) or with whether the herds were open or closed ($p = 0.88$). The distributions of other management practices in the study cohort are presented in **Tables 1–5** along with the percentage classified as VTEC-positive farms.

3.2. Farm-level exposures

Seven exposures had univariable associations with farm-level VTEC status (**Tables 1–3**). There was no evidence of serious collinarity or interactions among those variables and only three of them were retained in the final model (**Table 6**).

Table 6
Farm-level risk factors of VTEC O157 for young-stock on 255 cattle farms in England and Wales

Factors	Exposure level	*b*	S.E.	*p*-Value	OR	Lower 95% CI	Upper 95% CI
Frequency of assessing the wetness of the bedding	Daily				1.00		
	Less than daily	1.49	1.99	<0.01	4.43	1.8	10.7
Presence of springs in cattle grazing field	No				1.00		
	Yes	−1.11	0.14	0.01	0.33	0.14	0.78

Excluding missing values; intercept = −2.24; Hosmer–Lemeshow χ^2 for fit of final model: 0.24, $p = 0.89$.

Table 7
Group-level risk factors associated with VTEC O157 in young-stock on 255 cattle farms in England and Wales

Factors	Exposure level	*b*	S.E.	*p*-Value	OR	Lower 95% CI	Upper 95% CI
Number of animals in group (quartiles)	<10				1.00		
	10–15	1.00	1.79	0.13	2.72	0.75	9.9
	16–24	1.33	2.28	0.03	3.78	1.2	12.3
	>25	1.33	2.34	0.03	3.78	1.1	12.7
Squelch score of bedding (median)	1				1.00		
	2	1.23	1.76	0.02	3.43	1.3	9.4
	>2	1.44	2.68	0.02	4.24	1.2	14.6
Straw used as feed	No				1.00		
	Yes	0.89	1.02	0.03	2.43	1.2	5.5

Excluding missing values; intercept = −3.45; Pearson χ^2 for fit of final model: 2.49, $p = 0.96$.

3.3. Group-level exposures

Nine exposures had univariable associations with the group-level VTEC status of the 157 indoor-housed groups (**Table 5**). There was no evidence of serious collinarity or interactions among those variables and three of them were retained in the final model (**Table 7**).

Six exposures had univariable associations with the groups-level VTEC status of the 152 outdoors groups, but none was retained in the final model (**Table 5**).

4. Discussion

The study cohort was not chosen at random from the English and Welsh cattle population, but by a convenience approach dependent on volunteering and willingness by farmers, which can have introduced selection bias. The approach also favoured larger herds with a history of frequently submitting samples for diagnosis or screening to governmental regional laboratories. This suggested that either the farms might exhibit greater-than-average health problems or they might use a very pro-active management style with frequent screenings and herd-health planning. Despite potential selection bias, the prevalence of infected farms (32.2%) in this study resembled the national estimate of 38.5% [$CI_{95\%}$: 28.1, 50.4] obtained 3 years earlier in a structured prevalence survey (**Paiba et al., 2003**) and we consider our study cohort fairly representative.

VTEC survives well in the environment and transmission between herds of grazing cattle can occur if the herds share water-courses such as rivers or lakes (**Jackson et al., 1998; LeJeune et al., 2001**). Our model revealed that farms, where cattle had access to springs, were less likely to be VTEC-positive. Intuitively, this might be explained by the fact that springs do not carry pathogens from other farms. However, springs were rarely the only source of water on these farms and it is unlikely that the farm's total water supply originated from springs. The significant protective effect of springs can have occurred by chance, which is supported by other studies finding no association between water source (springs, rivers, mains) and VTEC status (**Rugbjerg et al., 2003; Synge et al., 2003; Eriksson et al., 2005**).

Wet and dirty deep bedding creates an optimal environment (temperatures between 15 and 45 °C and neutral pH) for growth and survival of VTEC (**Ward et al., 2002**). This can explain the significantly higher risk of VTEC isolation, if the squelch score was >1 and if bedding-assessment frequency was less than daily. **Rugbjerg et al. (2003)** found that access to straw

increased the risk of VTEC isolation from cattle (albeit not distinguishing between straw as feed or as bedding), and a Scottish study reported that introduction to indoor housing was a risk factor in beef cattle (Synge et al., 2003). If farmers adapted their daily routines to include assessment of the dryness of the bedding, it might also reduce the risk for other pathogens; a similar association between dirty bedding, dirty cows and mastitis (including *E. coli* mastitis) has also been found (Ward et al., 2002).

Larger groups of cattle had increased odds of being VTEC positive, but the increased odds were not associated with stocking density (i.e. animals per m^2) in pens. An underlying reason might be that more animals provide more hosts for the survival and recirculation of the pathogen or that having more animals in an enclosure also increases the probability that at least one animal was shedding at the time of sampling. Introduction of new animals during rearing is more likely in larger groups, which might disrupt the social stability and immune status in established groups and increase the risk of introduction of the agent. Young-stock that had recently been moved were reported at higher risk of shedding VTEC than less dynamic animals (Rugbjerg et al., 2003).

Groups of animals that were fed straw were more likely to excrete VTEC, which adds to the large group of studies that identify a feedstuff to be associated with VTEC excretion. Most risk factor studies have identified one or two feedstuff components as associated with VTEC, but only rarely are the same components identified. Access to straw was reported by Rugbjerg et al. (2003) to increase VTEC excretion in young animals, but several other studies found no association (Synge et al., 2003; Schouten et al., 2004). A feed component that reduces VTEC excretion could play an important role in a control programme because a diet could be designed to use before transport to abattoir, during transient mixing (i.e. shows) or during grazing. However, more research in this area is needed to provide more consistent evidence.

We used pat samples from the floor to identify positive groups and farms, which can have introduced some misclassification, because the sensitivity of environmental samples is not 100%. The results might have been affected by temperature change, drying, dilution or sunlight, despite the effort to collect mainly fresh pats and the VTEC organisms' ability to survive well in the environment (Ward et al., 2002). The sensitivity of a floor faecal sample was estimated to be 77% compared to rectal samples and our aggregate testing scheme used to identify group status further increased this sensitivity to 83% (Sanderson et al., 2005).

We did not include the VTEC status of the individual animal in our study, because we consider group status to be of more relevance to control policies in young-stock. VTEC infection in cattle is asymptomatic and the bacterium is shed intermittently and non-persistent by individual animals (Heuvelink et al., 1998; Schouten et al., 2005; Matthews et al., 2006b). Control measures aimed at the individual animal can be costly, because repeated testing is necessary to identify an infected individual. Furthermore, the time delay can allow the agent to colonise other animals in close proximity or to create an environmental reservoir, before action against the infected animal is taken. Social research show that laborious, ineffective and/or costly interventions are less likely to be implemented and sustained in practice, which influences the impact of an otherwise effective intervention (Panter-Brick et al., 2006). This suggests that group-level control measures, which can be incorporated into the farmer's daily practices with least possible disruption of routines, are more likely to successfully reduce VTEC in young cattle.

No single exposure could account for the VTEC status of a group or a farm, which supported the common census that VTEC shedding in cattle is multi-factorial and is explained by a complex web of factors, in which cattle, other animals, transmission vehicles and the immediate environment play important roles (Rugbjerg et al., 2003; Synge et al., 2003; Schouten et al., 2004). The complex epidemiology of VTEC complicates the prediction of effect of potential

control programmes; further research is needed to identify pragmatic interventions that can reduce VTEC at the farm-level.

5. Conclusion

Our main objective was to identify farm-management practices that were associated with the VTEC status of young-stock cattle. Cattle farms that had poor bedding hygiene and neglected bedding-assessment practices were more likely to be VTEC positive than farms that kept the bedding of young-stock dry and assessed the wetness of the bedding daily. Large groups of housed young cattle were more at risk than small groups (<10) and feeding straw also appeared to increase the risk of groups of young-stock housed indoors. Access to spring water protected against VTEC, but no management factors were associated with groups of young-stock 'housed' outdoors being VTEC positive.

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Temporal Patterns and Risk Factors for *Escherichia coli* O157 and *Campylobacter* spp. in Young Cattle

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ABSTRACT

Escherichia coli O157 and *Campylobacter jejuni* and *Campylobacter coli* are zoonotic pathogens originating from farm animals. Cattle are the main reservoir for *E. coli* O157 and also contribute to human cases of campylobacteriosis through contaminated milk, direct contact, and environmental contamination. Thirty groups of young cattle on 30 farms were observed for 7 months and sampled on 4 to 6 separate occasions for *E. coli* O157 and *C. jejuni/coli* to characterize shedding patterns and identify risk factors. The within herd prevalence of *E. coli* O157 per sampling occasion ranged from 0 to 60% (mean = 24%) and average *Campylobacter* spp. within herd prevalence was 47% ranging from 0 to 100%. The prevalence of *E. coli* O157–positive herds declined with a linear trend throughout the study from 100 to 38% (OR: 0.5, $P < 0.01$), whereas time in the study was not significantly associated with *Campylobacter* prevalence ($P = 0.13$). Larger herds were more likely to be positive with either or both agents, whereas the number of suckler calves on the farm reduced the risk of both organisms (OR: 0.4/0.6, $P < 0.01$). Poultry on the premises reduced the risk of *E. coli* O157, but was not associated with *Campylobacter*. Emptying and cleaning the water troughs more often than once monthly reduced the risk of detecting *Campylobacter* and cattle sourced by private water supplies were more likely to be *Campylobacter* positive. No drinking water management practices were associated with *E. coli* O157. The risk of detecting both organisms were almost five times higher when the cattle were housed indoors (OR: 4.9, $P = 0.03$).

Several zoonotic agents such as *Escherichia coli* O157, *Campylobacter, Cryptosporidium* spp., and *Salmonella* serovars can be carried by and multiply in the intestinal tracts of cattle, increasing the risk to public health *(3, 6, 27, 28)*. Cattle products, the environment, and direct contact between cattle and humans are well-recognized transmission routes for these agents *(16, 23, 24)*. Cattle are regarded as the main reservoir of *E. coli* O157, and 39% of all commercial cattle farms in England and Wales are estimated to be positive for the organism *(20)*. *Campylobacter* spp. are carried by a large proportion of asymptomatic cattle in England and Wales, but the role of cattle as a source for human campylobacteriosis is not well explored *(8, 19)*.

Because of the asymptomatic nature of *E. coli* O157 and *Campylobacter* infection in cattle, farmers often are unaware that animals present a risk to human health. Even when on-farm control is implemented, improvement cannot be observed or measured by the farmer and the farmer derives no direct economic gains or measurable benefits. Behavioral models indicate that these hurdles impact implementation of control practices negatively and thus result in poor reduction of zoonotic risk to humans *(2)*. Alternative incentives must be promoted to ensure implementation of a zoonotic control program.

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One way of promoting adoption of such programs could be to simplify the advice on how to control multiple zoonoses to enhance the perception of self-efficacy, which is known to be low in English and Welsh cattle farmers *(4)*. Cooperation could be obtained by recommending a control plan that can reduce several zoonotic agents simultaneously to minimize the work burden requested of farmers and optimize benefits obtained from inputs. Such a plan must include the knowledge of genus-specific evidence-based characteristics and risk factors, which can be combined to generate a general zoonotic control program. The reduction of several zoonotic agents by a few measures is likely to appeal to farmers because of the improved safety for family and staff on the farm achievable with a feasible additional workload.

The objectives of this study were (i) to characterize shedding patterns and individual risk factors for *E. coli* O157 and *Campylobacter* spp. in young cattle 6 to 24 months of age and (ii) to identify combined risk practices for the two zoonotic agents that can be targeted in a combined control plan.

MATERIALS AND METHODS

Study population. Thirty cattle farms in England and Wales were randomly selected from a group of 57 *E. coli* O157–positive farms with more than 60 cattle each. The 57 farms had been iden-

tified for a different study, and details of the selection process have been described previously *(6, 7)*.

One group of at least 20 cattle 5 to 18 months of age (young stock) was monitored on each farm, and fecal samples were collected every 4 to 8 weeks. The first visit was carried out in August 2003, and the final visit occurred 10 months later in June 2004. The groups were monitored for an average of 6.8 months (range, 4.5 to 8 months).

Sampling. At each sampling visit, 20 fresh fecal pats were collected from the enclosure(s) of the monitored cattle groups. To ensure that the samples represented the whole group, the enclosure was visually divided into five equal areas, and four samples were collected based on a compass-like approach radiating from the center of each area. When the group was housed in multiple enclosures, the number of samples collected in each enclosure was proportional to the number of animals in each enclosure, still yielding a total of 20 samples.

Data collection. Information about the farm and the monitored cattle group was collected at each visit using standardized questionnaires and forms (available on request) to evaluate farm characteristics: herd size, main enterprise; type of cattle present; and presence of other animal species. Characteristics of the monitored group were defined by number of animals in the group, average age, age range (maximum to minimum), and housing (indoor or outdoor) at each visit. General management practices were also recorded: buying in animals, use of quarantine for newly acquired cattle, cleaning and disinfection of pens between groups of young stock, frequency of routine emptying and cleaning of water troughs, dryness of bedding on the day of the visit (1, dry and clean; 5, wet and slippery or dirty *(6)*), drinking water supply (private or public), and whether the cattle in the herd had physical contact with animals from other herds. Presence of *Coccidia* spp. and ringworm also was recorded, and these data were used as exposure variables to assess general health status of the cattle. For ringworm, a visual assessment was undertaken and recorded as the number of animals in the monitored group affected, and the variable was tested both as a continuous variable (percentage of affected animals in the group) and binary variable (presence or absence of affected animals). *Coccidia* status was determined using a modified McMaster method combined with microscopy; the group was classified as positive for *Coccidia* when one or more oocysts were detected in at least one sample.

Detection of *E. coli* O157. One gram of feces from each sample was examined for the presence of *E. coli* O157 using a peptone buffered enrichment followed by immunomagnetic bead separation (Dynal Biotech, Oslo, Norway). After separation, 50 μl of culture was plated onto sorbitol MacConkey agar containing cefixime and tellurite and incubated for 24 h at 18°C. Colonies that did not ferment sorbitol were confirmed as *E. coli* O157 by a latex agglutination test (Oxoid, Basingstoke, UK). Colonies from visit 1 were further characterized using PCR to detect the genes *vt1 (25)* and *vt2 (12)*, and the amplicons were separated by electrophoresis.

The specificity of the immunomagnetic bead separation and latex slide agglutination to correctly and exclusively identify *E. coli* serotype O157 was very high at the first visit (92.5% at the strain level and 96.7% at the farm level), and isolates from visits 2 through 6 were not confirmed beyond the latex slide agglutination test.

Detection and characterization of *Campylobacter*. A total of 745 samples from visits 2 through 6 were analyzed for the presence of *Campylobacter* spp. This group comprised 4 samples (every fifth in numerical order) from visits 2 and 3 and 10 samples (every second in numerical order) from the fourth and last visits.

The samples were enriched in 10 ml of Bolton broth for 48 h under a normal atmosphere and then subcultured onto modified charcoal-cefoperazone-desoxycholate agar plates and incubated at 37°C in microaerobic atmosphere for 48 h. Presumptive *Campylobacter* strains were Gram stained, and curved gram-negative rods were subcultured onto blood agar plates. Strains were classified as human pathogenic *Campylobacter* by PCR, applying the species-specific primers for *Campylobacter jejuni (10)* and *Campylobacter coli (10)*.

Statistical analyses. Farm and cattle group characteristics and management practices were described at the farm level. The microbiological results for each sample were transformed into binary variables for each visit, and the outcome of interest was the *E. coli* O157 and *Campylobacter* status of the visit. Univariate logistic regression models were used to assess whether the farm and group characteristics and management practices were potentially associated with either of the outcomes at each visit. Variables with a P value of <0.25 were included in the multivariate logistic regression models. The multivariate logistic regression models included farm as a random effect to adjust for clustering and were built using a backward stepwise approach until all exposures in the models were significant ($P < 0.05$). The strength of the associations was reported as the odds ratio (OR), where an OR of >1 describes an increased risk of the zoonotic agent when the practices are carried out, and the larger the value, the higher the risk. An OR of <1 indicates a protective effect of the practice. When the value 1 was included in the confidence interval, the P value was >0.05 and the effect was nonsignificant.

All continuous and ordinal categorical variables, which were included in the initial multivariate model, were assessed as both linear and factor effects by likelihood ratio testing, and when no statistical difference was identified, the simplest form (linear) was favored. Time parameters (visit number, month, minimum age, maximum age, and average age) were evaluated in univariate logistic regression models, and when several of these parameters were associated with the outcomes, only time parameter with the strongest association was included in the model to avoid colinearity. For strict interpretation, the visit number variable was used as a continuous variable as time in study or time since the beginning of study, because all time variables overlap to capture underlying times such as maturing of animals and calendar time. When no time parameter was associated at $P < 0.25$, the time variable with the lowest P value was kept in the model throughout the stepwise exclusion. When the final model was identified, likelihood ratio tests were used to assess whether the time variable made a significant difference to the model. If not, the time variable was dropped. Interactions between biologically plausible variables in the final models were tested and kept as interaction functions only if they improved the fit of the model by more than 30%. Model fit was measured using Pearson χ^2 tests, where $P < 0.05$ indicates a poor model fit.

All descriptive and association analyses were carried out using Intercooled Stata 10.0 (StataCorp LP, College Station, TX).

RESULTS

Study population. The 30 farms were located in various places in England and Wales (Fig. 1). Dairy farming was the main cattle enterprise in 25 of the farms, and 10 of these also reared beef cattle. Beef cattle were the main products on five farms, with two of these farms produced mainly suckler calves, two others reared mainly fattening

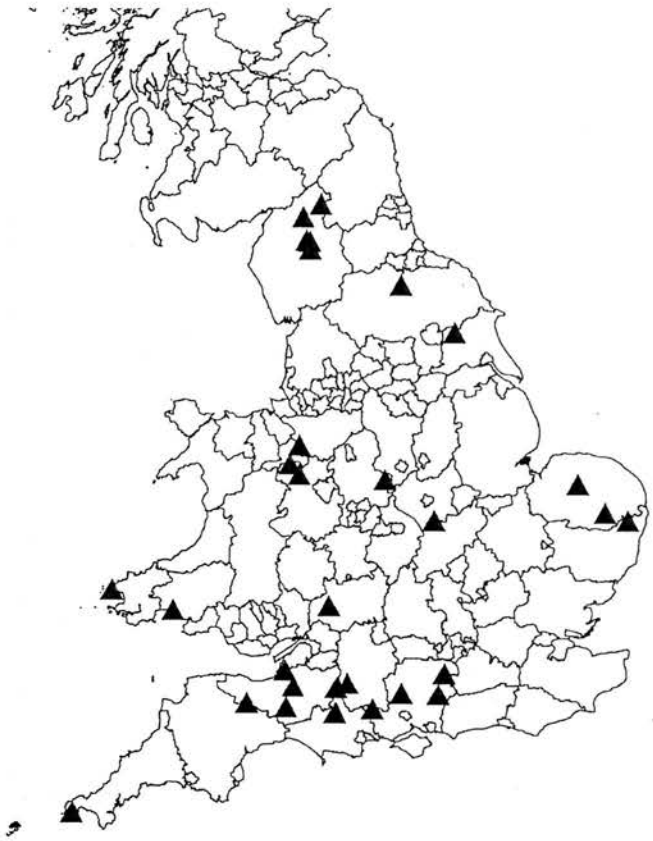

FIGURE 1. *Location of 30 cattle farms monitored for* E. coli *O157 and* Campylobacter *spp.*

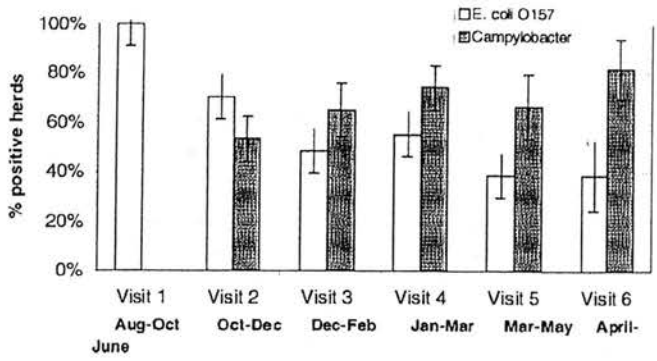

FIGURE 2. *Prevalence of cattle herds positive for* E. coli *O157 and* Campylobacter *spp. over time* (n = *30).*

cattle, and one farm allocated production equally to suckler and fattening cattle. The average herd size was 295 cattle (median, 259; range, 88 to 603), with dairy farms tending to have more cattle (300 on average) than beef farms (270 on average), although this difference was not significant ($P = 0.62$). Eight of the farms reported having sheep on the premises, six kept poultry, and eight kept horses, but only one farm kept all three species in addition to the cattle. Dairy and beef farms were equally likely to keep other animals species on the premises. The majority of farms claimed to keep closed herds, and only five reported the purchase of cattle from the outside and then only directly from other farms. Four of these farms used some kind of quarantine facility for new animals; however, the details of these practices were not examined or verified. Five farmers allowed their cattle to have contact with other cattle.

The monitored groups had an average of 50 cattle (range, 30 to 105). At enrollment, the average minimum age of the animals in these groups was 5 months (range, 1 to 12 months). Within the groups, the mean age was 6.5 months, (range, 3 to 17 months). On 23 of the farms, the monitored groups were housed indoors at every visit. Ringworm was observed in 14 of the monitored groups at least once during the study, and these groups were larger (mean, 52 cattle) than those in which animals did not have ringworm (mean, 44 cattle). *Coccidia* spp. were isolated from cattle in 14 of the monitored groups, but no symptoms were recorded. No clinical signs of disease were observed at the study visits. Straw was provided for 15 of the monitored groups all through the study, and another 9 farms provided straw as feed more than 50% of the study time. Seven farmers emptied and cleaned their water troughs at least monthly, some more frequently, and only two farmers had cleaned and disinfected the pens before housing the monitored group.

***E. coli* O157.** A total of 3,152 samples were collected and tested for *E. coli* O157, and 23.9% (753) of these samples were positive. The proportion of positive samples dropped from 37.7% (visit 1) to 8.9% (visit 6) during the period of monitoring, and the number of positive farms also declined by visit from 100 to 38% (Fig. 2). The largest proportion of positive samples was detected in August, September, and October, with a dip in prevalence in May and June (Fig. 2). The association of number of visits ($P = 0.001$) with the prevalence *E. coli* O157 was stronger than the effect of month ($P = 0.711$) or any age variable; thus, visit was included as a time variable.

***E. coli* O157 strain characterization at visit 1.** Presumptive *E. coli* O157 isolates were detected in 37.7% of the samples at the first visit, and 91.2% of the isolates were confirmed as *E. coli* O157 by somatic antigen serotyping. Of 103 confirmed isolates, 8.6% did not harbor any verotoxin (vt) genes, 1% had only *vt1* genes, 60.0% had only *vt2* genes, and 30.5% harbored both *vt1* and *vt2* genes. All strains with verotoxin genes also had *eae* genes.

The verotoxin profiles were clustered within the groups, and in 80% (24) of the groups only one verotoxin profile was detected. On 16 farms, all isolates carried *vt2* genes only, and on 8 farms only isolates harboring both verotoxin genes were detected. On the remaining farms, a mixture of the two patterns was found. Verotoxin-negative *E. coli* O157 isolates were detected together with *vt2* isolates on two farms, and isolates harboring both verotoxin genes and isolates that were negative for verotoxin were detected on one farm. One *vt1*-positive, *vt2*-negative strain was found on a farm that also had strains harboring both genes. Two farms had no confirmed isolates but were kept in the study because verotoxigenic *E. coli* O157 had been confirmed previously on the farms and verotoxigenic *E. coli* O157 was confirmed at the second sampling visits for both farms.

TABLE 1. *Farm characteristics and practices associated with the presence of* E. coli *O157 and* C. jejuni *or* C. coli *on cattle farms at sampling visits*

Dependent variable	Predictive variable	OR (95% confidence interval)	*P* value, logistic model
E. coli O157	Visit number	0.50 (0.36–0.69)	<0.01
	Herd size (per 10 animals)	1.16 (1.10–1.34)	0.02
	Presence of suckler calves	0.27 (0.11–0.62)	<0.01
	Presence of poultry	0.37 (0.15–0.88)	0.03
	Goodness of fit (Pearson χ^2)	0.223, df = 157	
Campylobacter	Visit number	1.46 (0.90–2.37)	0.13
	Herd size (per 10 animals)	2.00 (1.31–3.10)	<0.01
	Presence of suckler calves	0.61 (0.47–0.80)	<0.01
	Empty water troughs ≥monthly	0.16 (0.06–0.42)	<0.01
	Private water supply	3.29 (1.22–8.82)	0.02
	Goodness of fit (Pearson χ^2)	0.284, df = 99	
E. coli O157 and *Campylobacter*	Visit number	1.16 (0.76–1.77)	0.48
	Herd size (per 10 animals)	1.90 (1.25–2.89)	<0.01
	Presence of suckler calves	0.40 (0.25–0.62)	<0.01
	Indoor housing	4.90 (1.15–20.8)	0.03
	Goodness of fit (Pearson χ^2)	0.690, df = 99	

***Campylobacter* spp.** Human pathogenic *Campylobacter* strains were detected in 182 of the 745 samples; *C. jejuni* was isolated from 87.4% of the positive samples, and *C. coli* was isolated from 14.8%, with 4 samples harboring both species. The average proportion of positive samples at each visit was 47.0% (range, 0 to 100%), and the proportion of positive farms at each visit ranged from 53.3 to 81.8% (Fig. 2).

From a total of 68 (66%) of the 105 visits during which samples were collected for *Campylobacter* examination, the samples collected were positive for *Campylobacter* on 28 farms. Only eight farms were persistently positive, and the remaining 20 farms were intermittently positive or negative. *C. jejuni* was isolated from every positive farm at least once and was the only species isolated on 22 of the 30 farms. *C. coli* was present on six farms and only in combination with *C. jejuni.*

Coexistence patterns. On 18 sampling occasions for 13 groups, samples were negative for both *Campylobacter* and *E. coli* O157. The combination of *E. coli* O157 and *Campylobacter* was found in 21 groups, and in 11 of these the combination was detected more than once. On six farms, the combination was found in samples collected on three or four visits, suggesting that some of these groups could have remained positive for both pathogens for up to 6.5 months. Only one group was persistently infected with both pathogens throughout the study.

***E. coli* O157 risk factors.** A total of nine variables (contact with other herds, housing, herd size, visit, number of dairy cattle on farm, number of suckler calves on farm, emptying and cleaning of water troughs more often than monthly, main enterprise, and ringworm-affected animals in the group) were potentially associated with visits during which *E. coli* O157–positive samples were obtained. These variables were included in the initial full model. After adjustment, the multilevel multivariate model revealed that larger herds were more at risk of *E. coli* O157 infection (Table 1). The presence of poultry and number of suckler calves were associated with a decrease in risk, and a linear trend of decreasing risk per visit was also identified.

***Campylobacter* risk factors.** Herd size, month, number of dairy cattle, number of suckler calves, contact with cattle from other herds, housing, emptying and cleaning of water troughs more often than monthly, presence of ringworm, drinking water supply, and main enterprise were the variables included in the initial *Campylobacter* model. After the stepwise exclusion, water trough hygiene was associated with *Campylobacter* excretion, revealing that emptying and cleaning the water troughs more frequently than monthly strongly reduced the risk of *Campylobacter* infection (Table 1). The number of suckler calves present on the farm also reduced the risk. The risk increased with larger herds and when the drinking water originated from a private water supply rather than a public one. Visit was used to account for time and was forced into the model to improve the fit (*P* increased from 0.06 to 0.284) but was not significantly associated with the outcome.

Common risk factors. The initial model consisted of herd size, number of dairy cattle, number of suckler calves, presence of sheep, presence of poultry, housing, access to straw, and visit as a time indicator. Only visit, herd size, indoor housing, and number of suckler calves remained in the final model (Table 1). Young stock were almost five times more likely to be positive for *E. coli* O157 and *Campylobacter* when these animals were housed indoors instead of outdoors at the time of sampling. Presence of suckler calves reduced the risk and herd size increased the risk of carrying both zoonotic organisms, as observed in the individual models. Visit improved the model fit and was again forced into the model to account for time. However, no evidence of direct association of this variable with the outcome was observed.

DISCUSSION

E. coli O157 did not appear to be seasonal or associated with any particular month, and the strongest temporal pattern was associated with visit. The effect of visit may be a proxy indicator for any of several time effects. The risk of *E. coli* O157 infection decreases as the young stock get older, and the high-risk period is between 3 and 18 months of age *(19)*. Because we sampled at the group level, it was not possible to adjust for exact age at sampling, but the visit variable may have captured the maturing of the animals as the study progressed. The same visit number was carried out in 3-month blocks, and despite overlaps the visit number variable may also have captured some potentially underlying seasonal effects. Nevertheless, a convincing seasonal pattern of *E. coli* O157 excretion in Great Britain has not been reported by any statistically sound and representative studies. Because the length of the monitoring period was less than 2 years, we were not able to directly investigate seasonality *(6, 11, 19, 20)*. No deliberate changes in husbandry practices were adopted by the farmers during participation in the study, and sampling visits were purely observational. However, the awareness created by participation may have triggered unmeasurable changes in practices, resulting in a Hawthorn effect *(9)*. This effect would have increased over time, and this effect may have been reflected in the visit number variable.

The specificity of latex agglutination tests to identify *E. coli* O157 was estimated as 91.2%, and the high proportion of verotoxin gene carriage in *E. coli* O157 in this study (91.4%) is consistent with that reported by other researchers *(20, 26)*. Abandonment of the confirmatory test after visit 1 may have introduced a slight misclassification, and some of the strains isolated may not have been positive for the verotoxin genes. However, the risk of misclassification of the farm status at each visit was reduced by the aggregate approach of testing 20 samples to obtain farm status, and the risk of misclassification at the farm level due to the imperfect test was considered negligible.

The presence of poultry on the farm appeared as a protective husbandry practice for *E. coli* O157 infection. This effect was not intuitively easy to interpret and was considered a proxy predictor for something else that we did not measure. Farms that keep multiple species of animals may have had different biosecurity measures or barriers in place than those used on farms that only reared one type of animal. In the United Kingdom, some poultry companies have strict biosecurity regulations, and it is likely that some farmers inadvertently applied some of these principles to the cattle operation, thus reducing the incidence of *E. coli* O157 infection.

Campylobacter status was not associated with visit number, which suggests that the change from 4 to 10 samples after visit 3 did not introduce significant misclassification. The lack of association between visit number and *Campylobacter* isolation suggested that *Campylobacter* were less persistent and less associated with a particular farm or groups of animals than were *E. coli* O157 strains. Unfortunately, our level of strain characterization did not allow us to investigate this hypothesis, but other authors have suggested that *Campylobacter* strains are widely spread in the environment of cattle and that introduction of new strains continuously occurs *(14, 28)*.

Improved water trough hygiene was identified as a potential control measure against *Campylobacter* infection, suggesting that dirty standing water in water troughs may act as a reservoir for persistent reinfection within a group of cattle. This hypothesis is further supported by result of a study in which the use of individual waterers decreased the risk in cattle and the results of other studies highlighting the importance of providing farm animals with clean drinking water *(15, 17, 28)*. The occurrence and transmission of *Campylobacter* spp. through water is well known, albeit little is known about the original source of the *Campylobacter* transmitted through the water *(1, 14, 18)*. The private water supply, which is rarely disinfected before use, could be a source of continuous reintroduction of *Campylobacter* strains into a herd or may enhance survival of *Campylobacter* because of the lack of disinfectant in the standing water troughs *(15)*. It is not clear whether water-stressed organisms can cause infection, but human outbreaks from water sources occur frequently *(21, 22)*. Our study also revealed that larger herds had a higher risk of shedding *Campylobacter* spp., as has been reported previously *(28)*.

All three models identified the presence of suckler calves as protective against the zoonotic agents. Suckler calves and cows are outside the high-risk age group for *E. coli* O157 infection, and the presence of many less susceptible animals may have resulted a lower prevalence of *E. coli* on the farm and thus fewer organisms for reinfection *(19)*. *Campylobacter* also was detected significantly less often on farms that stocked suckler calves. Very little is known about the epidemiology of *Campylobacter* in cattle, but in a previous study we found that the presence of dairy cows increased the risk of detecting *Campylobacter* in the young stock and was potentially associated with milking practices *(5)*. The combination of stocking both suckler cows and milking cows is less common in the United Kingdom than stocking fattening cattle with a dairy herd, and we may have picked up the protective effect of sucklers rather than the risk of milking in our previous study. However, we did not find a risk associated with dairy cows in this study, and the exact mechanism underlying this association between suckler calves and *Campylobacter* remains unexplained.

To some degree, our combined model reflected the results from the other models, which also identified the associations with both herd size and suckler calves. However, the combined model also revealed that animals housed indoors are more likely to be infected with both organisms but not with the individual organisms. Water hygiene and source was no longer a risk factor because it was associated with only *Campylobacter* and appeared to have no effect on *E. coli* O157 *(7)*. Likewise, the effect of poultry disappeared because it was not associated with *Campylobacter*. However because these factors were associated with only one of the organisms, the strength of the associations

may have been reduced below the detection limited associated with our relatively small sample size.

Most current recommendations and control points target one agent at a time, bombarding farmers with advice and requests for adoption of multiple measures with one outcome. This approach is likely to create an information overload, which can lead to the perception that control of zoonotic agents is unrealistic, confusing, and complicated. Behavioral models recognize that the uptake of evidence leading to behavior change (in this case implementation of a control measure) is influenced by multiple factors, of which a very important one is the belief in self-efficacy as one's perceived ability to execute the behavior without difficulty *(2)*. This effect is well established in human medicine, where cessation of smoking and exercise as control measures are perceived as more difficult than taking a pill and thus have lower compliance rates *(13)*. The lack of multiple-agent control advice inhibits acceptance and implementation of control measures on cattle farms, leading to lack of reduction in risk to human health. More multi-agent research is needed to identify mutual control points for several pathogens to facilitate implementation of pathogen control recommendations on farms.

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Risk factors for *Campylobacter jejuni* and *Campylobacter coli* in young cattle on English and Welsh farms

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ABSTRACT

Campylobacter jejuni and *C. coli* are the most prevalent causes of bacterial diarrhoea in most of the Western World. In Great Britain, the source remains unknown for the majority of cases, though poultry is considered the main source of infection. Molecular typing methods identify cattle as a potential source of a proportion of the non-source-attributed cases, mainly through direct contact, environmental contamination or milk, but little is known about the epidemiology of *Campylobacter* in cattle. A cross-sectional study was undertaken on young cattle 3–17 months of age on 56 cattle farms in England and Wales to identify association between the presence of *C. jejuni* and *C. coli* and farm characteristics and management practices. *Campylobacter* was detected on 62.5% of the farms and the presence of dairy cows (OR: 3.7, $CI_{95\%}$: 1.2; 11.7), indoor housing (OR: 4.6, $CI_{95\%}$: 1.8; 12.0), private water supply (OR: 2.5, $CI_{95\%}$: 1.2; 5.4), presence of horses (OR: 3.2, $CI_{95\%}$:1.5; 6.9) and feeding hay (OR: 2.9, $CI_{95\%}$:1.6; 5.5) were associated with detection. The model's goodness-of-fit was improved when herd size was forced in the model without being statistically significant ($p = 0.34$).

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1. Introduction

Campylobacter jejuni and *Campylobacter coli* are the most commonly reported bacterial enteric pathogens in humans in the United Kingdom as in most of the Western world (**HPA**). Poultry meat is major source of human infection and a recent study has estimated that between 55 and 71% of human cases are attributable to chicken (**French et al., 2008**). This model was based on multi-locus sequencing types (MLST) results from three countries, where the species specificity of MLST have enabled source attribution studies (**French et al., 2005, 2008; Karenlampi et al., 2007**). These studies estimated that between 8 and 14% of human cases are attributable to cattle and a further 14–16% to sheep with slight intercountry variations.

Cattle contribute to human cases and outbreaks through several transmission routes such as direct contact, environmental contamination and milk (**Gillespie et al., 2003; Said et al., 2003; Kemp et al., 2005; Heuvelink et al., 2007**). *C. jejuni* and *C. coli* commonly colonize cattle without causing symptoms and other studies have reported that 40–60% of individual animals and 80% of herds in their study populations shed *Campylobacter* (**Wesley et al., 2000; Besser et al., 2005; Milnes et al., 2007**). Excretion of large quantities of fecal matter on large areas during grazing causes contamination of soil and watercourses and **Kemp et al. (2005)**, found that *Campylobacter*-contaminated water sources were associated with the density of cattle fecal pats in the surrounding area. Direct contact with cattle or their near-environment has also been associated with human Campylobacteriosis (**Kemp et al., 2005; Heuvelink et al., 2007**). Consumption of unpasteurized or inadequately pasteurized milk has also been identified as a source Campylobacteriosis and is

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believed to be associated with both outbreaks and sporadic cases (**Eberhart-Phillips et al., 1997; Gillespie et al., 2003**).

Very little is known about the dynamics and transmission of *Campylobacter* between cattle. *Campylobacter* are frequent in the environment, but certain MLST types have been associated with cattle rather than with their environment (**French et al., 2005; Karenlampi et al., 2007**). This suggests that certain genotypes either exhibit enhanced survival in cattle-specific environments or more effectively colonize the intestinal tracts of cattle. The latter could result in cattle acting as multipliers for the organisms, before they are excreted back into the environment. The exact nature of the relationship between the cattle host and *Campylobacter* is unknown, but even without the full biological understanding, knowledge of risk factors on farms, which enhance the probability of *Campylobacter*, can contribute to the design of feasible and practical interventions.

The objective of this study was to identify characteristics and management practices associated with the presence of *C. jejuni* and *C. coli* on cattle farms.

2. Materials and methods

2.1. Study population

The study population consisted of 56 Verocytotoxigenic *E. coli* O157 (VTEC O157) positive cattle farms, which were selected for a different study. The selection process was previously described in detail (**Ellis-Iversen et al., 2008a,b**), but in short, the studied farms originated from a pool of 411 farms in England and Wales, which were initially approached for participation through their private veterinary practices on the request of the Veterinary Laboratories Agency (VLA). A total of 255 farmers agreed to an initial VTEC O157 screening visit and eligibility check. Farms were eligible, if they stocked more than 60 cattle with a minimum of 20 young stock, were TB-negative and if the premises were not shared with any public access enterprises such as open farms, Bed & Breakfasts or farmshops and did not sell unpasteurized milk. Of the 87 VTEC O157 positive farms, 56 further agreed to a second visit and were enrolled for this *Campylobacter* study.

No nationally representative studies were available to provide a reliable within group prevalence estimate. Wesley et al. reported that 38% of all cattle tested were positive and a study from the US reported a within feedlot prevalence of approximately 45%, but both estimates included cattle from negative herds (**Wesley et al., 2000; Besser et al., 2005**). A high (80–100%) within flock prevalence is usually reported from poultry flocks, where the epidemiology of *C. jejuni* is better explored (**Evans, 2000; Hald et al., 2000**). The within group prevalence of *Campylobacter* in our study was assumed to be at least 50%, because of the close contact between our sampled animals. On this assumption, 4.2 samples were required from each herd to detect at least one positive with 95% confidence if the group was positive (calculated using Win Episcope 2.0). The number of herds was a convenience sample, but post-enrolment power calculations revealed that the 56 herds provided us a power of 80% to detect a twofold difference in proportions of exposures between negative and positive populations with 95% confidence, if 50% of herds were infected.

2.2. Data collection

Between 20 October 2003 and 1 February 2004, a sampler from the closest VLA regional laboratory visited each of the 56 farms once to collect data and 4–6 fecal samples. The sampler had been trained by information meetings and had previously carried out the screening visits to the farms. Information on farm characteristics and normal practices for management of young stock was collected on questionnaires, which were used in previous studies (**Paiba et al., 2003; Ellis-Iversen et al., 2008a**). A copy is available on request. Most questions were closed and used tick boxes to increase precision of the answers and the questionnaires were completed by the sampler in discussion with the farmer. Furthermore, to approximate hygiene, the sampler scored the wetness of the bedding in five different areas of the enclosure (1 = very dry and 5 = very wet, dirty and slippery) (**Hughes, 2001**) and the cleanliness of five animals using the well-established Meat Hygiene Service's Assessment Score system (**FSA**).

2.3. Sample collection

Fecal pat samples were collected from each indoor or outdoor enclosure, where a group of 20 or more healthy looking young stock (3–17 months of age) was housed on the day of sampling. In an attempt to avoid sampling one social group within the enclosure, the enclosure was visually divided into four areas and 1–2 samples of fresh-looking fecal pat(s) were collected in each area. In the laboratory, each sample was streaked onto a separate mCCDA plate and incubated at 37 °C in a microaerobic atmosphere for 48 h. Presumptive *Campylobacter* strains were Gram-stained and all Gram-negative spiral-formed rods were sub-cultured onto blood agar plates and incubated as above, before they were speciated into *C. jejuni* and *C. coli* by species-specific PCR (**Gonzales et al., 1997**).

The burden of *E. coli* O157 within the sampled group was determined using immuno-magnetic separation before culture on McConkey agar as previously described (**Paiba et al., 2003**). Furthermore, each sample was analyzed for the presence of *Coccidia* spp. using a modified McMaster method combined with microscopy. The farm was classified as *Coccidia* positive if one or more oocysts were detected in at least one sample. The burden of *E. coli* O157 and the presence of *Coccidia* spp. were included as exposure variables in the study.

2.4. Data analyses

The data and microbiological results were entered into an Access 97® database (Microsoft Corp., Redmond, WA) and visually checked for implausible or outlying values, which were traced back to the original questionnaires, the farmer or the laboratory for clarification and confirmation and amended accordingly in the database.

Binary variables were created from the hygiene scores for use in the multivariable model. For dryness of bedding, a cut-off between 2 and 3 was used, where the bedding went from "slightly soiled and dry" to "squelchy" because this cut-off was found to be of importance to *E. coli* O157 burden in young cattle and no information was found regarding *Campylobacter* spp. (**Ellis-Iversen et al., 2008b**). Animal cleanliness was also divided between 2 and 3 according to the legislation used at UK slaughter houses (FSA).

Analysis of farm characteristics and management variables and their association with *Campylobacter* was carried out as a two-step process. Initially, univariable logistic regression tests were used to investigate crude associations between *Campylobacter* status and each binary or categorical variable. Continuous variables were evaluated using *t*-tests. All exposure variables with a crude *p*-value <0.3 were included in a full multilevel multivariable logistic regression model using sample status as outcome and farm as random effect to adjust for potential clustering. Herd size was considered an important proxy for uncaptured variables such as production information, general health status of the animals, number of staff, etc. and was forced in the model independently of statistical significance, while a backwards stepwise elimination of all other non-significant variables was applied to obtain a minimal model containing only significant variables (Wald's $p < 0.05$). To assess confounding, the variables eliminated by the stepwise process were reintroduced one by one to the minimal model. A newly introduced variable was retained and included in the final model if it either improved the goodness-of-fit of the model by >10% or changed the odds ratio by >25% (confounding). The goodness-of-fit was assessed using Hosmer–Lemeshow tests for 10 groups. Biological plausible interactions between variables in the final model were assessed using likelihood ratio testing of nested models. All analyses were carried out using Intercooled Stata 10.0 (STATA Corp., College Station, TX).

3. Results

The study population consisted of 56 farms, where dairy was the main enterprise on 28 farms and beef was the main enterprise on the other 28 farms. However, dairy cows were present on 45 of the farms suggesting a high proportion of mixed farms. Fattening cattle were reared on 24 farms and suckler calves were present on twelve. Horses, sheep and poultry were present on 17, 19 and 6 farms, respectively. Only 12 of the farms had bought-in cattle in the last 12 months and 11 farms used only public water supply for their cattle. Private water supplies such as wells or boreholes supplied the cattle on 25 farms and the cattle were also supplied from open water courses on 30 farms. Fifty-one of the sampled groups were housed indoor at sampling, 23 were fed silage, 7 hay and 32 were fed straw. The age of the animals ranged from 3 to 17 months with an average of 8.7 months. *Coccidia* spp. was isolated from 14 groups and the average burden of *E. coli* O157 was 0.39 (0.29; 0.52).

The *Campylobacter* strains originated from 35 (62.5%, $CI_{95\%}$: 0.49; 0.76) of the farms and the proportion of positive samples within each farm varied from 16.7 to 100%. The majority of positive farms had ≤50% positive samples and the distribution of within herd prevalence is shown in **Fig. 1**.

All farms were visited once and 4–6 samples were collected on each farm yielding a total of 235 samples. *Campylobacter jejuni* or *C. coli* were isolated from 27.7% of the samples and the majority of the isolates (95.2%) were *C. jejuni*. *Campylobactor coli* was detected in five samples and three of these originated from farms, which also harbored *C. jejuni*. The two remaining *C. coli* strains originated from different farms and *C. jejuni* was not detected on these farms.

Tables 1–3 describe the distribution of exposures in the study population and identify the variables with a potential association with *Campylobacter* detection.

From the 17 variables initially included in the multivariable model 5 remained significant (**Table 4**). Reintroducing private water supply and housing into the model improved the fit from $p = 0.07$ to $p = 0.72$ and the decision to keep herd size in the model as a proxy confounder was justified by a 50% improvement in fit. No interaction between any of the variables in the final model improved the fit of the model significantly and all variables were considered to be independently associated with *Campylobacter* detection.

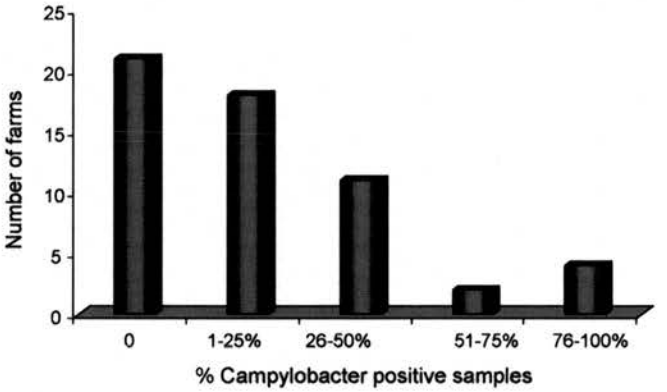

Fig. 1. The percentage of *Campylobacter* positive samples in young cattle on 56 cattle farms in England and Wales.

Table 1
Distribution of *Campylobacter* positive and negative samples collected from 56 cattle farms in England and Wales

Variable	Exposure level	Campylobacter status			
		Positive		Negative	
		n	%	*n*	%
Herd size (categorical)[a]	60–176	12	43	16	57
	177–260	18	21	66	79
	261–369	12	20	47	80
	370–1300	21	33	43	67
Main enterprise	Dairy	34	29	183	71
	Beef	29	25	89	75
Type of cattle on farm	Dairy[b]	56	29	135	71
	Sucklers[b]	12	21	46	79
	Fattener	24	24	78	76
Cattle bought in the last 12 months	Yes	11	22	40	78
	No	50	29	125	71
Horses[b]	Present	26	36	46	46
	Absent	37	23	126	77
Sheep	Present	21	26	60	74
	Absent	42	27	112	73
Poultry	Present	7	28	18	72
	Absent	56	27	150	73

[a] Included in model as a proxy confounder;
[b] Included in multivariable model ($p < 0.3$); Missing values omitted.

Table 2
Distribution of Campylobacter positive and negative samples according to management practices of young stock on 56 cattle farms

Variable	Exposure Level	Campylobacter			
		Positive		Negative	
		n	%	*n*	%
Contact with cattle from other herds in the last 2 months	Yes	7	28	18	72
	No	56	27	154	73
Period of at least 8 weeks between spreading of manure, slurry or dirty water on land and allowing grazing[a]	Yes	33	31	73	69
	No	30	23	99	77
Cattle drink from private water supply[a]	Yes	36	34	69	66
	No	27	21	103	79
Any water sources shared with other cattle herds[a]	Yes	15	22	54	78
	No	45	28	113	72
Housing[a]	Indoor	60	29	149	71
	Outdoor	3	12	23	88
Cattle fed silage[a]	Yes	21	23	70	67
	No	41	30	97	70
Cattle fed hay[a]	Yes	12	44	15	56
	No	50	25	152	75
Cattle fed calf-nuts[a]	Yes	38	32	79	68
	No	24	21	88	79
Cattle fed straw[a]	Yes	42	33	84	67
	No	20	19	83	81
Squelch score of bedding (median of group)[a]	1 or 2	11	24	35	76
	>2	44	28	108	72
Boot dip present and used[a]	Yes	9	26	16	64
	No	54	26	156	74
Over coat present and used[a]	Yes	9	43	12	57
	No	54	25	160	75
Coccidia spp.	Positive	6	33	12	67
	Negative	44	27	117	73

[a] Included in multivariable model ($p < 0.3$); Missing values omitted.

Table 3
Distribution of age and within herd VTEC prevalence on 56 groups of young cattle on English and Welsh farms and Campylobacter status at samplelevel

	Campylobacter positive				Campylobacter negative			
	n	Mean	P_{50}	Min–max	*n*	Mean	P_{50}	Min–max
Group size[a]	62	22	22	7–47	167	24	24	3–67
Age of cattle (months)	63	8.80	8	3–17	155	8.40	8	4–17
VTEC prevalence	63	0.41	0.5	0–1	172	0.35	0.25	0–1
Animal cleanliness[a]	63	N/A	2	1–4	165	N/A	2	1–5
Squelch score	55	N/A	2	1–5	146	N/A	2	1–5

[a] Included in multivariable model ($p < 0.3$); Missing values omitted.

Table 4
Husbandry practices and farm characteristics associated with *C. jejuni* and *C. coli* presence in young stock in England and Wales, when adjusted for other risk potential factors in a multilevel clustered logistic regression model

Variable		Coef	OR	95% CI	*p*-Value
Herd-size (priory confounder)		−0.2	0.8	0.5; 1.2	0.37
Presence of dairy cows	Yes	1.3	3.7	1.2; 11.7	0.02
	No		1.0		
Private water supply	Yes	0.9	2.5	1.2; 5.4	0.02
	No		1.0		
Feeding hay	Yes	1.1	3.0	1.6; 5.5	<0.01
	No		1.0		
Presence of horses	Yes	1.2	3.2	1.5; 7.0	<0.01
	No		1.0		
Housed indoors	Yes	1.5	4.6	1.8; 12.0	<0.01
	No		1.0		

$N = 229$; cluster effect = farm; constant = −5.7; goodness-of-fit = 0.72.

If dairy cattle were kept on a farm, the odds of *Campylobacter* detection increased by four times compared to farms only stocking beef cattle and the presence of horses appeared to have a similar increasing effect (**Table 4**). Drinking water supplied by a private water supply for any of the cattle in the herd also increased the odds and samples from indoor housed animals were more likely to be *Campylobacter* positive than samples from outdoor animals. The odds of cattle shedding *Campylobacter*, when they were fed hay, were three times higher than cattle that were not fed hay.

4. Discussion

Our study population was not a random selection of farms and did not directly represent the national cattle herd. All farms were previously classified as VTEC O157 positive and thereby represented around 10% of cattle herds in England and Wales (**Paiba et al., 2003**).

We were unable to identify any studies on a representative population comparable to the English and Welsh cattle population that would enable us to assess the validity of our findings. We found *Campylobacter* on 62.5% of the study farms and other studies have reported similar or even higher prevalence (**Wesley et al., 2000**; **Milnes et al., 2007**). However, some of these studies also included *Campylobacter* spp. other than *C. jejuni* and *C. coli* with less impact on human health in the prevalence estimate and are not directly comparable.

All our study farms were VTEC O157 positive and it is unknown whether the VTEC O157 status introduced selection bias in our study. *Campylobacter* has different survival and infection mechanisms than *Enterobactericeae* such as *E. coli* and *Salmonella* (**Murphy et al., 2006**). *E. coli*'s survival mechanisms and growth requirements are more similar to *Salmonella* than *Campylobacter* and we found no association between burden of VTEC O157 and *Campylobacter* presence in our study. Therefore, we consider any selection bias due to VTEC O157 status low or negligible.

In this study, we classified 62.5% of the study farms as *C. jejuni* and/or *C. coli* positive from 1 to 6 samples per farm. The limited studies of *Campylobacter* in cattle left us unable to estimate the sensitivity of the methods to adjust samples sizes during the study design and adjust for a potential misclassification bias in the analyses. In the study design phase, we assumed a high proportion of positive samples within the sampled group, because of reported high individual animal prevalence at slaughter, high within flock prevalence reported in chicken flocks and because we sampled from one group of cattle with close contact (**Evans, 2000**; **Hald et al., 2000**; **Milnes et al., 2007**). Surprisingly, we found a low proportion of positive samples (<50%) on the majority of the positive farms and in hindsight we acknowledge that positive farms might have gone undetected. False negative farms can have resulted in an underestimation of the strength of our reported associations. This potential misclassification was not considered differential, but because of the low proportion of positive samples within the groups, larger farms could have been more likely to be misclassified as false negatives. This could explain, why herd size was not significantly associated with *Campylobacter* status, despite improving model fit.

Risk behavior and practices might enhance the probability of *Campylobacter* shedding by either increasing the risk of introduction or sustaining the population of *Campylobacter* already present. Sustaining *Campylobacter* within a herd could be accomplished by either increasing susceptibility of the animals, by increasing infectious contacts, by increasing excretion or by enhancing environmental survival. Our study identified management practices and farm characteristics which might affect all these pathways, but without knowing the exact biological mechanisms of *Campylobacter* in cattle, we acknowledge that our explanations are hypotheses that need more investigation.

We identified private water sources, such as wells and boreholes as a risk factor for the cattle. Private water supplies usually pump up ground water to be used in the household or for the farm animals (**Said et al., 2003**). Ground water is normally considered free of pathogens and is subsequently rarely treated. However, leaks in pipes and pumps serve as a potential mechanism for introduction of organisms such as *Campylobacter* into the herd. Furthermore, *Campylobacter* could survive longer in non-treated water standing in water troughs, by biofilm formation or in protozoa, increasing the probability of contact with susceptible hosts (**Buswell et al., 1998; Snelling et al., 2006**). Waterborne human outbreaks are well documented from non chlorinated water supplies or if the chlorination fails and are also believed to account for some of the untraced human cases (**Duke et al., 1996; Engberg et al., 1998; Furtado et al., 1998**). However, *Campylobacter* in water often enter a non-culturable stress stage, which escapes traditional detection methods and might result in an unrecognized or underestimated contaminated source (**Baffone et al., 2006**). Associations between drinking water source and *C. jejuni* in farm animals has also been identified by other authors and the importance of providing farm animals with drinking water of proper microbiological quality should not be neglected (**LeJeune et al., 2001; Kemp et al., 2005; Arsenault et al., 2007**).

Campylobacter's ability to survive in the environment is well documented and isolation has been reported from beach sand, sewage and surface water amongst others, despite anti-bacterial properties such as UV-light, dry conditions and fluctuating temperatures (**Kemp et al., 2005; Murphy et al., 2006**). When the animals are housed indoors, these stress-inducing conditions for the bacteria are reduced and wet bedding, low hygiene and higher animal density potentially enhance survival of the bacteria (**Mason et al., 1999; Murphy et al., 2006**). The longer survival times of *Campylobacter* and the higher stocking density of cattle, which provide more agent–host contact, are plausible explanations for the increased risk associated with indoor housing.

Young stock (3–18 months of age) on UK farms are generally managed extensively in groups with very little handling of the individual animal. Our sampling approach was targeted at identifying group-level risk factors, because individual animal interventions are not feasible for the farmer and are thus, unlikely to be applied. It was surprising that the presence of dairy cattle on a farm increased the risk of *Campylobacter* in young stock, because beef and dairy cattle at the sampled age are usually managed in similar ways. The increased risk was only related to dairy cows, not the presence of suckler cows, suggesting that the risk was associated with management practices of dairy cows rather than with age or potential immune status. We failed to measure any association between *Campylobacter* status and the main cattle enterprise, which reflects the large proportion of mixed cattle farms in the UK and presence of dairy cows could be a proxy for the presence of a dairy enterprise. The daily or twice daily herding of dairy cows to the milking parlor causes increased movements of groups of animals on dairy farms compared to beef farms and the frequent mixing of fecal material between cattle groups can potentially enhance the transmission within the farms. The number of female animals in a dairy herd has previously been identified as a risk factor for *Campylobacter* excretion, which could have been a proxy for milking cows in their study (**Hoar et al., 2001**).

The effect of different feed components on bacterial excretion has been investigated particularly with *E. coli* O157, with the intention of recommending a pre-slaughter diet that will reduce shedding and hide contamination on the way to slaughter (**Hancock et al., 2001**). However, no consistent results are available and conflicting evidence regarding hay has been reported, such as hay having no effect on excretion of VTEC O157, but has also to be associated with reduced hide contamination (**Berry et al., 2006; Mather et al., 2007**). We found that groups, which were fed hay, had an increased risk of *Campylobacter*. Probable explanations could be enhanced survival and multiplication in the gut and/or increased excretion of facial matter due to a fibre rich diet. The underlying mechanisms cannot be explained by our study and because the study was carried out at group level, the attempt to interpret the results at animal level could result in an ecological fallacy.

The identified associations between management factors and *Campylobacter* shedding were all strong with an odds ratio of >3. Weaker associations could have been disguised by our small sample size. Nevertheless, this study provided some new insight into the epidemiology of *Campylobacter* in cattle and highlighted farm characteristics and management practices, which potentially increase the risk of contaminating the environment with *Campylobacter*. More research is needed to confirm the underlying biological mechanisms for the risk factors identified in this study and to further explore the dynamics of *Campylobacter* within and between cattle and the environment. We also recommend that the relative importance of introduction and sustainability of *Campylobacter* in cattle herds is investigated further, before interventions to reduce *Campylobacter* in cattle can be designed and implemented.

5. Conclusion

This study established that some cattle farm characteristics and management practices are associated with isolating *C. jejuni* or *C. coli* from young cattle aged 3–17

months. A total of 35 (62.2%) of our study farms were infected with *Campylobacter* and *C. jejuni* were more frequently isolated than *C. coli*. We identified five independent risk factors for *Campylobacter* in cattle: drinking water supplied by private water supplies, indoor housing, feeding hay, presence of dairy cows and presence of horses.

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Verocytotoxin-producing *E coli* O26 infection in calves

SIR, – Verocytotoxigenic *Escherichia coli* (VTEC) O157 is recognised as an important cause of severe illness in humans. Cattle provide the main reservoir of infection but remain clinically unaffected. In a recent field study of 75 farms in England and Wales by Paiba and others (2003), the prevalence of excretion by individual cattle was 4·2 per cent. Although VTEC O157 is the most important VTEC serogroup from human infections in Britain, verocytotoxins (VTs) can also be produced by other *E coli* serogroups, such as O26. Surveillance within the Veterinary Laboratories Agency (VLA) for new and emerging strains of *E coli* in animals has recently been enhanced to include extended testing for virulence factors, serotype and antimicrobial resistance in isolates conforming to specified selection criteria.

We would like to report the findings from a recent farm investigation in which VTEC O26 was recovered from clinically normal cattle following an initial isolation in faeces from a scouring calf. The isolate was submitted from a two-month-old calf in a 33-cow beef suckler and calf-rearing enterprise. The herd commenced calving indoors in a strawed yard in January 2006. Several of the suckler calves developed diarrhoea but only one was sampled and the sample submitted to the VLA for limited diagnostic testing. This animal subsequently died.

Examination of the faecal sample using the standard procedures adopted within the VLA did not identify any potential pathogens apart from *E coli*. The isolate was examined under the enhanced *E coli* surveillance programme and identified as *E coli* O26 with genes for VT1 and intimin (*eae*), which is associated with intestinal attachment and effacement.

At a follow-up visit to the farm, none of the cattle appeared to be scouring. Faecal samples were taken from the previously affected group of suckler calves housed with their dams and from two groups of heifers and steers that were kept in adjacent pens. The samples were screened for *E coli* O26 using an automatic immunomagnetic separation technique (DEFRA 2006). Eight of 10 samples from the calves and five of six from the cows were positive using this technique. Extended testing of three of the isolates revealed that they were also VT1 and *eae* positive by PCR. Four samples taken from cattle in unaffected groups were negative for *E coli* O26.

During the period May 2005 to May 2006, a total of 249 cattle isolates were included in the VLA enhanced *E coli* surveillance survey. Of these, 14 (5·6 per cent) were VTEC, of which serogroup O26 (six isolates [43 per cent]) was the most prevalent.

Pearce and others (2004) also reported that VTEC O26 was the most frequently detected VTEC serogroup in a longitudinal study on a cohort of beef cattle in Scotland. They found no association with housing or with diarrhoea. The results of our farm investigation have further confirmed a high prevalence of infection in clinically normal cattle. It is possible that VTEC O26 contributed to the demise of the initial calf sampled from the farm. There are other documented reports of diarrhoea in cattle associated with *E coli* O26 (Gunning and others 2001).

Like VTEC O157, VTEC O26 carries the potential for human infection and severe illness (Brooks and others 2005). There was no associated human illness on this farm, and human cases of VTEC O26 infection are rare in Great Britain (Willshaw and others 2001). However, other countries such as Austria, Germany and Italy report it as the second most prevalent enterohaemorrhagic *E coli* isolated from patients with haemolytic-uraemic syndrome and diarrhoea (Leomil and others 2005). All the VTEC O26 isolates identified from the VLA survey to date have contained the VT1 rather than the VT2 gene, which is consistent with many other surveys. However, strains of VTEC possessing VT2 have emerged in recent years as having increased pathogenicity in man, in both the UK and North America (Jenkins and others 2003, Brooks and others 2005), and there is some evidence that *E coli* O26 is continuing to evolve by uptake of VT2 genes (Zhang and others 2000).

In summary, VTEC O26 appears to be a frequent contributor to the gut flora of cattle and can be isolated from both scouring and clinically normal animals. There is clearly an occupational hazard associated with contact with farm animals and efforts should be made to reduce the risk by adhering to sensible hygiene precautions. Practitioners should also be aware that diarrhoeic calves may carry VTEC infection either coincidentally or associated with clinical disease.

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Prevalence of attaching and effacing *Escherichia coli* serogroup O103 in orphan lambs on an open farm in eastern England

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Escherichia coli O157:H7 is currently the most common serotype associated with complex sequelae such as haemorrhagic colitis (HC) or haemolytic-uraemic syndrome (HUS) following diarrhoeal disease in human beings (Keene and others 1994, Proulx and others 2003). However, a considerable proportion of cases are due to infections associated with non-O157 serotypes, particularly O103:H2, which has emerged as a causative agent of HC and HUS in many countries (Mariani-Kurkdjian and others 1993, Urdahl and others 2002, Jores and others 2005). Serogroup O103 *E coli,* with and without Shiga toxins (also known as verocytotoxin), has also been described in sheep and lambs as well as many other species, including calves, pigs, goats, chickens, partridges and rabbits (Duhamel and others 1992, Mariani-Kurkdjian and others 1993, Urdahl and others 2002, La Ragione and others 2004, Jores and others 2005). These pathogens are typified by their ability to colonise the mucosa of the gastrointestinal tract intimately by forming attaching and effacing lesions (Knutton and others 1997) and the production of Shiga toxins, which are believed to be the primary cause of the complex sequelae (Sandvig and van Deurs 1994).

The risk to human beings, via the food chain, of pathogenic *E coli* from animals, with *E coli* O157:H7 being the prime example, is of continuing veterinary public health concern (Tauxe 1997). However, direct contact with infected animals is also recognised as a significant risk, with a number of cases of HC and HUS arising from visits to petting or open farms (Chapman and others 2000, Duffy 2003, CDC 2005). The authors have previously shown that lambs of slaughter age in the UK can carry potentially zoonotic *E coli* (Aktan and others 2004). This short communication describes a study to assess the hazard that orphan lambs present as a potential source of *E coli* pathogenic for human beings. Orphan lambs are usually unwanted triplets or lambs whose mothers have died, and they are often artificially reared on farms open to the public. This study focuses on quantifying and determining the serogroups and pathotypes of *E coli* strains capable of intimate attachment and possessing Shiga toxins isolated from orphan lambs on a typical open farm in eastern England.

A total of 88 faecal samples were collected from 45 orphan lambs; 43 of the samples were pooled samples from pens of six lambs. The lambs ranged in age between one and 10 days. The samples were transported to the laboratory within two hours of collection and were plated on MacConkey's agar immediately or kept at 4°C until being plated the following day. After overnight incubation, five colonies from each sample with the typical appearance of *E coli* were selected at random and re-streaked onto non-selective Luria-Bertani agar. The isolates were confirmed as *E coli* by biochemical tests using API 20E miniaturised biochemical test strips (bioMérieux). PCR assays were used to detect virulence genes. A small quantity of the test organism was picked up with a loop and transferred directly to 50 µl of the PCR mixture comprising a multiplex PCR targeting the Shiga toxin 1 (*stx1*), Shiga toxin 2 (*stx2*) and intimin (*eae*) genes as described previously by La Ragione and others (2002). For isolates identified as *eae* positive, the specific intimin subtype was determined by PCR as described by Aktan and others (2004), and the serogroup was established by the *E coli* serotyping section at the Veterinary Laboratories Agency.

Of a total of 530 isolates confirmed to be *E coli* isolates, 61 (11·5 per cent) carried the *eae* gene. No *eae*-positive isolate possessed either *stx1* or *stx2* genes, and of the remaining 469 isolates, two were *stx1*-positive and two were *stx1* and *stx2*-positive. Twenty-one *eae*-positive isolates were serogrouped; in order of frequency, there were 17 O103K+ strains, two O8:K+ strains, one O118:K+ strain and one Rough strain. All of the *eae*-positive isolates identified as belonging to serogroup O103:K+ possessed the *eaeε* (epsilon) intimin type. No other serogroup recognised as a human pathotype was identified. In order to investigate the possible relationships between the *E coli* O103:K+ isolates obtained during the study, pulsed-field gel electrophoresis (PFGE) fingerprints of 10 isolates selected at random were determined. Each isolate was analysed by PFGE as previously described by Liebana and others (2001). There was no difference in the *Xba*1 (Promega) PFGE patterns for these isolates.

The clinical significance of *E coli* O103:K+ for the orphan lambs is unknown, although from a veterinary public health perspective it has potential zoonotic significance. A recent survey of the prevalence of non-O157 *E coli* on Scottish farms revealed a mean prevalence of 2·7 per cent for serogroup O103 (Pearce and others 2006) in cattle. Those authors stated that the carriage of *stx* was rare in the serogroup O103 isolates found in their study, and the absence of *stx* concurs with the present findings. The study of Pearce and others (2006) involved surveillance of 6086 samples from 338 farms across Scotland. While no equivalent survey data exist for England, the biased sample studied here and previous small surveys (Liebana and others 2001, Aktan and others 2004) indicate that *E coli* serogroup O103 may also be prevalent in sheep.

The present study showed that the *E coli* O103 isolates shared an identical PFGE profile, indicating clonality. Without more detailed investigation no conclusions can be drawn about the source of the organism, but it can be speculated that either a common clonal line is widespread and infected lambs arrived on-farm, or the clone is resident on-farm and the orphan lambs are readily colonised upon exposure on the farm. What is clear is that the orphan lambs studied were colonised with and shedding very high numbers of this organism, as proven by direct detection by plating without any selection. The environmental burden arising from this level of shedding would be of greater veterinary public health concern if the isolates possessed Shiga toxins.

A previous small survey conducted on animals at slaughter in the UK revealed that *E coli* serotype O103 lacking Shiga toxins comprised 0·6 per cent of the total number of isolates tested, and 8 per cent of the various attaching and effacing *E coli* detected in cattle, sheep and pigs (Aktan and others 2004). It has been suggested that *E coli* O103 lacking Shiga toxins from rabbits and *E coli* O103 possessing Shiga toxins from human beings have a common origin but different mechanisms of adaptation to their hosts (Mariani-Kurkdjian and others 1993). More recent data suggest that, while there is genetic diversity within serogroup O103 isolates, probably mediated through acquisition of different virulence genes and DNA rearrangements, certain subtypes (for example, multilocus sequence typing [MLST] profile II) from human beings and animals were very similar except for the presence or absence of the Shiga toxins (Beutin and others 2005). The authors also demonstrated that serogroup O103 isolates possessing the *eaeε* intimin type, as found in the present study, belong to the MLST profiles I and III, which elaborate Shiga

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toxins and are not only associated with human disease but are also geographically widespread. The isolates from orphan lambs lacked Shiga toxins, and it is possible that loss of the *stx* genes may have occurred, as has been observed during subculture (Karch and others 1992) and may well also occur for serogroup O103 (Urdahl and others 2002), possibly caused by the mobility and instability of bacteriophages that are known to encode the *stx* genes (Schmidt and others 1999).

In conclusion, *E coli* serogroup O103 isolates were prevalent in the orphan lambs examined in this small study. The levels of shedding from these young animals were very high, indicating increased potential for transmission. The intimin subtype of the O103 isolates examined was identical to a subtype that is both geographically widespread and associated with human disease. While none of the isolates studied here carried the genes encoding Shiga toxins, their relative abundance suggests that the risk of them acquiring *stx* genes by lateral transfer may be high.

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2.22a

Surveillance for Verocytotoxigenic E. coli (VTEC) in Farmed Animals in England and Wales 2005-2006

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INTRODUCTION

There is much information on VTEC O157 but data on non-O157 VTECs in England and Wales is lacking.

In 2003, an abattoir survey[a] in GB showed prevalence of VTEC O157 faecal carriage was 4.7 % in cattle, 0.7 % in sheep and 0.3 % in pigs.

Unlike VTEC O157, some non-O157 VTECs can cause animal disease[b] and like VTEC O157 are also potentially zoonotic.

In 2005, the Veterinary Laboratories Agency (VLA) revised its approach to selection and submission of *E. coli* isolates for surveillance testing of endemic disease cases from farmed livestock in England and Wales. Using standardised submission criteria, the aims were to:

- **Investigate association with specific disease syndromes**
- **Identify emerging potentially zoonotic strains**
- **Provide year on year comparisons to identify trends.**

MATERIALS AND METHODS

INSTRUCTIONS TO REGIONAL LABORATORIES:

Each Regional Laboratory (RL) is allocated a quarterly target number of *E. coli* isolates for Enhanced *E. coli* Surveillance Testing to provide representative coverage by species and geographical location. RLs submit *E. coli* isolates to VLA-Weybridge for Enhanced *E. coli* Surveillance Testing.

SELECTION OF ISOLATES:

- Isolates from endemic disease submissions to RLs
- Carcasses, viscera or faecal samples.
- Must be from outbreaks (at least two cases in individual animals)
- There must be no clear alternative diagnosis.
- Colonies selected must have biochemical and morphological characteristics typical of *E. coli*.
- Pure or basically pure growth on the primary culture plate.

CHARACTERISATION METHODS:

- Biochemical confirmation of *E. coli* by ONPG and indole
- Serological determination of O antigen type by microagglutination test
- Verocytotoxin (VT) , Cytotoxic Necrotising Factor (CNF) and Labile Toxin (LT) detection by Verocell assay[d]
- Multiplex-PCR to detect VTI, VT2 and eae genes
- Real-time, multiplex PCRs[e] to detect genes encoding Heat Stable toxin (Sta), Labile toxin (LT), K88 (F4), K99 (F5), 987p (F6), F17, F18 and F41.

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RESULTS

Table to show number of *E. coli* isolates submitted by submission category and species

Submission category	Cattle	Ruminants	Pigs
Attaching and effacing activity- detected by histopathology	7	11	0
Undiagnosed haemorrhagic diarrhoea- in mammals up to twelve months old	25	3	6
Other undiagnosed diarrhoea/enteritis outbreaks (pigs ≤ 3 months, other mammals ≤ 12 months)	101	11	20
Porcine bowel oedema	Not applicable	Not applicable	1
ß-haemolytic pig enteric isolate- untypable at RL	Not applicable	Not applicable	30
Porcine/ruminant K88 (F4), K99 (F5) or 987p (F6) Fimbrexc positive, or partly typed at RL	27	3	29
Colisepticaemia	36	9	8
Other categories at discretion of submitting RL	52	12	10

401 *E. coli* isolates were tested between May 2005 and May 2006

- 21 of the 401 isolates (5.2%) were VTEC
- Only one isolate was VTEC O157
- VTEC isolated only from cattle and pigs
- 14 (5.6%) of 248 cattle isolates examined were VTEC
- The predominant VTEC serotype isolated from cattle was O26 (6 isolates)
- 7 (6.7%) of 104 porcine isolates examined were VTEC
- The predominant VTEC serotype isolated from pigs was O2 (4 isolates)

VTECs isolated from cattle:

	VT1	VT2	eae	Reason for submission
O26	+	-	+	Attaching and effacing activity (3 isolates)
O26	+	-	+	Other undiagnosed diarrhoea (3 isolates)
O157	-	+	+	Other undiagnosed diarrhoea
O145	+	+	+	Other undiagnosed diarrhoea
O145	+	-	+	Other undiagnosed diarrhoea
O118	+	-	+	Attaching and effacing activity
O181	+	+	-	Undiagnosed haemorrhagic diarrhoea
O113	-	+	-	Undiagnosed haemorrhagic diarrhoea
Untypable	+	+	-	Colisepticaemia
O177	+	-	+	Other categories

VTECs isolated from pigs:

	VT1	VT2	eae	Reason for submission
O2	-	+	-	ß-haemolytic, untypable at RL (4 isolates)
O139, F18	-	+	-	Porcine bowel oedema
O139, F18	-	+	-	Partly typed at RL
O138, F18, Sta	-	+	-	Partly typed at RL

15 (3.7%) of the 401 isolates were VT negative but eae positive:

- Seven from cattle- O26 (2 isolates), O3, O64, O123, O145, O153
- Three from pigs- Untypable (2 isolates), O108
- Three from sheep- O26, O153, O158
- One from goat- O103
- One from alpaca- O15

DISCUSSION AND CONCLUSIONS

- This poster presents preliminary findings
- Limited results so far but looks promising
- Prevalence of VTEC O157 was 0.25% in diseased cattle, compared with 4.7% in cattle at slaughter from 2003 abattoir survey
- Non-O157 VTECs found in 5% of isolates from clinical cases.
- O26 was the most common VTEC isolated
- Diarrhoea and deaths in calves were associated with VTEC O26 and VTEC O145
- Findings demonstrate the presence of potentially zoonotic non-O157 VTEC in the animal population
- Non-O157 VTECs are the main cause of human illness elsewhere in Europe, however, non-O157 VTECs are infrequently reported as a cause of human illness in England and Wales

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2.22b

Surveillance for Verocytotoxigenic *E. coli* (VTEC) in Farmed Animals in England and Wales

Katherine Sprigings, Robert Davies, Felicity Clifton-Hadley, Ian McLaren, Charles Byrne, Louise Finch, Geoff Pritchard

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In 2005 the Veterinary Laboratories Agency (VLA) revised its approach to selection and submission of *E. coli* isolates from farmed animal endemic disease cases for surveillance testing, based on standardised submission criteria to permit investigation of association with specific disease syndromes, to act as a sentinel for emerging potentially zoonotic strains and to provide year on year comparisons to identify trends.

Between May 2005 and May 2006, 414 *E. coli* isolates were selected for OK serotyping and further characterisation by Verocell assay to detect verocytotoxin (VT), cytotoxic necrotising factor (CNF) and labile toxin (LT), and multiplex PCR to detect VT1, VT2 and eae genes. Of 414 isolates, 21 (5.1%) were VTEC. Of the 249 cattle isolates examined, 14 (5.6%) were VTEC (O26, O113, O118, O145, O157, O177, O181 and O untypable). O26 (6 isolates) was the predominant non-O157 VTEC isolated. VTEC were isolated from cattle presenting with 'undiagnosed-haemorrhagic diarrhoea' (O113, O181), 'other undiagnosed diarrhoea/enteritis outbreaks' (O26, O145, O157) and colisepticaemia (untypable). VTEC O26 and O118 were isolated from cattle with attaching-effacing activity detected by histopathological examinations on alimentary tract collected at necropsy. Of the 104 porcine isolates examined, 7 (6.7%) were VTEC (O2, O138 and O139). VT negative, eae positive O26 was isolated from cattle (one isolate) and sheep (one isolate). The ovine O26 isolate was positive by Verocell assay for CNF. Other VT negative, eae positive serotypes included O3, O64, O123 and O153 from cattle, and O108 and O untypable from pigs.

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Papers

Verocytotoxin-producing and attaching and effacing activity of *Escherichia coli* isolated from diseased farm livestock

J. P. Hutchinson, T. E. A. Cheney, R. P. Smith, K. Lynch, G. C. Pritchard

Between May 2005 and June 2008, strategically selected isolates of *Escherichia coli* obtained from clinical submissions to Veterinary Laboratories Agency (VLA) regional laboratories in England and Wales were serogrouped and examined by PCR for verocytotoxin (VT) production and attaching and effacing (*eae*) genes, both of which are zoonotic determinants. VT-encoding genes were detected in 54 (5.3 per cent) of the 1022 isolates examined. Only one isolate (0.1 per cent) was identified as verocytotoxigenic *E coli* (VTEC) O157. Non-O157 VTECs were present in 4.7 per cent of isolates from cattle, compared with 7.9 per cent in pigs, 2.3 per cent in sheep and 6.7 per cent in goats. The predominant serogroup identified in cattle was O26 and the predominant serogroup in pigs was O2. Attaching and effacing activity was attributed to 69 (6.8 per cent) of all isolates.

VEROCYTOTOXIGENIC *Escherichia coli* (VTEC) O157 is widely documented as causing a broad range of conditions in human beings, from asymptomatic infection or mild diarrhoea to severe haemorrhagic diarrhoea with complications such as haemolytic-uraemic syndrome (HUS) (Karmali and others 1983, Friedrich and others 2002, Tarr and others 2005).

The pathogenicity of VTECs relates mainly to the production of verocytotoxins (VTs) types 1 and 2, encoded by the bacteriophage vtx_1 and vtx_2 genes. VTs attach to globotriaosylceramide (Gb3) receptors on eukaryotic cells before being transported to the endoplasmic reticulum, where they inhibit protein synthesis. The resulting destruction of endothelial cells in the intestine causes diarrhoea, while in cases of HUS widespread thrombotic microangiopathy occurs due to toxins targeting kidney, brain, pancreas and lung cells (Jenkins and others 2008, Karmali and others 2010). Many VTEC strains also possess intimin, an outer membrane protein encoded by the chromosomal attaching and effacing (*eae*) gene, which facilitates the formation of attaching and effacing lesions in the intestine, increasing disease severity in both human beings and domestic animals (Gunning and others 2001, Jenkins and others 2003). Together, VTs and *eae* are the main virulence factors associated with zoonotic illness caused by *E coli*.

VTEC serogroups associated with human illness vary worldwide; whereas VTEC O157 predominates in the UK, other serogroups such as O26 are the main cause of VTEC-related illness in other countries (Willshaw and others 2001, Jenkins and others 2003, 2008, Blanco and others 2004, Brooks and others 2005). A study in the UK (Jenkins and others 2003) identified O26 and O145 as the serogroups most frequently isolated from patients with non-O157 VTEC-related disease. Of the strains isolated from patients with HUS, 82 per cent carried the vtx_2 gene, and intimin was detected more frequently in isolates from patients with HUS (50 per cent) than those with less severe disease (32 per cent).

VTEC O157 is not considered to be pathogenic in domestic animals and its occurrence in animals with enteric disease is considered to be incidental. It is, however, a relatively common gut commensal, particularly in cattle, and livestock can therefore act as reservoirs for human infection, with transmission either via consumption of contaminated food (Bonnet and others 1998, Khanna and others 2008) or through direct or indirect contact with animals or animal faeces (Milne and others 1999, Pritchard and others 2000). Other VTECs, such as O26, have been identified as animal pathogens (Pearson and others 1999, Gunning and others 2001, Milnes and others 2006). Most human cases of VTEC O26 infection appear to be foodborne or a result of direct person-to-person transmission, and there is little published evidence of human infection resulting from direct contact with infected animals (Hiruta and others 2001, Jenkins and others 2008).

Due to the difficulties and cost associated with the detection of VTECs in animals, investigation of the VTEC status of animals is normally undertaken only for surveys, or during investigations of human cases linked to animal contact (Pritchard and others 2000, 2001, Paiba and others 2003, Pearce and others 2006). Between May 2005 and June 2008, the Veterinary Laboratories Agency (VLA) operated a scheme to provide detailed information regarding the virulence profiles of *E coli* isolates associated with clinical disease in samples from animals submitted to regional laboratories for general disease diagnosis. The objectives were to obtain data on the occurrence of O157 and non-O157 VTECs, *eae* and other virulence factors. This paper describes the findings regarding identification of VTs and *eae*, the main virulence determinants associated with human illness.

Materials and methods

Submissions to VLA regional laboratories

Clinical material from cattle, sheep, pigs and goats submitted by private veterinary surgeons across the UK to VLA regional laboratories and surveillance centres in England and Wales between May 2005 and June 2008 formed the basis of the study. Postmortem examinations

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and other diagnostic tests were performed in accordance with standard operating procedures. Diagnoses and details of the clinical history provided by the submitting veterinary surgeon were recorded in accordance with Veterinary Investigation Diagnosis Analysis (VIDA) database case definitions (Gibbens and others 2008).

Case selection

Isolates associated with outbreaks of disease (defined as at least two cases in individual animals on the same premises) and where there was no clear alternative diagnosis to *E coli* infection were included in the study. Isolates were also required to originate from one of the following specific diagnostic scenarios: colisepticaemia, attaching and effacing activity as determined by histopathology, haemorrhagic diarrhoea in animals up to 12 months of age, non-haemorrhagic diarrhoea in pigs up to three months old or in other animals up to one month old, β-haemolytic colonies in pigs and/or OK-typed *E coli* isolates from pigs (Morris and others 1982) including cases of porcine bowel oedema. In order to achieve representative coverage by species and geographical location, each regional laboratory was allocated a quarterly target number of isolates from each species based on relative annual throughput in the previous year. Qualifying isolates from each regional laboratory were submitted for further testing on a weekly basis during the study period to permit efficient use of resources and avoid fluctuations in submission numbers. In cases where the quarterly target of a regional laboratory was exceeded, a veterinary decision was made on the most appropriate isolates to include in the study.

Culture and selection of isolates

Samples were initially plated on 5 per cent sheep blood agar and MacConkey's agar and incubated at 37°C for 48 hours. Suspect colonies identified as *E coli* by the indole spot test and cytochrome oxidase test using a standard operating procedure were subcultured on to sheep blood agar and MacConkey's agar for purity. Only samples meeting the case selection criteria and from which there was a pure growth of *E coli* on the primary culture plate were considered for the study. A single representative colony from the blood agar subculture was transferred to a Dorset egg slope and submitted to the *E coli* reference laboratory at VLA – Weybridge for serogrouping and examination for virulence determinants. Isolates were serogrouped by microagglutination and examined for the virulence factor genes vtx_1, vtx_2 and *eae* by multiplex PCR (Atkan and others 2004).

Data management

Data were recorded on two specific Microsoft Access databases, one with the results of the isolate testing and the other holding the submission information such as the age and species of animals and the region and size of the farm. The two datasets were combined using unique identifiers and tables and graphs were designed in Microsoft Excel to describe the data.

Results

Study population

E coli isolates recovered from 1022 (0.4 per cent) of the 16,000 carcases and 240,000 other samples received by VLA regional laboratories during the study period were examined by PCR as described above. These comprised 634 (62.0 per cent) isolates from cattle, 242 (23.7 per cent) from pigs, 131 (12.8 per cent) from sheep and 15 (1.5 per cent) from goats.

Material submitted

The majority of isolates (51.0 per cent) were recovered from faecal samples (Table 1).

Geographical distribution of samples tested

The geographical distribution of *E coli* isolates received from each species is shown in Table 2.

Presenting clinical signs

For all species, diarrhoea was noted as the major presenting clinical sign in cases from which *E coli* isolates were subjected to PCR testing, accounting for 642 (62.8 per cent) of all isolates tested. The presenting clinical signs associated with all isolates are shown in Table 3.

TABLE 1: Numbers of clinical samples of different types submitted from which *Escherichia coli* were isolated, broken down by species

Material	Cattle	Goats	Pigs	Sheep	Total (%)
Faeces	415	3	76	27	521 (51.0)
Small intestinal contents	52	4	81	30	167 (16.3)
Large intestinal contents	30	3	20	12	65 (6.4)
Intestinal mucosa	29	1	13	10	53 (5.2)
Liver	28	1	8	7	44 (4.3)
Other*	80	3	44	45	172 (16.8)
Total	634	15	242	131	1022

* Other tissues or body fluids sampled during postmortem examination

TABLE 2: Number of *Escherichia coli* isolates tested from each livestock species from each region of England, Scotland and Wales

Region	Cattle	Goats	Pigs	Sheep	Total (%)
East Midlands	42	2	16	5	65 (6.4)
East England	36	1	40	6	83 (8.1)
North-east	11	0	5	4	20 (2.0)
North-west	117	0	13	28	158 (15.4)
Scotland	3	0	0	1	4 (0.4)
South-east	24	2	9	6	41 (4.0)
South-west	196	0	51	16	263 (25.7)
Wales	102	0	7	36	145 (14.2)
West Midlands	57	0	13	20	90 (8.8)
Yorkshire and Humber	46	10	88	9	153 (15.0)
Total	634	15	242	131	1022

TABLE 3: Presenting clinical signs associated with *Escherichia coli* isolates from different livestock species

Presenting sign	Cattle	Goats	Pigs	Sheep	Total (%)
Diarrhoea	440	10	146	46	642 (62.8)
Found dead	59	1	31	29	120 (11.7)
Malaise	38	0	15	11	64 (6.3)
Other*	97	4	50	45	178 (19.2)
Total	634	15	242	131	1022

* Presenting signs included wasting, neurological disease, respiratory disease and dehydration

Seasonal distribution

Fig 1 shows the distribution of samples received by month. Cattle and sheep samples were more likely to be examined in the spring and samples from pigs were more likely to be examined in the autumn.

Age

Summary statistics for the age of animals from which material was collected are shown in Table 4. The data show that cattle and pig samples originated from relatively young animals compared with the samples from sheep and goats.

Size of herd

Mean and median herd and flock sizes from which samples originated are shown in Table 5.

VT and *eae* status of isolates

Of the 1022 *E coli* isolates, 27 (2.6 per cent) had *vtx* and *eae* genes, 27 (2.6 per cent) had *vtx* genes only and 42 (4.1 per cent) had *eae* genes only. Isolates testing positive for both *vtx* and *eae* genes came from cattle aged between two and 107 days old (mean 24.6, median 14, n=27); of these isolates, vtx_1 only was the most prevalent (81.5 per cent), followed by vtx_1+vtx_2 (11.1 per cent) and vtx_2 only (7.4 per cent). A breakdown of the isolates according to VT and *eae* status is shown in Table 6.

Serogroups of isolates testing positive for VT-encoding genes

Twenty different serogroups were identified from the 54 *vtx*-positive isolates. The most common VTEC serogroup was O26, which was identified only in cattle samples and accounted for 16 (2.5 per cent) of

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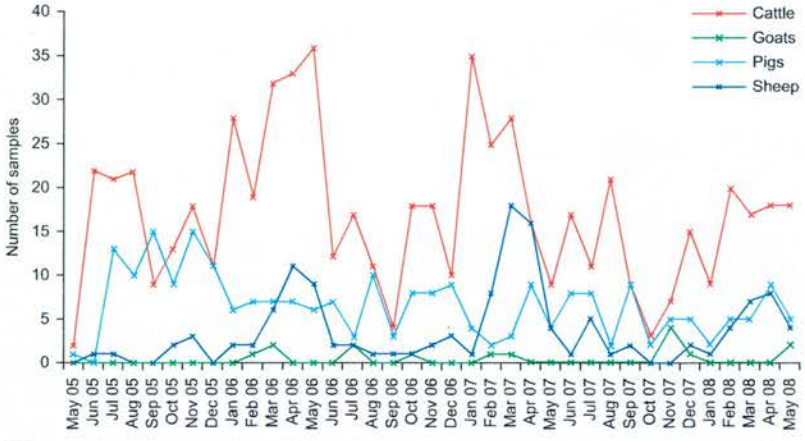

FIG 1: Number of samples from different livestock species received during each month of the study period for investigation for *Escherichia coli*

TABLE 4: Mean, median and range of ages (days) of animals from which samples were investigated for *Escherichia coli*

	Cattle	Goats	Pigs	Sheep
Minimum	1	3	1	1
Mean	92.6	194.3	53.3	147.1
Median	10	7	28	10
Maximum	3812	1460	1095	1825
Number of animals	605	15	223	120

TABLE 5: Mean, median and range of herd and flock sizes from which samples were submitted for investigation for *Escherichia coli*

	Cattle	Goats	Pigs	Sheep
Minimum	1	9	1	2
Mean	29.4	72.1	266.6	253.5
Median	18	11	100	107.5
Maximum	600	200	1800	1450
Number of isolates	439	7	139	88

TABLE 6: Verocytotoxin (VT) and attaching and effacing gene (*eae*) status of *Escherichia coli* isolates, broken down by animal species

Profile	Cattle (%)	Goats (%)	Pigs (%)	Sheep (%)	Total (%)
VT1+*eae*	22 (3.5)	0 (0)	0 (0)	0 (0)	22 (2.2)
VT2+*eae*	2 (0.3)	0 (0)	0 (0)	0 (0)	2 (0.2)
VT1+VT2+*eae*	3 (0.5)	0 (0)	0 (0)	0 (0)	3 (0.3)
VT1 only	0 (0)	1 (6.7)	0 (0)	2 (1.5)	3 (0.3)
VT2 only	2 (0.3)	0 (0)	19 (7.9)	0 (0)	21 (2.1)
VT1+VT2	2 (0.3)	0 (0)	0 (0)	1 (0.8)	3 (0.3)
eae only	24 (3.8)	1 (6.7)	10 (4.1)	7 (5.3)	42 (4.0)
None	579 (91.3)	13 (86.7)	213 (88.0)	121 (92.4)	926 (90.6)
Total	634	15	242	131	1022

all cattle isolates. Notably, only one (1.9 per cent) of the *vtx*-positive isolates was identified as serogroup O157. The serogroups of all *vtx*-positive isolates are shown in Table 7.

Isolates testing positive for both *vtx* and *eae* genes were identified in 27 (4.3 per cent) cattle samples. Of these, O26 was the predominant serogroup. The serogroups of all *vtx*- and *eae*-positive isolates from cattle are shown in Fig 2. No isolates with both virulence factors were identified from other species.

Discussion

This comprehensive survey involved over 1000 isolates of *E coli* selected from clinically diseased livestock cases submitted by veterinary surgeons to regional laboratories of the VLA over a three-year period. Isolates were selected for further testing by serogrouping and the presence of vtx_1, vtx_2 and *eae* genes by PCR using standardised selection criteria. The results provide a useful insight into the occurrence of *E coli* serogroups carrying zoonotic virulence factors in farmed livestock.

VT-producing capability was demonstrated by the detection of *vtx* genes in 5.3 per cent of all *E coli* isolates, representing 4.9 per cent of cattle isolates, 2.3 per cent of sheep isolates, 7.9 per cent of pig isolates and 6.7 per cent of goat isolates. A comparable study by Sherwood and others (1985) identified VTEC in nine (3 per cent) diarrhoeic calves. In another prevalence study involving healthy farm animals in England and Wales, Wray and others (1993) identified VTs in 2.8 per cent of cattle isolates, 6.1 per cent of sheep isolates and 4.7 per cent of isolates from pigs.

E coli isolates possessing both *vtx* and *eae* genes were indentified only in samples from cattle and accounted for 4.3 per cent of the submissions from cattle. The fact that six (0.9 per cent) of these isolates were recovered from liver tissue suggests possible bacteraemia involving VTEC in these cases. It is also noteworthy that *E coli* O26 with both VT-producing and attaching and effacing capability was recovered from 2.5 per cent of all cattle submissions, a finding that supports the view that this organism is common in cattle. Although there appears to be conflicting evidence with respect to the role of VTEC O26 as a cause of clinical disease in animals, it is interesting to note that diarrhoea was recorded as the main presenting sign in 13 (81 per cent) of the 16 cattle samples from which VTEC O26 was isolated (Pearson and others 1999, Gunning and others 2001, Pearce and others 2006, Jenkins and others 2008). This organism is also widely recognised as a cause of human illness, including HUS, and although human cases of VTEC O26 infection have been reported in the UK, the true prevalence may be underestimated as public health laboratories do not routinely screen samples for non-O157 VTEC (Jenkins and others 2008). Similarly, the *vtx*- and *eae*-positive strains of *E coli* O103 and O145 identified in 0.5 per cent of the cattle samples could be considered significant with respect to public health, as these serogroups have also been implicated as a cause of human illness (Pearce and others 2006, Karmali and others 2010).

As it is not considered to be a significant cause of disease in cattle, the single VTEC O157 isolate was likely to be a coincidental finding (Wray and others 2000). Although the prevalence of VTEC O157 in cattle samples was lower than that identified in previous studies, the discrepancy can be explained by differences in microbiological techniques, particularly the use of pre-enrichment concentration and semi-selective media to enhance recovery of the organism. Factors such as the stress of recent transport, concurrent illness and the site from which the organism was isolated may also have affected the isolation of VTEC O157, although it is interesting to note that, at 93 days, the mean age of cattle in this study was within the range of two to six months identified by Paiba and others (2003) as the peak age for VTEC O157 excretion in healthy cattle.

Among the other VTEC isolates identified in the present study, only serogroups O91 (recovered from one [0.8 per cent] sheep sample) and O118 (recovered from two [0.3 per cent] cattle samples) appear to be encountered relatively commonly as causes of human gastrointestinal disease (Bonnet and others 1998, Jenkins and others 2003, Karmali and others 2010). *E coli* O111, another VTEC serogroup commonly identified as causing human disease outside the UK (Jenkins and others 2003), was not recovered from any of the samples.

The majority (4.1 per cent) of porcine VTEC isolates belonged to serogroup O2. Serogroups O138, O139 and O141, which were identified as the most common VTEC isolates in porcine samples by Wray and others (1993), were detected in 3.3 per cent of samples from pigs. None of the porcine VTEC isolates identified in the present study belonged to serogroups commonly associated with human disease (Karmali and others 2010).

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TABLE 1: Serogroups of *Escherichia coli* isolates from different livestock species testing positive for *vtx* genes

Serogroup	Cattle	Goats	Pigs	Sheep	Total (%)
O2	0	0	10	0	10 (18.5)
O26	16	0	0	0	16 (29.6)
O38	0	0	0	1	1 (1.9)
O45	1	0	0	0	1 (1.9)
O65	0	1	0	0	1 (1.9)
O84	1	0	0	0	1 (1.9)
O91	0	0	0	1	1 (1.9)
O103	1	0	0	0	1 (1.9)
O113	1	0	0	0	1 (1.9)
O118	2	0	0	0	2 (3.7)
O138	0	0	2	0	2 (3.7)
O139	0	0	4	0	4 (7.4)
O141	0	0	2	0	2 (3.70
O143	1	0	0	0	1 (1.9)
O145	2	0	0	0	2 (3.7)
O157	1	0	0	0	1 (1.9)
O174	0	0	0	1	1 (1.9)
O177	3	0	0	0	3 (5.6)
O180	0	0	1	0	1 (1.9)
O181	1	0	0	0	1 (1.9)
UT:ND:ND*	1	0	0	0	1 (1.9)
Total	31	1	19	3	54

* Untypable isolate

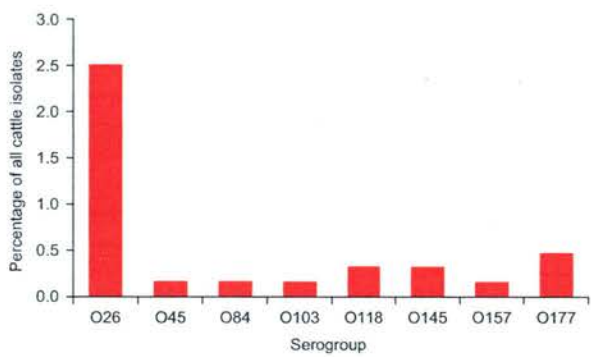

FIG 2: Serogroups of cattle isolates of *Escherichia coli* testing positive for both *vtx* and *eae* genes

Attaching and effacing capability, indicated by the presence of the *eae* gene, was demonstrated in 6.7 per cent of samples across all species. This is broadly comparable to the findings of a survey by Aktan and others (2004) in which 8.1 per cent of 1227 faecal *E coli* isolates taken from cattle, sheep and pigs at slaughter in England and Wales were identified as *eae*-positive.

Comparison of these data with the results of previous studies is complicated by the fact that this was not a randomised survey and it relied instead on opportunistic diagnostic material presented to VLA regional laboratories, most of which was from cattle.

The studied population was broadly representative of the livestock sector in England and Wales in that the regional distribution of samples appeared to correlate with UK livestock densities published by Defra (Defra 2009). The majority of cattle samples were received from north-west and south-west England, most of the pig samples came from eastern England, south-west England and Yorkshire, and submissions from Wales and western England accounted for most of the sheep samples. The mean cattle herd size of 29.4 in this study appears to correspond with the UK national averages of 71.1 and 25.3 for dairy and beef herds, respectively. Likewise, the mean sheep flock size of 253.5 was similar to the UK national average of 211.2 breeding ewes. However, possible bias towards smaller pig herds in this study is indicated by the fact that the mean herd size was 266.6, compared with the national UK average of 376.5 fattening pigs (Defra 2009).

Seasonal variation in sample submission may have had an effect on the recovery of VTEC, since the majority of cattle and sheep samples were received in the spring and samples from pigs were predominantly submitted in the autumn. In view of the fact that Paiba and others (2002) demonstrated higher rates of VTEC O157 carriage in healthy cattle and sheep in the summer months, the possibility that shedding of non-O157 VTEC in livestock may exhibit similar seasonal changes cannot be discounted. Likewise, the extent to which concurrent disease may have influenced recovery of VTEC remains uncertain.

As the rectoanal junction and faeces are generally considered to be the optimal sites for recovery of VTEC from ruminants (Davis and others 2006), it is possible that the other sampling sites used in the present study, such as liver and small intestine, may have had lower detection rates. Although *vtx*- and *eae*-positive samples were identified in all classes of clinical material received, the number of both samples and positive isolates was too low to investigate the possibility of bias.

Due to its public health significance, it is understandable that the majority of work examining VTEC prevalence in the UK livestock population has focused on VTEC O157, particularly in healthy cattle. Although it is widely acknowledged that O157 remains the most important VTEC serogroup with respect to human disease in the UK (Willshaw and others 2001), the results of the present study provide further evidence for the role of livestock, and particularly cattle, as potential reservoirs of non-O157 VTEC of public health significance. It is essential that both public health professionals and those involved in the livestock industry are aware of the risks posed by these organisms through direct and indirect contact with animals.

Acknowledgements

The data in this paper were compiled over a three-year period and the authors wish to thank the many colleagues within the VLA who were involved. The work was funded by Defra through project FZ2100.

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2.24

Virulotyping *Escherichia coli* of veterinary origin to assess the link between virulence profiles and pathotypes

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Introduction

Farm animals have been implicated as the main reservoirs of emerging *E.coli* infections. These infections are a result of transfer of mobile genetic elements harbouring *E.coli* virulence determinants. Rapid screening techniques that give high throughput results are needed to routinely monitor and identify any changes in virulence profiles of *E.coli*. Appropriate intervention strategies can then be timely implemented in the advent of threatening outbreaks.

The aim of this study was to virulotype about 200 isolates, submitted to the VLA Enteric Reference Laboratory between 2004-2007, to assess virulence patterns displayed in different *E.coli* strains.

A miniaturised microarray system, suitable for high throughput use, with 55 virulence and 16 control genes, was employed in this study. The 55 virulence genes on this chip can be classified as toxins, fimbrae, adhesins, siderophores and bacteriocins.

Method

•Strains were randomly selected from our database, growth from a single colony lysed, extracted DNA labelled with Biotin, hybridisation performed, and finally images scanned and analysed.

1

Array Preconditioning

2

Sample preparation with biotin labelling

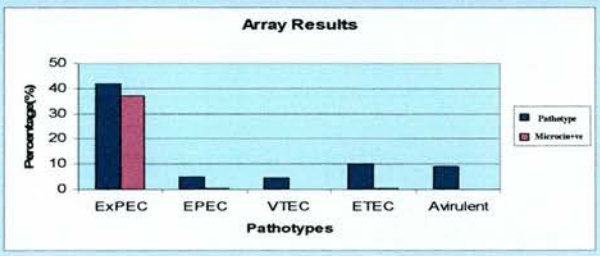
3

Hybridisation with signal detection

4

Result Presentation

Results



Array results from the 200 strains clustered into 6 groups according to virulence determinants. These were ExPEC(*prfB, sfaS, iron, ireA*), EPEC(*bfpA*, eae, *hlyA*), VTEC(*stx1A, stx2A, eae, hlyA)*, ETEC(*ltcA, stb, stA, fim41, K88, fedA, fedF, fanA, fasA*) and avirulent groups. 37% of the ExPEC strains showed presence of microcin genes

Species Gene	Cattle (%) n=98	Pig(%) n=39	Sheep(%) n=18
ExPEC	**45**	**39**	**36**
ireA_20	12	10	0
iroN_10	23	42	21
prfB_30	25	12	21
sfaS_10	0	13	5
f17-A_40	7	10	0
f17-G_20	9	0	5
cnf1_20	4	18	0
astA	10	5	15
VTEC	**7**	**3**	**0**
stx1A	3	0	0
stx2A	5	3	0
eae	4	0	0
espB	1	0	0
exhA	5	0	0
astA	3	0	0
fedF	0	3	0
ETEC	**9**	**28**	**0**
astA	0	18	0
fanA	7	0	0
fim41	7	0	0
K88	0	24	0
lthA	0	21	0
sta1A	7	5	0
fedA	1	5	0
fedF	1	5	0
stb	1	21	0
EPEC	**3**	**0**	**11**
eae	3	0	11
espB	2	0	11
exhA	3	0	5
negative	**9**	**5**	**11**

Conclusion

There was a 99.4% correlation between array results and phenotypic characterisations done at the VLA Enteric Reference Labaratory.

The miniaturised array system was able to sort out strains into different pathotypes of *E. coli*.

There was a confirmation of pathotype and species association.

This project was funded by Department of Food, Environment and Rural Affairs, GB

2.25 A

Pan-genome and Miniaturized Virulence and Antimicrobial Resistance Microarrays to Study the Pathogenicity of *Escherichia coli*

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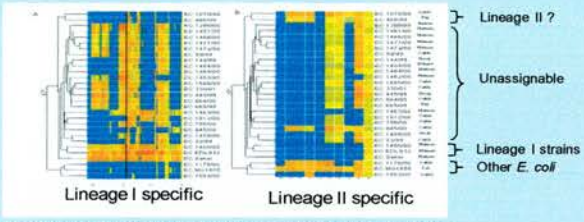
Veterinary Laboratories Agency

Introduction

Most *Escherichia coli* strains are harmless commensals that reside in the intestines of human and animals, but some can cause serious diseases, especially those that produce Vero toxins and other virulence factors. In order to gain a better understanding of these pathogenic *E. coli*, a pan-genome microarray based on the published sequences of *E. coli* was constructed and used to study the diversity among *E. coli* O157 strains. We have also constructed miniaturized microarrays suitable for routine diagnostic purposes to detect the presence of virulence determinates among *E. coli* isolates and antimicrobial resistance genes among gram negative bacteria.

pan-genome microarray

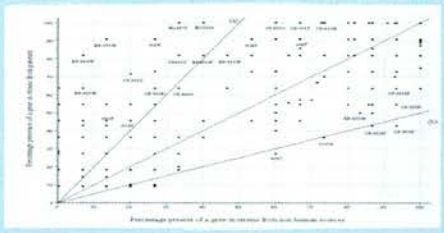
Distribution of lineage specific genes in strains studied



The presence of a gene in a strain is represented in warm colours (*e.g.* yellow and orange) and the absence of a gene is represented in cold colour (*i. e.* blue).

The figure shows that ***E. coli* O157 are much more diverse than previously thought.** Genes included in Fig. A and B are those identified as specific for lineage I and II according to Zhang *et al* (2007). Most *E. coli* O157 strains cannot be assigned to a lineage. See Wu, *et al.*, 2008

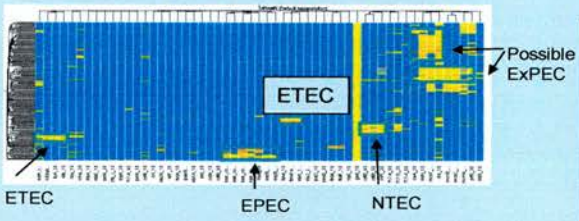
The association of genes with strains from human and non-human



Genes close to the 45-degree line were distributed equally among isolates from human and non-human sources. Line (a) and (b) are 2-fold lines drawn to help the visualization. **Some genes are more commonly found in human isolates than in animal isolates.**

Miniaturized microarray

Virulence typing of the *E. coli* strains



Miniaturised microarray classifies strains into different pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), verotoxigenic *E. coli* (VTEC), necrotoxigenic *E. coli* (NTEC), possible extra-intestinal pathogenic *E. coli* (ExPEC). The bacteriocin gene *cba* was more frequently found in isolates from cattle with haemorrhagic diarrhoea (p=0.04, non-parametric test, with Bonferroni multiple test correction).

Distribution of AMR genes within each pathotype (%)

Antimicrobial resistance genes	ExPEC	VTEC	ETEC	EPEC	Undesignated	Virulence undetected
0	13.6	87.5	5	25	22	28
1	15.1	0	20	37.5	33	22
2	19.2	0	15	12.5	22	11
>or =3	52.1	12.5	60	25	22	39

The miniaturized microarray is described in details by Anjum *et al.* (2007). A new version of virulence *E. coli* miniaturised array including 70 new VTEC genes is available.

Conclusions:

1. *E. coli* O157 were much more diverse than has been recognized previously and most of them do not belong to either of the two lineages of *E. coli* O157 reported by Zhang *et al.* Some genes were found more often in human than in animal isolates, which could be used as markers for detection of human pathogens.
2. *E. coli* strains were classified into different pathotypes according to the carriage of virulence genes using the miniaturised microarray.
3. Antibiotic resistance genes were common among those isolates. However, more antimicrobial resistance genes were identified in ETEC and ExPEC than VTEC strains.

This work was funded by the UK Department for Environment, Food and Rural Affairs (Defra) through the VLA's Seedcorn project SC145, the Endemic Diseases and Welfare programme and the non-statutory zoonoses project (FZ2100).

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2.25b

Pan-genome and miniaturized virulence and antimicrobial resistance arrays to study the pathogenicity of *Escherichia coli*

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Most *Escherichia coli* strains are harmless commensals that reside in the intestines of human and animals, but some can cause serious diseases in human and animals, especially those that produce Vero toxins and other virulence factors. In order to gain a better understanding of these pathogenic *E. coli*, a pan-genome microarray based on the published sequences of *E. coli* was constructed and used to study the diversity among the *E. coli* O157 and other Verotoxigenic *E. coli* (VTEC). We have also constructed miniaturized arrays for routine diagnostic purpose in order to detect the presence of virulence determinates among *E. coli* isolates and antimicrobial resistance genes among gram negative bacteria.

Our comparative genomic hybridization results showed that the gene contents of the *E. coli* O157 were much more diverse than has been recognized previously and most of them do not belong to the two lineages of *E. coli* O157 reported by Zhang *et al.* (1). Some genes were found more often in human than in animal isolates, which could be served as a marker for detection. The genetic differences between sorbitol fermenting *E. coli* and non-sorbitol fermenting *E. coli* have also been identified.

Miniaturized microarray was used to study the virulence and antimicrobial properties of 200 *E. coli* isolates from our reference collection. Strains were categorized into difference pathotypes: enterotoxigenic *E. coli* (ETEC), verotoxigenic *E. coli* (VTEC), necrotoxigenic *E. coli* (NTEC), and extra-intestinal pathogenic *E. coli* (ExPEC). Among cattle isolates, iron acquisition genes (*ireA* and *iroN*) were frequently associated with isolates from non-haemolytic diarrhoea and extra-intestinal infections, but were less common in isolates from haemorrhagic diarrhoea. The bacteriocin gene *cba* was found significantly more frequently ($p = 0.04$, non-parametric test, with Bonferroni multiple testing correction) in isolates from cattle with haemorrhagic diarrhoea. The distributions of antimicrobial resistance genes among difference pathotypes of *E. coli* were analyzed. More antimicrobial resistance genes were identified in ETEC than either ExPEC or VTEC.

1. Zhang, Y., C. Laing, M. Steele, K. Ziebell, R. Johnson, A. K. Benson, E. Taboada, and V. P. Gannon. 2007. Genome evolution in major Escherichia coli O157:H7 lineages. BMC Genomics 8:121.

2,26

Short Communications

Genes associated with *Escherichia coli* isolates from calves with diarrhoea and/or septicaemia

G. Wu, M. Mafura, B. Carter, K. Lynch, M. F. Anjum, M. J. Woodward, G. C. Pritchard

NEONATAL calf diseases have important economic impacts on the livestock industry worldwide. In the USA, for example, the National Animal Health Monitoring System has estimated the preweaning mortality of dairy calves to be 10.8 per cent, with diarrhoea accounting for 52.2 per cent of the losses (McGuirk and Ruegg 2008). *Escherichia coli* septicaemia, with or without diarrhoea, is also an important cause of death in young calves, associated with colostrum deprivation (Fey 1971) and other management and husbandry factors. A range of pathogenic microorganisms have been identified as aetiological agents of diarrhoea and septicaemia in calves; *E coli* has been recognised as an important cause of these diseases for over 100 years (Sojka 1965). Enteric colibacillosis ('white scours') is usually associated with enterotoxigenic *E coli* (ETEC) (Smith and Halls 1967), but can also occasionally be associated with verocytotoxigenic *E coli* (VTEC) (Chanter and others 1984, Milnes and others 2006). This short communication describes an investigation of the genetic characteristics of *E coli* isolates from calves with diarrhoea and/or septicaemia in the UK.

Diagnostic samples (carcases, blood, viscera or faeces) from diseased calves were submitted by private veterinary surgeons to the regional laboratories of the Veterinary Laboratories Agency (VLA) for a range of examinations, including tests for ETEC as appropriate. Under an additional enhanced screening programme, *E coli* isolates implicated in the presenting disease were forwarded to a central reference laboratory (VLA – Weybridge) for further analysis. During 2006 and 2007, a total of 260 *E coli* isolates associated with calf diarrhoea were serogrouped according to the established criteria (Sojka 1965). The common serogroups were O101 (24 per cent), O8 (12 per cent), O9 (7.7 per cent), O26 (5 per cent), O2 (3.5 per cent) and untypable (15.4 per cent); other serogroups were much less commonly encountered. During the same period, a total of 60 *E coli* isolates associated with calf septicaemia were also serogrouped; these were mainly O8 (10 per cent), O153 (6 per cent), O78 (5 per cent) and untypable (28 per cent), and other serogroups were less common.

TABLE 1: Association of selected genes with isolates associated with haemorrhagic diarrhoea, non-haemorrhagic diarrhoea and extraintestinal infections (Fisher's exact test)

	Odds of presence of marker for haemorrhagic versus non-haemorrhagic diarrhoea 95% CI (OR)				Odds of presence of marker for diarrhoea versus extraintestinal infections 95% CI (OR)			
Gene	OR	Lower	Upper	P	OR	Lower	Upper	P
gad	0	0.0	Inf	1.000	0.00	0.0	Inf	1.000
astA	1.86	0.3	13.4	0.477	0.86	0.2	4.4	1.000
cdtB	2.32	0.3	27.4		0.80	0.2	5.4	0.713
cnf1	2.32	0.3	27.4	0.428	0.56	0.1	3.0	0.458
f17A	0.40	0.0	8.2	0.583	0.51	0.1	6.6	0.600
f17G	1.97	0.4	10.8	0.498	0.89	0.2	3.9	1.000
prfB	0.38	0.1	1.9	0.185	0.97	0.2	4.9	1.000
cba	4.84	0.5	248.3	0.198	Inf	0.4	Inf	0.327
celb	1.71	0.1	107.1	1.000	1.45	0.1	76.0	1.000
cma	0.58	0.1	4.0	0.684	Inf	0.5	Inf	0.176
mchB	0.00	0.0	32.5	0.455	0.21	0.0	2.0	0.107
mchC	0.00	0.0	32.5	0.455	0.21	0.0	2.0	0.107
mchF	0.18	0.0	0.9	0.021	0.34	0.1	1.2	0.075
mcmA	0.38	0.1	1.9	0.185	0.62	0.2	2.5	0.530
ireA	3.70	0.3	196.6	0.356	0.80	0.2	5.4	0.713
iroN	0.12	0.0	0.6	0.004	0.59	0.2	2.2	0.381
iss	0.40	0.1	1.7	0.213	0.57	0.1	2.2	0.547

CI Confidence interval, Inf Infinity, OR Odds ratio

The virulence profiles of a representative cross-section of isolates from the two disease categories, excluding ETEC, were analysed using a miniaturised microarray containing 56 virulence-related genes, including those for fimbriae, adhesins, toxins, microcins and iron acquisition; the details of the genes and the experimental procedures have been described previously by Anjum and others (2007). It is well established that the presence of certain virulence genes defines the pathotype of an isolate (for example, *stx*=VTEC). For virulence typing by the array, only isolates with clear clinical histories were included: non-haemorrhagic diarrhoea (22 strains), haemorrhagic diarrhoea (24 strains) and extraintestinal infections (colisepticaemia, 17 strains) of calves, and representing the diversity of serogroups. In addition, three enteropathogenic *E coli* (EPEC), three VTEC and four ETEC were used as control strains.

The results were used to generate a hierarchical clustering demonstrating the distance between similar genes and isolates, shown in Fig 1 (Pearson's correlation with an average linkage algorithm). Two of the three VTEC and three EPEC strains clustered together, agreeing with the authors' expectation, since both groups are known to contain a similar adhesin (*eae*) gene, with the VTEC group also harbouring verocytotoxin genes. Another *eae*-negative VTEC was not within this cluster. The four ETEC strains harboured a different set of adhesin and toxin genes from those in the EPEC and VTEC groups, and were clustered together. Nine strains harboured genes for cytotoxic necrotising factor (*cnf1*) and are defined as necrotoxigenic *E coli* (NTEC) in Fig 1. This group of strains also contained genes for cytolethal distending toxin (*cdtB*), six of the nine strains also harboured *f17G* and two of those contained the fimbrial gene *f17A* as well. Among them, some strains also harboured microcin genes *cma, cba, mcmA*, the iron acquisition gene *iroN* or the P-related fimbriae gene *prfB*. This group was composed of strains isolated from both cases of diarrhoea and extraintestinal infections. Four strains harboured *f17G* with or without *f17A*. Another group contained genes for *pfrB*, iron acquisition (*iroN* and *ireA*) or microcin (*mch*). No virulence factors were detected in three strains. For many strains studied here (Fig 1) it was not possible to distinguish between isolates from diarrhoea and isolates from extraintestinal infections on the basis of carriage of any specific genes on the array.

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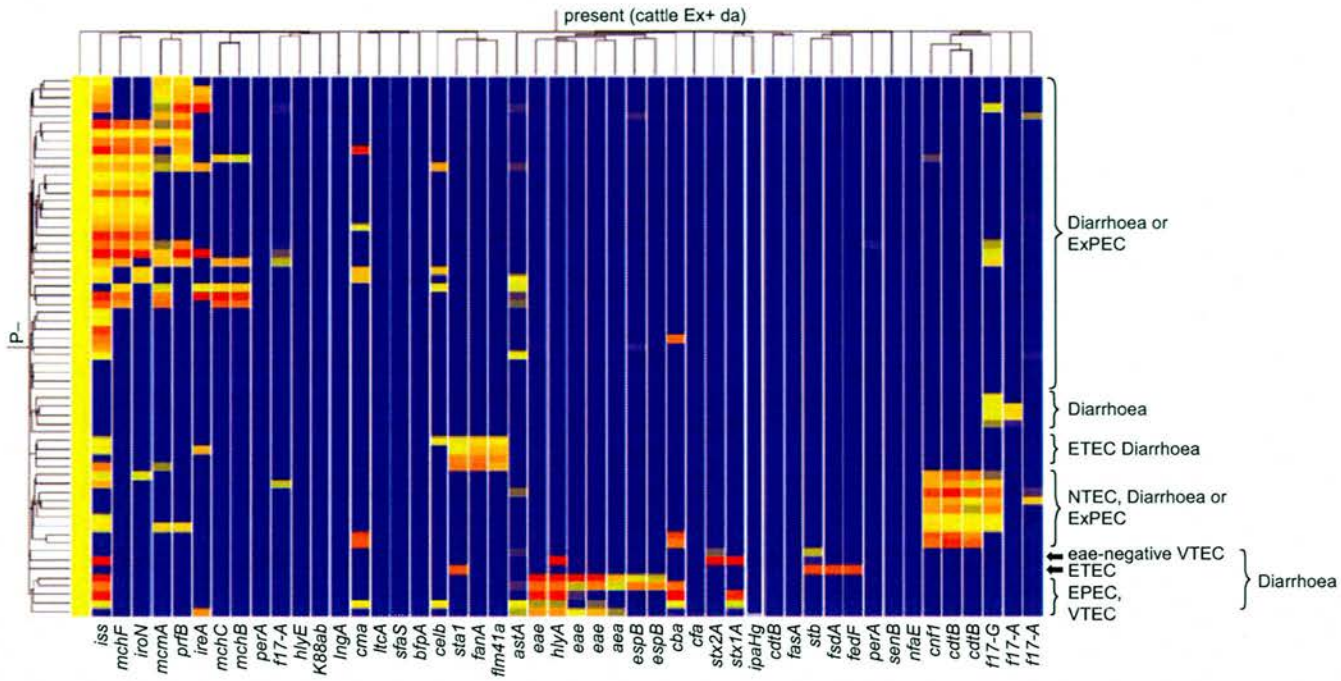

FIG 1: Virulence gene profiles of isolates from diseased calves from England and Wales. Both strain (dendrogram on the left) and genes (dendrogram on the top) were clustered with Pearson's correlation and the average linkage algorithm. Yellow represents a gene that is present and blue represents a gene that is absent. Multiple copies of genes are shown with brighter colours and genes with partial homology are shown in darker colours. Multiple probes were used for some genes, for example, *eae*. EPEC Enteropathogenic *Escherichia coli*, ETEC Enterotoxigenic *E coli*, ExPEC Extraintestinal pathogenic *E coli* that were isolated from extraintestinal locations, VTEC Verocytotoxigenic *E coli*

Additionally, the carriage of virulence genes varied within the same serogroup.

It is interesting to note the similarities between strains from extraintestinal infections and diarrhoea cases, which is consistent with an earlier report (Van Bost and others 2001). At the moment, gene probes that can differentiate them are not available, and further studies to characterise the virulence determinants of these isolates are needed in order to achieve better differentiation.

Fisher's exact test was used to test the association between genes detected in the strains and the associated diseases: haemorrhagic diarrhoea versus non-haemorrhagic diarrhoea (group 1), and diarrhoea versus extraintestinal infection (group 2). Examples of the results are presented in Table 1. The *iroN* and *mchF* genes were found to have a significant association with non-haemorrhagic diarrhoea ($P=0.0037$ and $P=0.021$, respectively). The *iroBCDEN* gene cluster has been described for extraintestinal strains, with *iroN* being orthologous to a catecholate siderophore receptor gene identified in *Salmonella* species (Baumler and others 1998, Russo and others 1999). *IroN* expression was shown to be regulated by the ferric uptake regulator (Fur) and increased by incubating the respective *E coli* strains in human urine, ascitic fluid, or blood (Russo and others 1999, 2002). Russo and others (2002) demonstrated that *iroN* enables the uptake of the catecholate siderophore enterobactin and contributed significantly to the virulence of *E coli* using a mouse infection model of ascending urinary tract infection. *mchF* forms part of a type I secretion apparatus together with *mchE* for the export of microcin *MccH47* (Poey and others 2006). The role of *iroN* and *mchF* in non-haemorrhagic diarrhoea has not been described and is worth further study. No significant difference in terms of virulence gene carriage was found among strains from diarrhoea and from extraintestinal infections.

In conclusion, isolates from diarrhoeic and septicaemic calves belonged to diverse serogroups and the carriage of virulence genes varied within the same serogroup, supporting the view that serogroup cannot be used to infer pathotype (Butler and Clarke 1994). NTEC strains and strains harbouring genes for microcins and iron acquisition or P-related fimbriae were found among *E coli* associated with both diarrhoeic and septicaemic disease. It is interesting to note the significantly biased distribution of the *iroN* and *mchF* genes among *E coli* associated with non-haemorrhagic diarrhoea. Further studies are required to elucidate the pathogenic mechanism of these organisms.

Acknowledgements

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Cryptosporidiosis and farm visits

SIR, – Since the mid 1980s there has been a steady increase in the number of exhibition farms open to the public and working farms hosting 'open days'. The latter events frequently take place over Easter during the lambing period and can attract large numbers of visitors. Cases of human cryptosporidiosis have been epidemiologically linked to these events. Young children have been particularly affected, often after bottle feeding or handling very young – frequently orphaned – lambs.

Cryptosporidium is an enteric protozoan parasite carried by many species of mammals, birds and reptiles. Subclinical infection is commonly encountered in young farm animals, particularly calves and lambs less than three or four weeks old. Infection can result in diarrhoea, usually in association with other enteric pathogens and particularly if colostrum deprived. However, even non-scouring animals can excrete oocysts and act as a source of infection.

Guidelines for the control of zoonoses among visitors to such farms have been prepared by the Department of Health, the Ministry of Agriculture, Fisheries and Food, and the Health and Safety Executive. The last has particular responsibility for open days at working farms, whereas exhibition farms come within the remit of the environmental health department of the local authority.

With the main lambing season approaching, veterinary surgeons with clients who are proposing to open their farms to the public can play a useful role by alerting them to the potential problems and suggesting they contact the Health and Safety Executive or local authority for advice. Veterinary surgeons can also help by inspecting stock before open days to ensure that the visiting public are not exposed to sick animals or to zoonotic diseases such as ringworm, orf and salmonellosis.

Visits to farms serve a very useful educational role but it is important that the risks of transmitting zoonotic disease are reduced to a minimum.

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Cryptosporidium parvum infection in orphan lambs on a farm open to the public

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A longitudinal survey was undertaken on an open farm to investigate the occurrence of *Cryptosporidium* species infection in orphan lambs obtained from three local flocks. During an initial pilot study, *Cryptosporidium* oocysts were detected by a fluorescent antibody test (FAT) in the faeces of two of 21 lambs aged between one and three weeks derived from one flock (flock A). Pooled pen samples of faeces were collected weekly from lambs derived from each flock; oocysts were detected by FAT in 24 (49·0 per cent) of 49 samples from lambs from flock A, 18 (30·5 per cent) of 59 samples from lambs from flock B and 14 (29·8 per cent) of 47 samples from lambs from flock C. Oocyst counts of 1 x 10^3 to more than 2 x 10^6 per gram of faeces were detected in lambs up to 12 weeks old, with the peak counts occurring at six weeks of age in the lambs from flocks A and B and at four weeks of age in those from flock C. The oocysts were confirmed by molecular analysis as *Cryptosporidium parvum*. Virtually all the infections were subclinical.

Cryptosporidium is an important protozoan parasite that is widespread in farmed livestock, particularly neonatal domestic ruminants; it causes diarrhoea, weight loss, dehydration and occasionally death. It is also the third most commonly reported non-viral cause of gastroenteritis in people in the UK (Health Protection Agency [HPA] 2007, Health Protection Scotland [HPS] 2007). Outbreaks have been linked to contaminated drinking water, recreational exposure to water (particularly swimming pools), children's nurseries, institutions, contaminated food, and farm visits. Secondary spread from person to person often occurs. In otherwise healthy people the infection may be asymptomatic or result in self-limiting diarrhoea, abdominal pain, nausea, vomiting, mild fever and weight loss, generally lasting between one and two weeks (Palmer and Biffin 1990). It can also cause severe, persistent or chronic life-threatening diarrhoea in immunocompromised individuals (Hunter and Nichols 2002).

Seventeen species and 30 genotypes have been identified, but *Cryptosporidium parvum* (formerly *C parvum* genotype 2) and *Cryptosporidium hominis* (formerly *C parvum* genotype 1) predominate in human cases in Britain. The incidence varies spatially and temporally (McLauchlin and others 2000, Anon 2002). *C parvum* has a broad mammalian host range, a very low infectious dose (Dupont and others 1995, Okhuysen and others 1999) and is widely associated with zoonotic transmission (Hunter and others 2004). *C hominis* is largely restricted to human beings although naturally occurring infections have been described in livestock, including cattle in Scotland (Smith and others 2005).

Human cases of cryptosporidiosis have been variously associated with seasonal farming events and practices, particularly lambing, bottle-feeding of lambs and attending open farms (Casemore 1989, Pritchard and Fleetwood 1995, Sayers and others 1996, Elwin and others 2001, Chalmers and others 2005). A serious outbreak of cryptosporidiosis with 120 confirmed human cases was linked to contact with orphan lambs at a wildlife centre in Perthshire, Scotland, in April 2005 (Baker 2005, W. J. Reilly, personal communication). The work described here evaluated the zoonotic hazard quantitatively by surveying the patterns of shedding of *Cryptosporidium* oocysts and the numbers of oocysts in samples of faeces from orphan lambs on an open farm in eastern England.

MATERIALS AND METHODS

Open farm

The survey was carried out between January and June 2005 on a well managed open farm in eastern England that has approximately 100,000 visitors annually, mainly in school parties or family groups. In addition to orphan lambs brought in for bottle-feeding by the public, there was also a wide range of animals of varying ages, including other sheep, donkeys, ponies, goats, pigs, cattle, rabbits, guinea pigs and assorted poultry. Confirmed human cases of *C parvum* infection had previously been linked to visits to this farm (Chalmers and others 2005) and various preventive measures had been adopted.

Eighty healthy Suffolk or Texel cross orphaned lambs or unwanted triplets up to two weeks old (mainly approximately one week old) were obtained from three local commercial crossbred flocks (A, B and C), which lambed consecutively, thereby providing a continuous supply. The lambs were identified by numbered plastic ear tags and arrived from each flock in batches of between approximately 10 and 30. Groups of up to six lambs of approximately the same age were kept together on straw bedding in one of five numbered pens in either of two separate modern purpose-built buildings constructed of wood and metal cladding, with smooth concrete floors. The buildings (houses 1 and 2) had been used by lambs and calves the previous year, but had been pressure-washed, steam-cleaned, disinfected thoroughly with Virkon-S (Antec) and left empty for approximately four months before they were used in January 2005.

For the first four to five weeks, the lambs were kept together in house 1 and bottle-fed exclusively by the farm staff with proprietary lamb milk powder (Volac International) made up with water from the general mains supply and fed at body temperature. The bottles were washed out with hot soapy water and sterilised with chlorine-based disinfectant (Milton). The lambs also received mains drinking water, proprietary creep pellets and hay. They were subsequently moved to house 2 (keeping the same pen groups) for bottle-feeding three times daily by the visiting public until they were eventually weaned at approximately four months of age. After the pens had been vacated, house 1 was cleaned out and pressure-washed (but not steam-cleaned) using an all-in, all-out approach, pending the arrival of lambs from the next flock.

A disinfectant footbath containing Virkon-S was kept outside house 1 for boot-dipping by the farm staff, and the contents were changed two or three times a week. No specific hygiene precautions were taken by personnel when moving between individual pens of clinically normal lambs from the same flock in the same building. However, all diarrhoeic lambs were moved promptly into a separate pen at one end of the house and handled last to reduce the risk of transmitting disease. The occupancy of the pens and signs of clinical disease (particularly diarrhoea and deaths) were recorded. There were good knee-operated hand washing facilities for

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TABLE 1: Relationships between the fluorescent antibody test (FAT) score of pooled pen samples of faeces from orphan lambs, the average number of oocysts per field of view (estimated from 10 fields at x 400) and the estimated numbers of oocysts/g of faeces

FAT score	Number of oocysts/field of view	Oocysts/g of faeces
0	0	$<1 \times 10^3$
1	<1	1×10^3 to 1×10^4
2	1	$>1 \times 10^4$ to $1{\cdot}5 \times 10^5$
3	2-5	$>1{\cdot}5 \times 10^5$ to 7×10^5
4	6-10	$>7 \times 10^5$ to 2×10^6
5	>10	$>2 \times 10^6$

use by the public located next to house 2, and numerous signs warning them about the need for hand washing after contact with the lambs. The farm staff also closely supervised children engaged in bottle-feeding and gave an introductory talk about hygiene.

Sample collection

Samples of freshly voided faeces were collected by farm staff from all the lambs with overt diarrhoea, using disposable gloves or wooden tongue depressor spatulas. In addition, during a preliminary pilot exercise carried out between January 13 and February 3, 2005, faecal samples of normal consistency were similarly collected opportunistically from individual lambs in house 1 while they were being fed or when their bedding was being renewed. This labour-intensive approach to sample collection was discontinued because it proved too impractical under commercial conditions, and it was replaced by collecting pooled samples of faeces from each pen.

The pooled samples were collected weekly in 150 ml plastic screw-top pots from each pen containing flock A lambs during the 12 weeks between January 13 and April 5, from flock B lambs for 15 weeks between February 21 and June 1, and from flock C lambs for 10 weeks between April 13 and June 14. The samples were collected in the morning after all the bedding and faeces had been removed from the pens the previous afternoon and replaced by clean straw. Most of the pens contained five or six lambs, but when new lambs were not available some pens in house 1 were left unoccupied and were therefore not sampled. Lambs from two different flocks (A and B or B and C) were sometimes present on the open farm at the same time, but they were always kept in separate buildings.

Preliminary laboratory examinations

All the faecal samples were mixed thoroughly with wooden spatulas, and homogenates were examined by a fluorescent antibody test (FAT) (Crypto-cel; Cellabs) that is considered to be much more sensitive than conventional staining methods (Casemore 1992, Arrowood 1997). The FAT results were recorded on a scale from negative (0) to strong positive (5) using a scoring system (Table 1) that has been adopted as a standard procedure within the Veterinary Laboratories Agency (VLA) after comprehensive in-house validation, including oocyst counting and repeatability studies (J. A. Marshall, unpublished data). The FAT score for each pooled faecal sample was recorded together with the date, the age of the lambs, the pen number and the flock of origin, and the average scores by week of age were calculated for the lambs from each source flock.

In addition to the FAT examinations, samples of faeces from diarrhoeic lambs were examined for common causes of diarrhoea using the standard procedures adopted within the VLA for the diagnosis of clinical enteric disease in lambs. These included standard bacteriological cultures and the use of phenol auramine-stained smears to detect *Cryptosporidium* oocysts; modified Ziehl-Neelsen-stained smears are also widely used within the VLA to detect *Cryptosporidium* oocysts, but they were not used for this study.

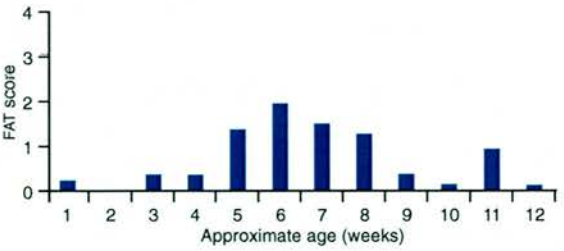

FIG 1: Relationship between the average fluorescent antibody test (FAT) score of pooled pen samples of faeces for *Cryptosporidium* oocysts and the age of the lambs from flock A

Molecular examinations

The species of *Cryptosporidium* in the FAT-positive faecal samples were identified by molecular examination. DNA was extracted either from the oocysts after they had been separated from faecal matter by salt flotation or from FAT slide material. The floated oocysts were disrupted by boiling (Elwin and others 2001), and those separated from slides were disrupted by three rapid cycles of freezing with dry ice in methanol and thawing at 100°C (Robinson 2005). The DNA was then extracted by using a DNA mini kit (Qiagen) and stored at 4°C until use. The DNA was used as a template for a nested PCR-restriction fragment length polymorphism (RFLP) analysis developed on the 18S rRNA gene (Xiao and others 2000), which detects and assigns all currently known *Cryptosporidium* species, and visualised on a 2 per cent agarose gel stained with 10 µg/ml ethidium bromide. Representative positive isolates were selected for confirmation of the RFLP results by DNA sequence analysis. The amplified PCR product from the 18S rRNA gene was sequenced on both the sense and antisense strands using an ABI sequencer (Lark Technologies). The consensus sequences of both the sense and antisense strands were analysed using Lasergene software and BLASTN searches on the NCBI databases.

RESULTS

Individual lambs

Four of the 80 lambs died during the study; none of them had a history of diarrhoea and they were not examined post-mortem. Three lambs from flock A had overt diarrhoea; one of them had clinical cryptosporidiosis, as confirmed by phenol auramine-stained faecal smears and a FAT score of 4. This lamb was in the first batch from farm A and became diarrhoeic soon after it arrived; it was put into the isolation pen. No *Cryptosporidium* oocysts were detected in the faeces from the other two diarrhoeic lambs; one had an enterotoxigenic *Escherichia coli* K99 (F5) infection, but no diagnosis was reached with the other. *Cryptosporidium* oocysts were detected by FAT in two of the 21 lambs aged between one and three weeks sampled individually during the pilot study; they had FAT scores of 1 and 4.

Pooled pen samples

Cryptosporidium oocysts giving a FAT score of at least 1 were detected in 24 (49·0 per cent) of the 49 pooled pen samples collected weekly from flock A lambs, in 18 (30·5 per cent) of the 59 weekly pooled pen samples from flock B lambs, and in 14 (29·8 per cent) of the 47 weekly pooled pen samples from flock C lambs. *Cryptosporidium* was identified in lambs up to 12 weeks of age in flock A, up to 11 weeks in flock B and up to eight weeks in flock C. The maximum FAT score of 5 was recorded in one or more pooled pen samples from each of the three flocks during the survey. The peak period of oocyst shedding (with average FAT scores of between 2 and 3)

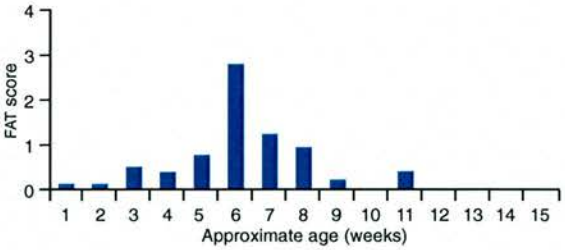

FIG 2: Relationship between the average fluorescent antibody test (FAT) score of pooled pen samples of faeces for *Cryptosporidium* oocysts and the age of the lambs from flock B

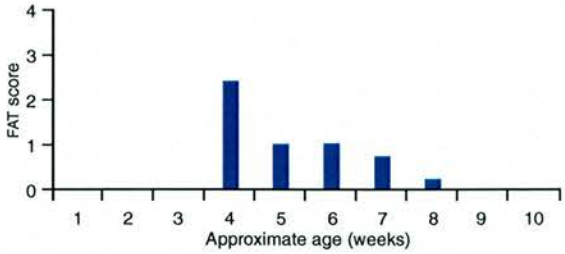

FIG 3: Relationship between the average fluorescent antibody test (FAT) score of pooled pen samples of faeces for *Cryptosporidium* oocysts and the age of the lambs from flock C

occurred at approximately six weeks of age in the lambs from flocks A and B (Figs 1, 2) and at approximately four weeks of age in the lambs from flock C (Fig 3).

Molecular analysis

Sixteen pooled pen samples of faeces and three samples using DNA extracted from the FAT slides were examined by PCR-RFLP. Amplified product identified as *C parvum* was detected in 14 of the faecal samples and two of the slide samples. In the other three samples either no amplified product was detected or there was insufficient for the RFLP analysis. Sequence analysis of the product from three samples showed 100 per cent homology with the *C parvum* 18S rRNA gene sequences deposited in the NCBI DNA database.

DISCUSSION

It has been suggested that bottle-fed lambs are more prone to cryptosporidiosis than naturally reared lambs, either because of a lack of colostrum intake or the stress of artificial rearing (Fayer and Ungar 1986, Casemore 1989). However, despite the extensive *Cryptosporidium* infection of these lambs, the incidence of clinical disease was extremely low. Unrelated research work at VLA – Weybridge has indicated that experimentally infected neonatal lambs are seldom diarrhoeic, even when they are excreting over 10^5 oocysts/g faeces for a long period (M. Giles, personal communication). Sturdee and others (2003) also found little evidence of diarrhoea in infected lambs in a six-year field study on a mixed-species lowland farm in England. In the present study, the lambs appeared to thrive on bottle-feeding and the mortality rate of 5 per cent was well within acceptable standards for conventionally reared lambs.

The prevalence of faecal shedding of *C parvum* oocysts (9·5 per cent in the individual lambs and 30 to 50 per cent in the pooled pen samples) was broadly comparable to that described elsewhere; for example, Ortega-Mora and others (1999), Majewska and others (2000) and Sturdee and others (2003) recorded prevalences of 71, 10·1 and 12·9 per cent, respectively, and Ryan and others (2005) detected oocysts in 2·6 per cent of samples of lamb faeces tested by microscopy, compared with 26 per cent by PCR. The lambs may have acquired the infection from their dams during the periparturient increase in oocyst excretion (Ortega-Mora and others 1999) or after their arrival on the open farm. The lambs from flock A, which included the only case of clinical cryptosporidiosis, may have infected later batches of lambs through the movement of personnel and/or other fomites. However, adopting stringent biosecurity measures to prevent or reduce the transmission of oocysts between adjacent pens of apparently normal lambs in the same building would not have been feasible.

The oocysts were identified as *C parvum*, which is consistent with animal-associated cryptosporidiosis incidents and outbreaks investigated elsewhere in Britain (Elwin and others 2001, Chalmers and others 2005). However, in Australia, Ryan and others (2005) found one *C hominis* isolate but no evidence of *C parvum* in 60 sequenced *Cryptosporidium* isolates from sheep in an abattoir study, thereby raising doubts about the importance of sheep as a zoonotic reservoir. However, farming conditions in Australia are different from those in Britain, and, moreover, those authors did not examine samples from unweaned lambs. Chalmers and others (2002) observed that sheep sampled during a waterborne outbreak of human cryptosporidiosis in Britain were shedding a distinct genotype, now known to be homologous with the cervine genotype, which demonstrated the relevance of species identification.

Although clearly not ideal, the pooled pen samples of faeces provided a practical alternative to sampling individual animals and a measure of the potential environmental contamination. Using the sensitive FAT scoring system, very high counts of *Cryptosporidium* oocysts were recorded in clinically normal orphan lambs. Some counts per gram of faeces exceeded 2×10^6, compared with only 20 to 440/g in asymptomatic sheep sampled by Ortega-Mora and others (1999). The counts were very high in relation to the median infective dose of 132 oocysts (Okhuysen and others 1999) and an ID50 of between nine and 1042 oocysts (Dupont and others 1995) for human disease, which further emphasises the potential zoonotic hazard presented by *Cryptosporidium* via faecal and environmental exposure, particularly in view of the stability of the oocysts (Caccio and others 2005). Oocysts were shed by lambs aged between one and 12 weeks, with a peak between four to six weeks. This period coincided with when the public were allowed to bottle-feed lambs on this farm. Much younger lambs (from one to two weeks old) are sometimes bottle-fed by visitors on open farms elsewhere, but in the present study the lambs of this age had lower oocyst counts than the slightly older lambs. However, their faeces were generally more liquid in consistency and more likely to cause contamination, a risk that may outweigh any potential advantage, in terms of a lower zoonotic risk, in the use of neonatal lambs for bottle-feeding by the public. Very young lambs are also physically less able to withstand handling by small children.

The results show that *Cryptosporidium* in clinically normal orphan lambs can be a significant zoonotic hazard, supporting observations from investigations of human outbreaks. The data also provide quantitative information on the extent of the shedding of oocysts by orphan lambs on a commercial open farm and the period of potentially increased risk to the visiting public. In recent years there have been considerable increases in the numbers of open farms and farm open days (Pritchard and others 2000); these fulfil an important educational role and are an integral part of the leisure and tourism industries. Farm operatives and the visiting public should be aware of the potential zoonotic risks from *Cryptosporidium* and other pathogens, such as verocytotoxigenic *E coli* (VTEC) O157 (Pritchard and others 2000), and manage them accordingly. However, despite an estimated 60,000 visitors to this

farm during the period of the survey, no cases of human cryptosporidiosis were detected by an enhanced surveillance programme operated by the East of England Health Protection Unit (R. Eyles, personal communication). This was probably because of increased awareness, high standards of supervision and good compliance with the hygiene guidelines provided by the Health and Safety Executive (2002).

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2.29

Sub clinical *Cryptosporidium parvum* infection in lambs: an underestimated zoonotic hazard?

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Introduction

Cryptosporidiosis caused by *Cryptosporidium parvum* infection is an important cause of diarrhoeic disease in young ruminants
Cryptosporidiosis is also a common cause of human gastroenteritis
Human infection is predominantly either *C. hominis* (derived from human sources) or *C. parvum* (human or animal sources)
Man is usually infected from exposure to contaminated water (particularly drinking water and swimming pools)
Contact with farm animals or their environment is also an important source of human infection

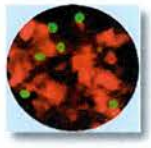
- Human outbreaks of cryptosporidiosis have been linked to contact with lambs on farms, particularly during lambing and bottle-feeding
- Infection is known to occur in clinically normal lambs but this aspect has not been extensively evaluated in relation to the zoonotic hazard
- The introduction of more sensitive laboratory methods for detecting oocysts has facilitated studies of infection in clinically normal lambs
- Two investigations into sub clinical infection in young lambs using completely different sample populations are presented here

Fluorescent antibody test scoring system

- A commercially available fluorescent antibody test (FAT) (CryptoCel-TCS Biosciences Ltd) was used to detect faecal oocysts in lambs
- This method is much more sensitive than conventional MZN stained smears[1]
- A method was developed to link FAT scores (graded 0 -5) to oocyst counts[1]

FAT scores and oocyst counts in animal faecal samples

Fat score	Oocysts per field of view (x40)	Oocysts/gm of feaces
0	None seen	$<1 \times 10^3$
1	<1	1×10^3 to 1×10^4
2	1	$>1 \times 10^4$ to 1.5×10^5
3	2-5	$>1.5 \times 10^5$ to 7×10^5
4	6-10	$>7 \times 10^5$ to 2×10^6
5	>10	$>2 \times 10^6$



The fluorescent antibody test showing *Cryptosporidium* spp oocysts in faeces (x 40)

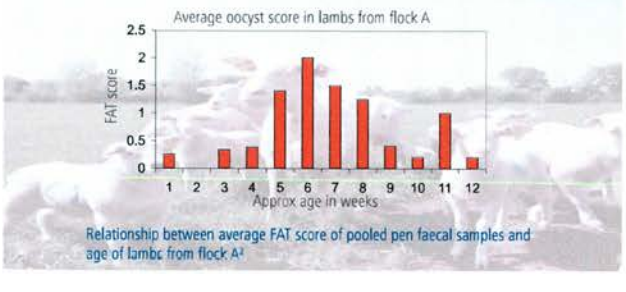
Investigation 1: Lambs on a farm open to the public

Materials and methods

- Longitudinal sampling survey on an open farm[2]
- 80 bottle-fed orphan lambs from three local flocks (A, B, C)
- Faecal samples collected from lambs up to three months old
- Samples were scored for oocyst shedding using the FAT

Findings

- Two (9.5%) of 21 individual lambs had positive FAT scores
- Positive FAT scores (to 5) were found in weekly pooled samples:
  24 (49. 0%) of 49 from flock A
  18 (30. 5%) of 59 from flock B
  14 (29. 8%) of 47 from flock C
- Peak oocyst excretion occurred at 4-6 weeks depending on source
- Shedding continued at least until 12 weeks old
- Oocysts were confirmed as *C. parvum* using nested PCR-RFLP at the 18srRNA locus and DNA sequence analysis[2]
- Lambs were healthy (only three deaths occurred overall)
- Clinical cryptosporidiosis was diagnosed in only one lamb



Relationship between average FAT score of pooled pen faecal samples and age of lambs from flock A[2]

Investigation 2: Lambs submitted for diagnostic necropsies

Materials and methods

- Sub clinical infection with *Cryptosporidium* spp. was explored in lambs submitted to VLA regional laboratories for general disease diagnosis
- 266 lambs < 3 months were scored for oocyst shedding by FAT
- Faecal consistency was graded as pelleted, pasty or diarrhoeic
- Multivariate analysis was used to examine the relationship between FAT score, age, faecal consistency and eventual disease diagnosis

Findings

- *C. parvum* was confirmed by FAT, PCR and DNA sequencing in 43 (16.2%) of the 266 lambs
- Other *Cryptosporidium* spp. were identified in a further five samples
- Strong association between FAT scores and confirmed diagnoses (13) of cryptosporidiosis ($p<0.001$)
- Maximum FAT scores (5) were seen in 12 of the 13 lambs with cryptosporidiosis
- A diagnosis other than cryptosporidiosis (eg coccidiosis or clostridial disease) was reached in 30 (70%) of the 43 FAT positive lambs
- Overall, sub clinical *C. parvum* infection was detected in 30 (11.9%) of the 253 lambs diagnosed with a disease other than cryptosporidiosis
- There was no association of the above with any particular condition
- No significant association between FAT scores and faecal consistency
- Positive FAT scores were significantly associated with lambs aged four weeks or less ($p<0.001$) with peak prevalence at 3-4 weeks

General Conclusions

- The FAT scoring system readily quantified sub clinical infection in lambs
- Both investigations revealed extensive *C. parvum* faecal shedding
- Positive FAT scores indicative of sub clinical infection were detected in:
  9.5% of individual lambs,
  30 - 50% of pooled pen samples
  11.9% of lambs without clinical cryptosporidiosis
- Peak shedding was at three to six weeks
- Some lambs had an FAT score of 5 (>2 million oocysts/g)
- **Even FAT scores of 1 can cause human illness[3,4]**

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Permission was given by parents to photograph the children feeding lambs in investigation 1.

Cryptosporidium species in lambs submitted for diagnostic postmortem examination in England and Wales

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CRYPTOSPORIDIOSIS is a common enteric disease in human beings and a wide range of animal species. *Cryptosporidium parvum* is the main species responsible for zoonotic infection in Britain. In a previous study, Pritchard and others (2007) evaluated the zoonotic hazard associated with subclinical infection with *C parvum* in orphan lambs on a farm open to the public. This short communication describes a follow-up study to evaluate the extent of subclinical infection with *Cryptosporidium* species in lambs submitted to the Veterinary Laboratories Agency (VLA) for diagnostic postmortem examination.

Between January and July 2006, faecal samples were collected from the rectum or colon of lambs aged three months or younger that were submitted to regional laboratories of the VLA by private veterinary surgeons for general diagnostic postmortem examination. All lambs submitted were included in the survey, regardless of the presenting clinical signs or reason for submission. Data were collected on the location of the receiving laboratory, the age of the lamb, the consistency of rectal faeces (graded as pellets, pasty or diarrhoeic) and the disease diagnosis, based on the examination protocols and diagnostic criteria adopted by the VLA and as recorded in its VIDA database (Hall and others 1980). *Cryptosporidium* oocysts were visualised and counted using a fluorescent antibody test (FAT) and scored on a scale from 0 ($<1 \times 10^3$ oocysts per gram of faeces [opg]) to 5 ($>2 \times 10^6$ opg) as described by Pritchard and others (2007). The species were identified by PCR-restriction fragment length polymorphism at the 18S RNA locus (Xiao and others 2000) and the HSP70 locus (Morgan and others 2001), and by bidirectional DNA sequence analysis of PCR products.

Multivariable analysis, using a main effects logistic regression model, examined the relationship between location of the regional laboratory, FAT score, age, faecal consistency and VIDA disease diagnosis. All variables in the logistic regression were categorised with the dependent variable FAT diagnosis (negative/positive). Age was grouped as zero to two, two to four, four to six, six to eight and more than eight weeks, thus avoiding the need to fit a function. A likelihood ratio test was used to examine the significance of the effects in the logistic regression. Only univariable associations were examined with FAT scores. The non-parametric Kruskall-Wallis test examined the association of the FAT scores with age category, and the linear-by-linear association test evaluated the trend with faecal consistency.

Positive FAT scores were recorded in 48 (18·0 per cent) of the 266 samples examined in this survey. Samples from postmortem examinations carried out at VLA – Aberystwyth (which serves mid-Wales) recorded the highest proportion (29 per cent) of FAT-positive samples, although multivariable analysis showed no statistical association with the geographical location of the regional laboratory that received the carcase. *C parvum* was confirmed in 43 of the 48 FAT-positive samples. These ranged from 14 (32·6 per cent) with a FAT score of 1 (1×10^3 to 1×10^4 opg) to 22 (51·2 per cent) with the highest score of 5 ($>2 \times 10^6$ opg). Clinical cryptosporidiosis was diagnosed in 13 (30 per cent) of the 43 *C parvum*-positive cases (of which 12 had a FAT score of 5); there was a strong association between clinical cryptosporidiosis and FAT score ($P<0·001$). Notably, a diagnosis other than cryptosporidiosis (mainly coccidiosis or clostridial disease) was reached in the remaining 30 (70 per cent) of the FAT-positive lambs, with no associations with any particular diagnosis. These diagnoses were based on the well established case definitions, which utilise clinical, pathological and other laboratory findings, adopted by the VLA for the VIDA database (Hall and others 1980). These results indicated that subclinical infection with *C parvum* was present in 11·3 per cent of the 266 lambs surveyed, compared with the 4·8 per cent that had a confirmed diagnosis of clinical cryptosporidiosis. There was no significant association between FAT score and faecal consistency. However, scores were significantly ($P<0·001$) associated with lambs aged four weeks or less; the peak prevalence of *C parvum* infection was at three to four weeks of age.

Cryptosporidium species other than *C parvum* (with FAT scores between 1 and 2) were confirmed in the faeces of five of the 48 FAT-positive lambs (1·9 per cent of all 266 lambs surveyed). These lambs were all diagnosed with conditions other than cryptosporidiosis by the VIDA case definitions. *Cryptosporidium hominis*, which is an important cause of cryptosporidiosis in human beings, was detected in a 10-day-old diarrhoeic lamb from a flock in Wales, in which a diagnosis of lamb dysentery was reached. This case has been described by Giles and others (2008). Four lambs, two from flocks in North Yorkshire and one each from Shropshire and Cornwall had *Cryptosporidium bovis* infection; one of these lambs was also infected with *C parvum*. The identification of *C parvum* in this lamb was initially made using sequence analysis of the HSP70 locus and was confirmed using species-specific real-time PCR (Tanriverdi and others 2003). Three of the lambs with *C bovis* infection (age range: five to eight weeks) were diarrhoeic but the cause was attributed to coccidiosis; the fourth lamb (aged three months) had normal faeces and was diagnosed with *Mannheimia haemolytica* pneumonia. *C bovis* does not appear to be zoonotic and has previously been reported in sheep by Ryan and others (2005) in Australia, by Santin and others (2007) in North America and in Britain by Mueller-Doblies and others (2008). In cattle, *C bovis* is much less pathogenic than *C parvum* (Starkey and others 2006), and there was no evidence from the present study that *C bovis* infection in sheep was anything other than an incidental finding.

Although this was not a formally structured random epidemiological survey of the national flock, the lambs submitted to the regional laboratories came from across the whole of England and Wales; over 65 per cent came from the major sheep-rearing areas of Wales, the Welsh Borders, Yorkshire and Cumbria. The results support the findings from the study in orphan lambs (Pritchard and others 2007) by confirming the extent of subclinical infection with *C parvum* in lambs, although the peak prevalence (up to four weeks of age) was slightly earlier than seen in the study of orphan lambs (four to six weeks). There was a strong correlation between FAT scores and a diagnosis of clinical cryptosporidiosis, although this test also demonstrated how much subclinical infection is present at lower oocyst concentrations (corresponding to FAT scores <3), which are very difficult to detect with conventional modified Ziehl-Neelsen-stained smears (Arrowood 1997) but nevertheless present a significant zoonotic hazard (Dupont and others 1985, Okhuysen and others 1999). The survey also confirmed that *C parvum* is by far the major species of *Cryptosporidium* found in lambs in Britain and that the situation differs from that described in older (slaughter age) lambs in Australia by Ryan and others (2005). In the present study, the prevalence of subclinical infection (11·3 per cent) was almost three times that of clinical cryptosporidiosis,

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which further emphasises the zoonotic hazard from non-diarrhoeic lambs. The survey also demonstrated the presence of other *Cryptosporidium* species (*C bovis* and *C hominis*) in approximately 2 per cent of the lambs surveyed.

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Short Communications

2.31

Cryptosporidium species infection in pigs in East Anglia

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CRYPTOSPORIDIOSIS is a common enteric disease in human beings and a wide range of animal species; *Cryptosporidium parvum* is the main species responsible for zoonotic infection in the UK (Xiao and others 2004). In ruminants, *C parvum* is widely acknowledged as a common endemic infection, which is often associated with clinical disease (de Graaf and others 1999) and is a source of zoonotic infection for human beings (Xiao and others 2004). However, the role of *C parvum* in causing disease in pigs, and its zoonotic potential in pigs, is far less clear. VIDA surveillance data (Hall and others 1980) collected by the Veterinary Laboratories Agency (VLA) from diagnostic samples submitted by veterinary surgeons in England and Wales between 2005 and 2007 show an average of only one case of cryptosporidiosis per year in pigs, compared with approximately 560 cases in calves and 50 in lambs.

Previous studies have demonstrated a widely varying prevalence of infection of between 0.1 per cent and 33 per cent (Quilez and others 1996, Izumiyama and others 2001, Epe and others 2004); weaner and grower pigs are most commonly infected (Guselle and others 2003). Most infections are asymptomatic or cause only mild, non-specific colitis (Higgins 1999), but the clinical signs can be much more severe if there is concomitant infection with other enteropathogens, such as rotavirus (Enemark and others 2003). Genotypic studies in pigs have identified *Cryptosporidium parvum* and *Cryptosporidium suis* (formerly *C parvum* pig genotype 1) (Morgan and others 1999), and more recently *Cryptosporidium* pig genotype II has emerged (Ryan and others 2003). Experimental infection with *Cryptosporidium hominis,* an important human pathogen, has also been demonstrated in gnotobiotic pigs (Widmer and others 2000, Ebeid and others 2003, Giles 2006).

Pritchard and others (2008) surveyed *Cryptosporidium* infection in lambs in England and Wales, using the fluorescent antibody test (FAT) on samples from routine diagnostic submissions to VLA regional laboratories in England and Wales. This short communication reports the findings of similar investigations into the occurrence of *Cryptosporidium* infection in pigs, using samples submitted from herds in East Anglia, one of the main pig-producing areas of Great Britain.

Between January and December 2007, faecal samples from pigs submitted by private veterinary surgeons to VLA – Bury St Edmunds for general disease diagnosis and from Zoonoses Action Plan (ZAP) *Salmonella* investigations (Twomey and others 2007) were examined using the FAT, and the faecal oocyst count was scored on a scale from 0 (<1 x 10^3 opg) to 5 (2 x 10^6 opg) as described by Pritchard and others (2007). Where several pigs were submitted from the same farm for diagnostic investigation, a maximum of three was examined. The use of samples from ZAP investigations provided an opportunity to include more fattening and adult pigs in the study. Most ZAP visits involved the collection of at least 30 samples, but only six were selected at random for use from each herd for the present study. Data were collected on the age of the pigs, the consistency of rectal faeces (graded as normal, soft or diarrhoeic), whether the pigs were housed or outdoors, and the final disease diagnosis, based on the examination protocols and diagnostic criteria adopted by the VLA and recorded in the VIDA database (Hall and others 1980).

Thirty-nine FAT-positive samples, from pigs with a broad variation in age, faecal consistency and housing, were speciated and genotyped using PCR-restriction fragment length polymorphism analysis and confirmatory sequencing at the 18S rRNA locus (Xiao and others 2000). Multivariable analysis using a main effects logistic regression model was used to examine the relationship between the FAT score (dichotomised as 0 and >0) and the pigs' age class, faecal consistency, type of housing, the source of the sample and geographical location. Likelihood ratio tests were used to assess the significance of individual factor variables. The associations of genotype with age class and type of housing were tested with the Kruskal-Wallis test and Fisher's exact test, respectively.

Positive FAT scores were recorded in 119 (38.6 per cent) of the 308 samples examined. Of the 119 positive samples, 103 had a FAT score of 1 (1 x 10^3 to 1 x 10^4 opg) but only four had the highest score of 4 (>7 x 10^5 to 1 x 10^6 opg). The 308 samples that were tested came from a total of 72 farms, 50 of which yielded at least one positive sample, giving a farm prevalence of 69.5 per cent. A wide range of VIDA clinical diagnoses was recorded from infected pigs, including *Pasteurella multocida* pneumonia (10 cases), enteric colibacillosis (eight cases), *Salmonella* Typhimurium infection (seven cases) and porcine reproductive and respiratory syndrome virus infection (seven cases), but clinical cryptosporidiosis was not recorded.

Age was the only variable significantly associated with the detection of *Cryptosporidium* (P=0.038); the highest proportion of positive samples originated from growers (Table 1). A smoothed plot of *Cryptosporidium* prevalence suggested that the prevalence increased until pigs were 10 weeks of age, after which it declined gradually.

There was no significant association between FAT status and either faecal consistency or clinical diagnosis; the FAT scores did not differ significantly between outdoor and indoor-housed pigs, or between samples sourced from submissions for disease diagnosis or from ZAP visits.

Cryptosporidium pig genotype II was detected in 25 (64.1 per cent) of the 39 isolates that were sequenced. *C parvum* was identified in eight (20.5 per cent) of the genotyped isolates, and *C suis* was identified in six (15.4 per cent) isolates. There was no significant association between genotype and the pigs' age or housing system.

The findings of this study indicated a higher proportion of infection (38.6 per cent) with *Cryptosporidium* among pigs in East Anglia, compared with the prevalence in lambs (18.0 per cent) observed in similar studies (Pritchard and others 2008). However, the pigs had generally lower oocyst counts, and in view of the complete absence of clinical cryptosporidiosis (based on the strict diagnostic criteria used by VLA), the infections appeared to be entirely subclinical and incidental. The increasing risk of infection until 10 weeks of age is in broad agreement with the published literature, and this study also showed a high prevalence of infection among adult pigs. The zoonotic association between infection with *C parvum* in animals and illness in human beings is well accepted and a study of human cases of cryptosporidiosis with reported associations with farms in England and Wales matched the subtypes from human beings with those in pigs (Smith and others 2010). Pigs excreting *C parvum* constitute a potential zoonotic risk. *C suis* is of minor zoonotic importance and infection in immunocompetent people has been reported on only two occasions (Xiao and others 2002, Leoni and others 2006). *Cryptosporidium* pig genotype II, which was most com-

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TABLE 1: Occurrence of *Cryptosporidium* species in 307 pigs of different age groups in East Anglia*

	Positive	Negative	Odds ratio†	95% CI	P
Age					0.038
Sucklor (0-3 weeks)	4	16	1.00		
Weaner (4-7 weeks)	14	22	2.37	0.64-8.73	0.196
Grower (8-13 weeks)	45	48	3.74	1.13-12.4	0.031
Finisher (14-24 weeks)	44	75	2.00	0.58-6.86	0.269
Adult (>24 weeks)	12	27	1.34	0.35-5.22	0.670

*308 samples were examined; one observation was removed from analysis due to incomplete age data
†Adjusted odds ratios from logistic regression
CI Confidence interval

monly identified in the present study, is generally considered to be of little public health significance (Xiao and others 2004). On the basis of the findings of this small-scale study, the authors conclude that infection with *Cryptosporidium* species in pigs is of little clinical importance. Unlike in sheep (Pritchard and others 2008), *C parvum* – the main zoonotic species – was not the main species present in the pigs, and generally the faecal oocyst counts were relatively low ($<1 \times 10^4$ opg), which suggests that pigs present a lower zoonotic hazard for *Cryptosporidium* than ruminant species. This fact is particularly important in view of the large numbers of pigs being kept outdoors in East Anglia and elsewhere in Great Britain, and the potential for environmental contamination.

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Short Communications

2.32

Cryptosporidium species in calves submitted for postmortem examination in England and Wales

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CRYPTOSPORIDIOSIS is a common enteric disease in human beings and a wide range of animal species. In ruminants, *Cryptosporidium parvum* is widely acknowledged as a common endemic pathogen, which is often associated with clinical disease in young animals (de Graaf and others 1999) and is a source of zoonotic infection for human beings (Xiao and others 2004). Previous studies have used the fluorescent antibody test (FAT) on samples from routine diagnostic submissions to the Veterinary Laboratories Agency (VLA) to survey *Cryptosporidium* species infection in lambs (Pritchard and others 2008) and pigs (Featherstone and others 2010). This short communication describes a similar study to evaluate the extent of infection with *Cryptosporidium* species in calves.

Between January and December 2008, faecal samples were collected from the rectum of calves aged three months or younger that were submitted to the regional laboratories of the VLA by private veterinary surgeons for general diagnostic postmortem examination. All calves submitted were eligible for the survey, regardless of the presenting clinical sign(s). If more than one calf was received from the same farm, only one calf was included in this survey. Data were collected on the location of the receiving laboratory, the age, production purpose and disease presentation of the calf, and the consistency of the rectal faeces (graded as normal, soft or diarrhoeic), in addition to details of farm management. The final disease diagnosis was also recorded using the examination protocols and diagnostic criteria adopted by the VLA and as recorded in its VIDA database (Hall and others 1980).

Cryptosporidium species oocysts were visualised and counted using the FAT and scored on a scale from 0 (<1 x 10^5 opg) to 5 (>2 x 10^6 opg) as described by Pritchard and others (2007).

Representative isolates from calves with a broad variation in age, faecal consistency, production purpose, housing and FAT score were speciated and genotyped using PCR-restriction fragment length polymorphism at the 18S rRNA locus, followed by confirmatory sequencing (Xiao and others 2000).

All statistical analyses were conducted using Stata/IC 10.0 for Windows (Stata Statistical Software Release 10.0; StataCorp). Univariate analysis examined the potential association of each variable with FAT status (with the FAT score dichotomised as 0 and >0) using a chi-squared test, Fisher's exact test or the Kruskal-Wallis test for the equality of ranks where there were ordinal groups within a factor. This analysis was used to help assess variables for inclusion into a multivariate model to estimate association of FAT status with various risk factors. A logistic regression model with FAT status as the response variable was then fitted using a stepwise method, with variables significant at $P<0.05$ retained in the final model.

Positive FAT scores were recorded in 103 (45 per cent) of the 229 samples examined. Of the 103 positive samples, 34 (33 per cent) had a FAT score of 1 (1 x 10^5 to 1 x 10^4 opg) and 43 (42 per cent) had the highest FAT score of 5 (>2 x 10^6 opg). Clinical cryptosporidiosis was diagnosed in 46 (45 per cent) of the 103 FAT-positive calves, of which 32 (70 per cent) had a FAT score of 5. A diagnosis other than cryptosporidiosis was reached in the other 57 (55 per cent) FAT-positive calves, indicating that subclinical infection with *Cryptosporidium* species was present in 25 per cent of the 229 calves surveyed. Subclinical infection was significantly associated with lower FAT scores than the FAT scores of calves with clinical cryptosporidiosis ($P<0.001$). There was no association between the presence of subclinical infection and any particular clinical disease diagnosis (mainly navel ill, colisepticaemia or pneumonia). A LoWess smoothed plot of prevalence showed that the calves' peak oocyst excretion occurred between 10 and 20 days of age, after which it declined gradually.

C parvum was detected in 49 (91 per cent) of the 54 isolates that were sequenced. *Cryptosporidium bovis*, which is not considered to be zoonotic and is less pathogenic than *C parvum* in cattle (Starkey and others 2006), was identified in one eight-week-old calf, which was diagnosed with pneumonia and had a FAT score of 1. The four remaining isolates were untypable; all had low FAT scores of 1 or 2, and only one was from a calf diagnosed with clinical cryptosporidiosis, which had concurrent pneumonia.

Table 1 shows the final multivariable model with adjusted odds ratios (OR) and 95 per cent confidence intervals for factors associated with the detection of *Cryptosporidium* species oocysts in faeces. Of the 229 calves included in the survey, suckler calves (n=80) were significantly less likely to test positive for *Cryptosporidium* oocysts (OR 0.37) than calves from dairy (n=129), calf rearer (n=13) and beef finisher (n=7) units. *Cryptosporidium* species was more likely to be detected in calves that had diarrhoea (OR 2.88) than in calves whose faeces was described as normal or soft. The risk of finding *Cryptosporidium* species in faeces also varied significantly according to the age of the

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TABLE 1: Multivariate analysis of variables associated with the detection of *Cryptosporidium* species oocysts in faecal samples from 229 calves

	Odds ratio	95% confidence interval	P
Production method			
Non-suckler	1		
Suckler	0.37	0.19-0.72	0.001
Faecal consistency			
Normal or soft	1		
Diarrhoeic	2.88	1.54-5.40	0.003
Age of calf (days)			
0-7	1		
8-14	4.05	1.78-9.17	0.001
15-28	2.34	1.01-5.40	0.047
29-56	0.78	0.30-2.02	0.605
57-84	0.15	0.03-0.72	0.018

Hosmer-Lemeshow goodness of fit test P=0.6219

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calf, with calves between one and three weeks of age at greater risk. There was no statistical association in the model between FAT status and geographical location, month of submission, water source, housing, group penning or concurrent disease.

Cryptosporidiosis is an intestinal infection, and therefore the association of diarrhoeic faeces with oocyst shedding was not unexpected. However, the model also identified that suckler calves were at a lower risk of cryptosporidiosis compared with other types of calves, particularly dairy calves. This effect may be due to different management factors on dairy and calf rearing units, such as higher stocking density of animals of similar age in non-suckler units and possibly different breed susceptibilities to *Cryptosporidium* species infection. The non-suckler calves in this survey were also almost exclusively housed. Although sufficient colostrum intake has been shown to have a significant effect on the prevention of disease associated with viral and bacterial enteropathogens, Harp and Goff (1998) have suggested that adequate colostrum intake does not appear to confer significant protection against cryptosporidiosis in calves, probably due to the intracellular life cycle of *Cryptosporidium*, which means it is largely protected from the effects of antibodies. Operating calf rearing systems with high levels of hygiene that minimise exposure to *Cryptosporidium* species oocysts and reduce the level of environmental challenge has been recommended by many researchers as the best approach to controlling cryptosporidiosis (Harp and Goff 1998, Scott and others 2004, Trotz-Williams and others 2007). In the present model, the age of the calf was also shown to be an important determining factor for the risk of testing positive for *Cryptosporidium* species oocysts, with calves older than two months of age having the lowest risk. This age related information is of particular use when assessing the zoonotic risk posed by young calves.

Although this was not a formally structured random epidemiological survey covering all herds in the UK, the calves submitted to the VLA regional laboratories came from across the whole of England and Wales in approximate proportion to the local cattle densities. The survey confirmed that the zoonotic genotype *C parvum* was by far the major species of *Cryptosporidium* in calves of this age range in England and Wales; this concurs with the findings of a similar study in Northern Ireland, which surveyed calves up to one month of age (Thompson and others 2007). The present study indicated a higher proportion of infection (45 per cent) with *Cryptosporidium* species in young calves compared with the prevalence in pigs (39 per cent) and lambs (18 per cent) observed in similar studies (Pritchard and others 2008, Featherstone and others 2010). In the present study, the prevalence of subclinical infection (25 per cent) was greater than that of clinical cryptosporidiosis, which further emphasises the zoonotic hazard from non-diarrhoeic young calves.

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2.33

SHORT COMMUNICATIONS

Cryptosporidium hominis in a goat and a sheep in the UK

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IN THE UK, human diarrhoeal disease associated with the protozoan parasite *Cryptosporidium* is caused mainly by *Cryptosporidium parvum* and *Cryptosporidium hominis*, which together account for 96 per cent of cases, in approximately equal proportions (Nichols and others 2006). *C parvum* is considered to be zoonotically acquired, while there is strong epidemiological evidence that *C hominis* is primarily spread by person-to-person transmission (Hunter and others 2004).

There are few reports of naturally occurring *C hominis* infections in animals; until 2005, the infection had been reported in a rhesus monkey (Xiao and others 1999), a dugong (Morgan and others 2000) and Canada geese (Zhou and others 2004). Reports of *C hominis* in shellfish described low numbers of oocysts, with no evidence of other stages of the life cycle or active infection (Gomez-Couso and others 2004, Graczyk and others 2001). Since 2005, *C hominis* has been reported twice in farmed animals: a sheep in Australia (Ryan and others 2005) and two cattle in the UK (Smith and others 2005). Although experimental infections have shown that *C hominis* is not infectious for mice, rats, cats or dogs (Morgan-Ryan and others 2002), other studies have shown that it can successfully infect, colonise and be excreted by lambs (Giles and others 2001, Ebeid and others 2003), piglets (Widmer and others 2000, Akiyoshi and others 2002, Giles 2006), calves (Akiyoshi and others 2002, Tanriverdi and others 2003, Guk and others 2004, Giles 2006) and gerbils (Baishanbo and others 2005).

During epidemiological studies into possible animal sources of sporadic cases of human cryptosporidiosis in south-west England, one pygmy goat (*Capra hicus*) was found to be shedding moderate numbers (5×10^5 oocysts per gram of faeces [opg]) of *Cryptosporidium* oocysts by modified Ziehl-Nielsen microscopy. The species was determined as *C hominis* using a nested PCR-restriction fragment length polymorphism (RFLP) assay targeting the small subunit (SSU) rRNA gene (Xiao and others 2000) and the multiplex allele-specific PCR assay targeting the *Dhfr* locus (Giles and others 2002). This was confirmed independently by PCR-RFLP of the *COWP* locus (Spano and others 1997) and PCR-RFLP and sequencing of an 830 base pair (bp) fragment of SSU rRNA gene (Xiao and others 2001). The pygmy goat was from a petting farm that kept a variety of other animal species, including goats, sheep and cattle. PCR analysis of faecal samples from a proportion of animals on the farm showed that *C parvum* was also present. The petting farm had been sampled for *Cryptosporidium* species after a 10-month-old child was diagnosed with *C hominis* infection having visited the farm in the two weeks before the onset of symptoms.

C hominis was also identified in the caecal contents of a diarrhoeic 10-day-old male Texel lamb that was investigated as part of a different survey to examine the prevalence of *Cryptosporidium* species in lambs. The animal was one of three lambs from a flock of 100 in Wales that were found dead after being turned out on to pasture shortly after birth. *Cryptosporidium* oocysts ($1{\cdot}5 \times 10^5$ opg) were detected by immunofluorescence microscopy.

The oocysts were characterised by PCR-RFLP and sequencing of 830 bp SSU rRNA gene (Xiao and others 2001), a species-species real-time PCR at the *Lib13* locus (Tanriverdi and others 2003) and by DNA sequence analysis of 448 bp of the *HSP70* gene (Morgan and others 2001) and 830 bp of the *GP60* gene (Alves and others 2003). The oocysts were found to be *C hominis* subtype IbA10G2. This is the most common *C hominis* subtype found in cases of human cryptosporidiosis in the UK (Chalmers and others 2008). *C hominis* was thought to be an incidental finding, and the cause of death was attributed to lamb dysentery.

The sequences for the two *C hominis* isolates were deposited in GenBank under accession numbers EU186152 to EU186156.

The number of *C hominis* oocysts shed by the goat and the lamb described in the present study is comparable to that found in cattle ($<2 \times 10^4$ oocysts per ml of faeces) reported by Smith and others (2005). These are lower counts than the count of at least 2×10^6 reported by Pritchard and others (2007) in a study of naturally occurring *C parvum* infections in healthy orphan lambs. However, it is unknown at which stage of infection the goat and the lamb described here were sampled, and the numbers of *C hominis* may have been in decline. Experimental transmission of *C hominis* in lambs, calves and pigs has demonstrated that although counts in excess of 5×10^6 opg have been detected, shedding is always for a much shorter duration than with *C parvum* infections (Giles 2006). The concentrations detected are well above the median infective dose (ID50) (10 oocysts) for human *C hominis* infection (Chappell and others 2006), indicating that infection of animals are a potential source of *C hominis* infection for human beings. Molecular tools offering greater discrimination are required to fully characterise *C hominis* isolates, as there is evidence that not all isolates are capable of causing infection in all animals (Giles 2006).

Tanriverdi and others (2003) suggested that a subpopulation of *C hominis* may persist within *C parvum* infections and may be undetected by PCR protocols in which DNA templates compete for primers. Those authors also suggested that animals may play an important role in the maintenance of *C hominis*. This is corroborated by experimental evidence that, following the coinoculation of water or faeces with counted suspensions of *C hominis* and *C parvum* oocysts, both species are not always detected by PCR-based methods (Giles and others 2002, Reed and others 2002). Akiyoshi and others (2003) demonstrated in piglets that rapid displacement of *C hominis* infection occurred after the subsequent inoculation of *C parvum*, providing evidence for possible subpopulations of *C hominis* within *C parvum* infections.

The epidemiological evidence suggests that people may become infected with *C hominis* from other people (Hunter and others 2004). The detection of naturally occurring *C hominis* infections in farmed animals requires further investigation to fully understand all the potential sources of *C hominis* for human beings. The maintenance of infection within and between herds, the minimum infectious dose in susceptible animals, and the likelihood of transmission to human beings all need to be evaluated.

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These findings extend further the host range in which C *hominis* has been naturally reported. To the best of the authors' knowledge, this is the first documented evidence of C *hominis* in sheep in the UK and the first evidence of C *hominis* in goats worldwide.

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HPA Zoonoses Network Newsletter

Health Protection Agency

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Seasonal update: infections linked to open farms

Visits to open farms are highly popular with families and schools at this time of the year. Farm open days may be arranged independently or as part of the nationwide Open Farm Sunday initiative www.farmsunday.org. Unless appropriate hygiene precautions are taken there may be a risk of gastrointestinal infection; two such incidents are highlighted here.

1. Cryptosporidium

A large outbreak of cryptosporidiosis (at least 155 suspected cases, including 41 confirmed) was linked to a visit by 382 children from 8 local primary schools to a dairy farm in Carlisle at the end of April. The children had visited various work stations on the farm. They came into direct contact with a cow (which they milked), four calves (some had previously had diarrhoea), a nanny goat and four baby kids, and they walked through a cow cubicle house.

The children were provided with alcoholic hand gel (which is not effective against *Cryptosporidium*) before receiving refreshments in the farm office.

Under the direction of the Outbreak Control Team, a joint farm visit was carried out by an Environmental Health Technician and a Veterinary Investigation Officer from the Veterinary Laboratories Agency. *Cryptosporidium* oocysts (up to 2 million/g) were found by immunofluorescent microscopy in 3 of 7 faecal samples from the calves and their environment. The goats had been brought in from

another farm for the open day, and had since returned. This farm was also visited by VLA and goats were sampled. Up to 750,000 oocysts/g were detected in 7 of 20 samples.

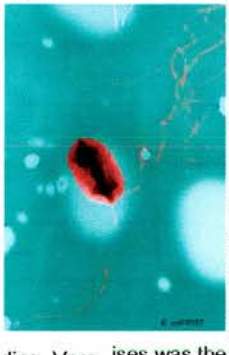
The positive animal samples were all identified by PCR-RFLP as *C. parvum* at VLA Weybridge, and gp60 sequencing showed that the calf samples were of the same subtype (IIaA17G1R1) as detected in human cases by the National Cryptosporidium Reference Unit in Swansea. This suggested that the calves may have been the source of the outbreak, however a preliminary epidemiological analysis showed that petting and feeding goats was the greatest exposure risk. Further analysis of goat samples is planned.

Although *Cryptosporidium* can cause diarrhoea in neonatal farm animals, it is often subclinical. However, infected animals frequently shed oocysts in numbers greatly above the minimum infectious dose for people. Efficient hygiene precautions are thus needed to prevent faecal-oral transmission from healthy animals.

Contributed by: Geoff Pritchard (VLA Bury St Edmunds), Tracey Wood (HPA Cumbria & Lancs), Andy Holliman (VLA Penrith), Robert Hogg (VLA Preston), Rachel Chalmers (UK Cryptosporidium Reference Unit, Swansea), Mathew Proudfoot (Community Services, Carlisle City Council), Michaela Giles & Jackie Marshall (VLA Weybridge).

2. *E. coli* O157

Two children aged 19 and 20 months developed *E. coli* O157 infection after visiting an open farm in Lancashire. Infection was transmitted to six family members with three cases complicated by haemolytic uraemic syndrome. A joint investigation by the Health Protection Agency, Environmental Health Department, Hospital Microbiology Departments and the Veterinary Laboratories Agency identified faecal contamination of the public areas at the farm and close contact with goats as the likely cause of the outbreak.



The outbreak strain was confirmed as *E. coli* O157, phage type 21/28, with genes encoding Vero cytotoxin 2. It was recovered from cases, environmental sampling, and from animal faeces. Five of 31 environmental samples were heavily contaminated by *E. coli* O157 and 41/125 floor faeces samples (including goat, deer, llama, sheep, horse, donkey and pig) were also positive. Isolates from all the human infections were indistinguishable by pulsed field gel electrophoresis (PFGE). Environmental strains (5), and isolates (9), representing all the different positive animal species also had the PFGE profile of the human isolates or a minor variant of it. Typing by variable number tandem repeat analysis confirmed the results of PFGE and supported the epidemiological findings that faecal contamination on the premises was the probable source of the infections. A similar prevalence of animal infection has been found in other on-farm investigations.

The farm was closed for remediation, including resurfacing of public areas to allow easy cleaning, secure fencing of goats to prevent free roaming, and increasing the number of wash basins with improved "wash your hands" signage. Along with many similar farms, there was a children's ball pool play area. These are difficult to clean effectively and so physical separation of the shoe removal area with adjacent hand washing facilities was introduced.

Contributed by: Ken Lambden (CCDC Cumbria & Lancs), with thanks to Geraldine Smith (HPA Centre for Infections), Kate Whittaker (VLA Preston), Michael Tasker (West Lancs District Council), Andrew Fox (HPA Food, Water and Environmental Lab) and Judith Bowley (Southport & Ormskirk Hospital NHS Trust)

(Continued on page 2)

ORIGINAL ARTICLE

Quantitative Risk Assessment for Zoonotic Transmission of *Cryptosporidium parvum* Infection Attributable to Recreational Use of Farmland

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Impacts

- *Cryptosporidium parvum* causes severe gastroenteritis in humans. Cattle are thought to be one of the main reservoir of infection, which poses a theoretical risk to humans through environmental exposure.
- *Cryptosporidium parvum* outbreaks are primarily related to failures of mains water supply and direct contact with young animals such as at open farms, but the burden of infection from the environment is unknown and could be significant for sporadic cases of infection.
- We have developed a worst-case scenario using camping to estimate an upper level for the risk of human infection from environmental exposure to *C. parvum*. We also provide information on the use and efficiency of control strategies.

Keywords:
quantitative risk assessment; *Cryptosporidium parvum;* risk mitigation

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Summary

Cryptosporidiosis caused by *Cryptosporidium parvum* infection is a major cause of enteric illness in man and there is a significant reservoir in animals, particularly young ruminant species. To preliminary assess the magnitude of the risk posed by contact with faeces produced by infected livestock, two microbiological risk assessments have been developed: one for the risk of human infection with *C. parvum* while camping on contaminated land recently grazed by infected suckler cattle and a comparable risk assessment for camping on land recently spread with contaminated cattle slurry. Using a worst-case scenario approach, the upper level of risk was estimated to be one infection in every 6211 person-visits for a camping event on land recently grazed by infected cattle. Translated into camping events of 100 persons, this risk estimate would most likely lead to zero (98% likelihood) or one infection (1% likelihood). The results for cattle slurry model are similar despite different pathways. Sensitivity analysis was conducted for the grazing cattle model only. This suggested that the time between grazing and camping was the most important control strategy, but increasing hand-washing frequency and the removal of cattle faeces before camping would also be beneficial. If the upper level of risk were to be judged unacceptable then further data would be required to more accurately estimate the risk of infection through these scenarios. Further research would also be required to assess the fraction of cases attributable to camping and/or environmental contact with *Cryptosporidium* oocysts.

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Introduction

Cryptosporidium parvum is a protozoan parasite of mammals and is capable of transmission to humans through a number of routes, including contact with farm animals, recreational waters, food and water consumption and person-to-person contact (Casemore, 1990). The resulting disease, cryptosporidiosis, is the third most commonly reported infectious intestinal disease in humans and there were over 4300 laboratory confirmed cases of *Cryptosporidium* spp. infection in the UK during 2006 (Defra, 2007), of which about half are estimated to be caused by *C. parvum* (Chalmers et al., 2009). Although not all *Cryptosporidium* spp found in livestock are zoonotic, there is a large reservoir of *C. parvum* (including clinical and subclinical infections) within the cattle and sheep populations in the UK [Charlotte Featherstone, UK Veterinary Laboratories Agency (VLA), personal communication]. This reservoir is thought to be a major source of zoonotic infection in human *C. parvum* cases, either through direct or indirect contact with animals (or their faeces), or faecally-contaminated water supplies (Meinhardt et al., 1996). In addition, contact with farmed animals has been identified as a significant risk factor for *C. parvum* in sporadic cryptosporidiosis in England and Wales (Hunter et al., 2004).

Outbreaks of cryptosporidiosis include those associated with drinking water or contact with recreational water. However, recent epidemiological and microbiological evidence support the hypothesis that direct contact with cattle is a major route for sporadic human infection with *C. parvum* (Hunter et al., 2003, 2004, 2007). Typically cattle, and young calves in particular, are thought to pose the highest environmental burden of any livestock species (as opposed to sheep), because of routinely high (up to 100%) point prevalence estimates for very young cattle (less than 3 months old) from a number of studies (Naciri et al., 1999; O'Handley et al., 1999; Sturdee et al., 2003), accompanied with high shedding rates of up to 2.4×10^6 oocysts/g of faeces (Sturdee et al., 2003; Charlotte Featherstone, VLA, personal communication). *Cryptosporidium parvum* oocysts have been shown to be hardy organisms, persisting in ambient conditions for months after being shed into the environment (Hutchison et al., 2005), and hence potentially pose a zoonotic transmission risk to humans visiting pasture even months after infected livestock have been removed from the land.

The effectiveness of control policies is dependent on knowing which transmission routes are most important, and as such it is crucial to have an estimate of the relative contribution of contaminated water, environmental or other exposure to the burden of human *Cryptosporidium* infection. Surveillance data for England and Wales showed a decrease in the number of reported cases of human cryptosporidiosis in 2001 compared with previous years, particularly *C. parvum* cases in the spring, which coincided with the restricted access to the countryside during the UK foot and mouth disease outbreak that year (Hunter et al., 2003; Smerdon et al., 2003; Strachan et al., 2003). Furthermore, continued reduction in the number of *C. parvum* cases in the spring may also be linked to improved catchment protection from animal faeces and enhanced water treatment (Sopwith et al., 2005). However, the environmental reservoir of *C. parvum* in sheep and cattle may contribute to human infection through more direct contact with contaminated faeces during rural pursuits (whether occupational or recreational) (Hunter and Thompson, 2005) and recent studies have shown that living within an area spread with animal manure represented a higher risk of *C. parvum* infection (Lake et al., 2007). However, the attributable contribution of this route of transmission to the total burden of human *C. parvum* infection in the UK is unknown.

Because of the lack of suitable data, it is not possible at this stage to accurately estimate the burden of human *C. parvum* infection from rural land exposure. However, it is possible to describe the upper level of risk from zoonotic *Cryptosporidium* spp., that is, principally *C. parvum*, by using microbiological risk assessment, a methodological tool to evaluate the health risks from human or animal exposure to pathogens (WHO/FAO, 1999). Consequently, a quantitative risk assessment was developed to estimate the probability of human *C. parvum* infection from what is considered a 'high-risk' rural activity, such as camping on pasture recently vacated by suckler cattle infected with *C. parvum*, or spread with *C. parvum*-contaminated slurry/manure. We used a similar framework to that used by Strachan et al. (2002) to estimate the risk of vero-cytotoxin producing *Escherichia coli* (VTEC O157) infection from a similar exposure route.

Materials and Methods

Overview and aim of model

Two risk assessment models are presented here, one for the risk of *C. parvum* infection following camping on a field recently grazed by infected cattle, and one for the risk of infection following camping on pasture on which contaminated stored cattle slurry has been spread. For both models, the end-point is the number of infections that would occur following a camping event of 100 people. A schematic outline of both models is given in Fig. 1 and a summary of the parameters/variables and their distributions are shown in Table 1.

Where possible, probability distributions have been assigned to parameters/variables. However, in areas of the model where a lack of data is present, we have taken a

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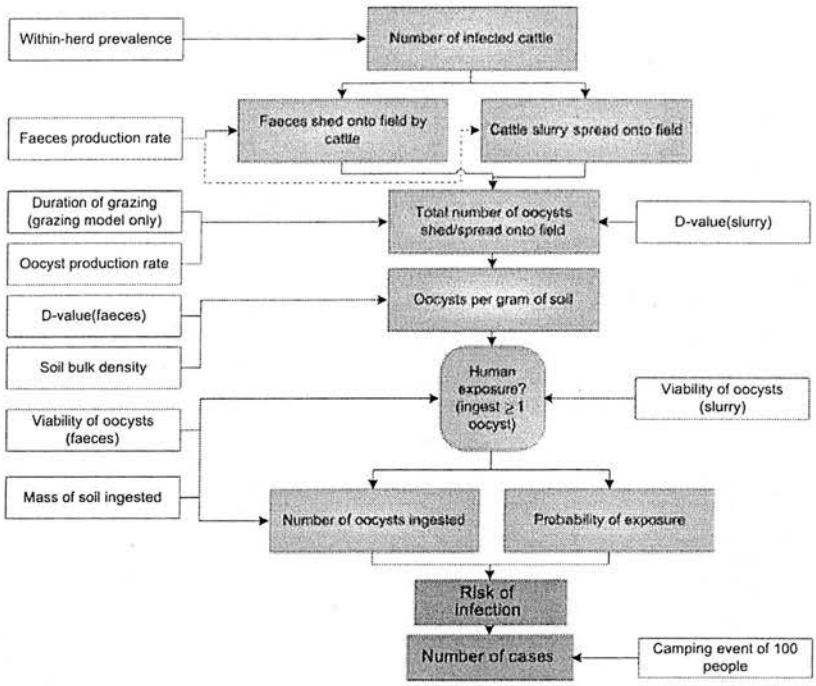

Fig. 1. Schematic diagram of grazing and slurry spread models. (Grey boxes represent calculations within model, white boxes represent data required to parameterise each stage. *D*-value refers to the time required for a one-log reduction in oocyst numbers to occur).

worst-case approach, such that the risk estimate represents a realistic upper limit of the risk.

Summer grazing model

For the summer grazing model, we took a similar approach to Strachan et al. (2002) for a quantitative risk assessment of VTEC O157 infection in children during camping on pasture. We consider a field of size H ha on which an infected herd of B_a adult cattle and B_c calves under 1-month old (of which N_a and N_c are infected according to within-herd prevalence w_a and w_c respectively). Each of the N_a and N_c cows shed oocysts for a period $T_{c,g}$, producing faeces at a rate f_a (adults) and f_c (calves) g/day. *Cryptosporidium parvum* oocysts are shed at a rate γ_a and γ_c (oocysts/g) according to age, and decay at a fixed decimal reduction time in the field, $D_{w,g}$. Because of a lack of data on transmission of infection and intermittent shedding, we assume a worst-case scenario where all infected cattle excrete oocysts for the maximum shedding duration ($T_{c,g}$) immediately prior to the end of the grazing period. Oocyst loads in the soil/faeces will depend on a variety of environmental factors (e.g. hydrology, weather patterns and season); the incorporation of this wide variability in oocyst load and distribution is beyond the scope of this model because of the lack of data to parameterize the complex pathway that exists in reality. In addition, we deal with a limited timeframe (16–50 days), which would therefore limit any dispersion of oocysts. Hence, we also assume faeces are spread homogeneously in the top 1cm of the field, which has a soil bulk density of ρ (g/cm^3). The oocysts then decay at rate $D_{w,g}$ until the arrival of the campers at time $T_{c,g} + T_{w,g}$, where $T_{w,g}$ is the time in days between the cattle leaving the pasture and the campers arrival. The proportion of oocysts that remain viable at the camping event is denoted as υ_g.

Stored waste model

As with the grazing model, we consider the same field size of H ha and soil bulk density ρ. However, in this case, the source of *C. parvum* oocysts is the spreading of cattle slurry from a storage tank filled with S kg/day/cow of excreta from $B_a + B_c$ cattle over $T_{c,s}$ days, where $N_a + N_c$ infected cattle shed *C. parvum* oocysts for $T_{c,g}$ days immediately prior to the spreading of slurry onto land. Cattle are assumed to shed faeces and oocysts at the same rates as for the grazing model. We assume a storage time of this slurry applicable to a spreading rate of 25 m^3/ha using volumes of cattle excreta produced quoted by government guidelines (Defra, 2002a). The oocysts in the cattle slurry decay during storage at a rate $D_{c,s}$. For simplicity, we assume the slurry is agitated prior to land-spreading on grazing land at the end of the housing and hence the oocysts are uniformly distributed

Table 1 Parameters and inputs of the risk assessment model, including initial values, equations and data sources

Notation	Description	Units	Value/distribution	Source
Grazing model				
B_a	No. adult cattle in herd [1 livestock unit (LU)]		25	Defra (2009)
B_c	No. young cattle under 3 months in herd		25	Assumed value
$T_{c,g}$	Duration of shedding	days	16	Fayer et al. (1998); Castro-Hermida et al. (2002)
$T_{w,g}$	Time between end of grazing and camping	days	1	Assumed value (worst case scenario)
$D_{w,g}$	Decimal reduction time of *viable* oocysts in fresh faeces/faecal material on field	days	17.5	Hutchison et al. (2005a)
υ_g	Viability of oocysts in fresh faeces	%	100	Hutchison et al. (2005a)
Stored waste model				
B_a and B_c	No. cattle in herd (adults, calves)		As for grazing model	–
$T_{c,s}$	Storage period	days	100	Estimated using Defra (2002a,b)
$D_{c,s}$	Decimal reduction time of oocysts in stored waste	days	250	Hutchison et al. (2005b)
$D_{w,s}$	Decimal reduction time of oocysts in slurry spread on land	days	17.5 (assume same as for grazing model)	Hutchison et al. (2005a)
$T_{w,s}$	Time between spreading of slurry and camping	days	21	SE Health Dept/FSA (Scotland) (2001)
υ_s	Viability of oocysts in stored waste	%	60	Hutchison et al. (2005b)
Cattle factors and human exposure				
A	Area of field	ha	10	Assumed value
w_a, w_c	Within-herd prevalence (adults, calves)	–	0.03, 0.50	Sturdee et al. (2003)
f_a, f_c	Faecal production rate (adults, calves)	g/day	Adults: Pert (min: 11 000; most likely: 17 000; max 23 000)[a] Calves: extreme value (233.46, 164.92, truncated minimum: 0)[b]	Strachan et al. (2002) Jackie Marshall, VLA, personal communication (2007)
γ_a, γ_c	Oocyst shedding rate (adults, calves)	oocysts/g	Adults: exponential (1760) (truncated min: 1; max 4000) Calves: exponential (107 040) (truncated min: 1; truncated max: 2.4×10^6)	Sturdee et al. (2003)
ρ	Soil bulk density	g/cm^3	Normal (1.3, 0.05)	Strachan et al. (2002)
m	Soil ingestion rate	g/day	0.13	Simon (1998)
R	Probability of infection from single oocyst	–	0.00419	Haas et al. (1996)

[a]Pert() represents a Pert distribution, which is formulated to place a physically realistic shape to a variable with limited information, using the assumed minimum, mode and maximum of the distribution.

[b]Extreme value() is a version of the Weibull distribution, and is used to more accurately reflect distributions with wide ranges. Using BestFit © (Palisade Corp., NY, USA) the extreme value distribution was the best fitting for the calf faecal production rate data available.

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within the slurry. We also assume the slurry is homogenously spread on the pasture by surface broadcast, and mixes in with the soil to a depth of 1 cm by the time of camping. From this time, the oocysts decay on the field at rate $D_{w,g}$ until the arrival of campers at time $T_{c,s} + T_{w,s}$, where $T_{w,s}$ is the time in days between the spreading of cattle slurry on the field and the arrival of the camping party. We assume there would be at least a 21-day wait between spreading of animal slurry onto the surface of land and a camping event, as recommended for spreading of organic wastes by the VTEC task force [Scottish Executive Health Department/Food Standards Agency (Scotland), 2001]. The proportion of viable oocysts after storage is denoted as υ_s.

Model equations

At this point, we introduce the sub-notation y to denote the grazing model ($y = g$) or the cattle slurry model ($y = s$). It is assumed that the field is used as a campsite $T_{w,y}$ days post-grazing/spreading, and we estimate that μ_y oocysts are ingested through an average soil ingestion rate m. We assume an exponential dose response in order to estimate the probability of infection (see Table 1).

The number of infections from a camping event of 100 people, β_y, can be given by Equation 1:

$$\beta_y = Binomial(100, R_y) \tag{1}$$

Where the risk of infection is given by Equation 2:

$$R_y = (1 - \exp(-r \cdot \bar{\mu}_y))(1 - \exp(-\mu_y)) \tag{2}$$

where r is the coefficient of the exponential dose-response model. The number of ingested oocysts per camper, μ_y, and the average number of oocysts ingested over those campers exposed, $\bar{\mu}_y$,can be estimated by

$$\mu_y = m\delta_y$$

$$\bar{\mu}_y = E(\mu_y \notin 0)$$

where m is the average mass of soil ingested over all exposed people and δ_y is the average concentration of oocysts within the top 1 cm of soil of the field, which can be derived as shown in Equations 3a and b for grazing and stored waste models respectively:

$$\delta_g = \frac{\upsilon_g}{10^5 \rho A} \cdot \sum_{t_{w,g}=1}^{T_{w,g}} \left[\eta_g \cdot 10^{-\frac{(T_{w,g} - t_{w,g})}{D_{w,g}}} \right] \tag{3a}$$

$$\delta_s = \frac{\upsilon_g}{10^5 \rho A} \cdot \sum_{t_{w,s}=1}^{T_{w,s}} \left[\eta_s \cdot 10^{-\frac{(T_{w,s} - t_{w,s})}{D_{w,s}}} \right] \tag{3b}$$

[1]Where the conversion factor 10^5 is used to convert hectares into cm^2, and η_g and η_s represent the number of oocysts on the field or within the slurry pit at the point where cattle are removed from pasture/slurry is spread onto grass. η_g and η_s are estimated as follows:

$$\eta_g = \left(\sum_{t_{c,g}=1}^{T_{c,g}} \left[\left(\sum_{1}^{N_{a,g}} \text{Poisson}(f_a \cdot \gamma_a) + \sum_{1}^{N_{c,g}} \text{Poisson}(f_c \cdot \gamma_c) \right) \cdot 10^{-\frac{(T_{c,g} - t_{c,g})}{D_{c,g}}} \right] \right)$$

$$\eta_s = \left(\sum_{t_{c,s}=1}^{T_{c,s}} \left[\left(\sum_{1}^{N_{a,s}} \text{Poisson}(f_a \cdot \gamma_a) + \sum_{1}^{N_{c,s}} \text{Poisson}(f_c \cdot \gamma_c) \right) \cdot 10^{-\frac{(T_{c,s} - t_{c,s})}{D_{c,s}}} \right] \right)$$

Parameter estimation

All parameter estimates are provided in Table 1. For both the stored waste and summer grazing models, there are a number of farm management parameters. However, a lack of data hampered the estimation of these farm management parameters, especially those which would reflect variability between farms. As such, a number of assumptions have been used to describe what is thought to be 'typical' of a UK suckler cattle herd, in particular for $B_{(a \text{ and } c)}$, $T_{c,(a \text{ and } c)}$, $T_{w,(a \text{ and } c)}$ and A. Where data are lacking, maximum (worst-case) estimates have been used to provide an upper end to the parameter estimate (and hence the overall risk estimate).

For the summer grazing model, we required estimates for the following biological and management parameters: $w_{(a \text{ and } c)}$, $\gamma_{(a \text{ and } c)}$, $T_{c,g}$ and $f_{(a \text{ and } c)}$. In addition, an estimate is required for the D-value of oocysts in fresh faeces/soil (D_g). The amount of faeces excreted by adult cattle (f_a) is estimated as described in Strachan et al. (2002) [using the same data from Smith and Frost

[1]Using current parameter estimates, all waste stored from 100 cattle could be spread onto the assumed 10 ha of land [given maximum recommended spreading rates of 50 kg waste per ha (Defra, 2003)], hence assuming the worst-case scenario where all waste is spread onto land there is no need to factor in the dilution of contaminated faeces within the total amount of excreta produced by 100 cattle over 100 days).

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(2000)]. The amount of faeces produced by calves less than 1 month old (f_c) is taken from an experimental study observing the shedding rates of oocysts in young cattle (Jackie Marhsall, Veterinary Laboratories Agency, personal communication, 2007). There are limited data to estimate the within-herd prevalence of *C. parvum* infection for UK cattle, and is a major area of data paucity (as is UK herd prevalence). However, some data have been collected by Sturdee et al. (2003) for both young and adult cattle over a 6-year period. Within the model, the end of grazing could occur at any point in the cycle of infection within the herd, and so we take the average prevalence over this 6-year period as the best measure of the prevalence at any one point in time. Given camping is much more likely in the summer, then we excluded samples taken during the winter season. For model simplicity, the duration of infection ($T_{c,g}$) is set at a plausible maximum of 16 days for all cattle, including adults: this value is based on two studies, where sixteen days represented the maximum and 90th percentile respectively (Fayer et al., 1998; Castro-Hermida et al., 2002). The oocyst shedding rates, γ_a and γ_c, have also been estimated from Sturdee et al. (2003), assuming the distinction between calves and adults is 3 months of age, as is common in practice, and that the calves are around 1 month old when taken off of the pasture.

Cryptosporidium parvum oocysts are very resistant organisms, but will decay according to the environmental conditions they face on pasture (e.g. temperature, water activity, UV) (Nicholson et al., 2004). Hutchison et al. (2005a) have experimentally observed the inactivation of *C. parvum* oocysts on pasture and in soil, and estimated the decimal reduction time and percentage viability of oocysts in different solid animal wastes spread onto the plots. Hutchison et al. do not directly quote the decimal reduction time for cattle faecal waste, hence we have estimated $D_{w,g}$ from the raw data provided by the first author.

For the stored waste model, similar biological information was needed to describe the survival of oocysts in the different environment presented by the slurry tank. Hutchison et al. (2005b) measured the decline of zoonotic agents introduced into liquid livestock wastes in on-farm storage tanks. They found that, in winter, the decimal reduction time for *C. parvum* oocysts in high dry matter slurry (10% w/v) was 250 days. In addition, Hutchison et al. also inoculated liquid slurries with *C. parvum* oocysts: the viable proportion of oocysts after 94 days of storage in pig slurry (during summer) was 60%. For S_{ha}, we assumed the maximum recommended slurry application rate by Defra (2003).

The stored waste and summer grazing models are identical once the number of oocysts remaining on the field at time $T_{c,y}$, δ_y, is estimated. Hutchison et al. (2005a) estimated the viability of oocysts in animal wastes spread on field plots. Although the viability of oocysts from cattle faeces was not reported, the viability of oocysts over a period of 2 months from pig waste was 100% and hence we use this estimate as a worst-case scenario for υ_g. Recent data suggest this assumption is correct (King and Monis, 2007; Peng et al., 2008). The soil bulk density rate, ρ, was estimated as in Strachan et al. (2002) from a study by Paul and Clark (1996). We assumed that a daily soil ingestion rate (m) estimated by Simon (1998) for adults with rural and suburban lifestyles was applicable to the stated camping scenario (this distribution was considered more biologically plausible than a similar distribution derived by Strachan et al. (2002).

Sensitivity analysis

A (rank) regression analysis was carried out for each parameter within the model against the risk of infection, R_y (Saltelli et al., 2000). The risk of infection was identified as a more reliable measure of sensitivity given the large number of null results for β_y (see *Results section*).

A number of parameters within the baseline model are point estimates, which meant they would not be captured within the baseline sensitivity analysis. We therefore carried out additional sensitivity analyses to assess the response of the risk estimate from values taken over the range of plausible probability distributions for those (worst-case) point estimate parameters we believe to be very uncertain, that is, D_g, $T_{w,y}$, m and r. The uncertainty distributions are listed in Table 2 and are based on available data.

Table 2. Plausible distributions used to conduct sensitivity analysis over range of values for uncertain point-estimate parameters within the model

Notation	Description of parameter	Unit	Distribution	Source
$D_{w,y}$	Decimal reduction time of oocysts on field	days	IntUniform(8,30)	Hutchison et al. (2005a)
$T_{w,g}$	Time between grazing and camping	Days	IntUniform(1,90)	Assumed value
m	Soil ingestion rate	g/day	LogNormal(0.1,3.2)	Simon (1998)
υ_g	Viability of oocysts in fresh faeces	%	Uniform(60,100)	Assumed value
R	Probability of infection from single oocyst	–	Minimum: 0.0048 Maximum:0.05	Haas et al. (1996) Teunis et al. (2002)

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Model simulation

The baseline simulation model was run for 50 000 iterations to ensure convergence using the Microsoft Excel (Version 2003, Microsoft, Washington, DC, USA) add-in @Risk 5.0.0 (Palisade Corp., Ithaca, NY, USA). Sensitivity analysis was carried out in the freely available statistical package R (version 2.8.1, http://www.r-project.org).

Results

Cattle grazing model

The average risk of infection for persons that camp on pasture the day after infected cattle have been grazing for a 16-day period was estimated to be 1.61×10^{-4} (fifth percentile: 6.9×10^{-5}, 95th percentile: 2.84×10^{-4}). This distribution of risk would not result in infection in any of the 100 campers in 98.5% of camping events. For those events where infection did occur by far the most common result would be one infection (1.49%) followed by two cases (0.01%).

Stored waste model

The risk of infection from camping on land spread with contaminated cattle slurry was estimated to be 1.12×10^{-4} (4.84×10^{-5}, 1.97×10^{-4}). This risk estimate would produce similar results for infection in 100 campers: no cases in 98.49% of camping events, 1.49% resulted in one case and two cases occurred 0.01% of the time.

Sensitivity analysis

Given the unlikely assumption of a camping event being held on land recently spread with slurry, the actual risk posed by the slurry spreading is likely to be much lower, to the point where no infections would occur. A brief investigation of the model verifies this assumption, as a more realistic time from spreading to camping (50 days) reduces the number of infections to zero. Therefore, we present only the grazing scenario sensitivity analysis in detail.

The sensitivity analysis for the baseline cattle pasture (worst-case scenario) model output contains five variable inputs: f_a, f_c, γ_a, γ_c and ρ. The results of this regression analysis are shown in Fig. 2. The concentration of oocysts in young calves' faeces, γ_c, dominates the estimate for the risk of infection, R_g, whereas the faecal production rate of adults, f_a, is the second most important parameter.

We also carried out an additional sensitivity analysis for each of the parameters which were given point-value estimates: $D_{w,g}$, $T_{w,g}$, m and r. The results of these analyses are shown in Fig. 3. Based on this analysis, the time

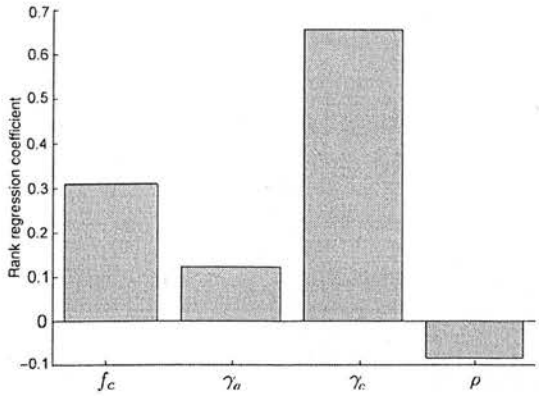

Fig. 2. Sensitivity analysis of baseline (worst-case scenario) grazing model. The model output is most sensitive to the concentration of oocysts within calf faeces and the mass of adult faeces shed onto the field.

to grazing $T_{w,g}$ and the average mass of soil ingested over all persons, m, had the greatest effect on the risk of infection; the risk is negligible if there is 50 days between grazing and camping, and the risk rises significantly with increasing mass of soil ingested. The time to achieve a log-reduction in the number of *C. parvum* in the faeces/soil ($D_{w,g}$) and the exponential dose-response parameter both had positive linear relationships with average risk of infection r, although not in the same order of magnitude of m and $T_{w,g}$.

Control strategies

Feasible control strategies need to take into account the resistance of oocysts to common disinfectants and include removing visible cattle faeces, washing hands regularly while camping or removing animals from the pasture and leaving it fallow for a period before people camp on the land. Removing visible faeces would affect the concentration of oocysts in the soil/faecal matter and hence would likely reduce the risk given the sensitivity of the risk estimate to γ_a, γ_c and f_c. The UK's Health Protection Agency and Health and Safety Executive (2006) recommend washing hands as a precaution against a wide range of pathogens when visiting farms or camping, and this would presumably reduce the amount of soil/faecal matter ingested by campers. Therefore, according to the sensitivity analysis, hand-washing with running water and soap would be a significant control measure[2]. However,

[2]Note that hand gels based on alcohol are not recommended given a farm setting as they will only work on visibly clean hands. Alcohol hand gels are also ineffective disinfectants against *Cryptosporidium spp*.

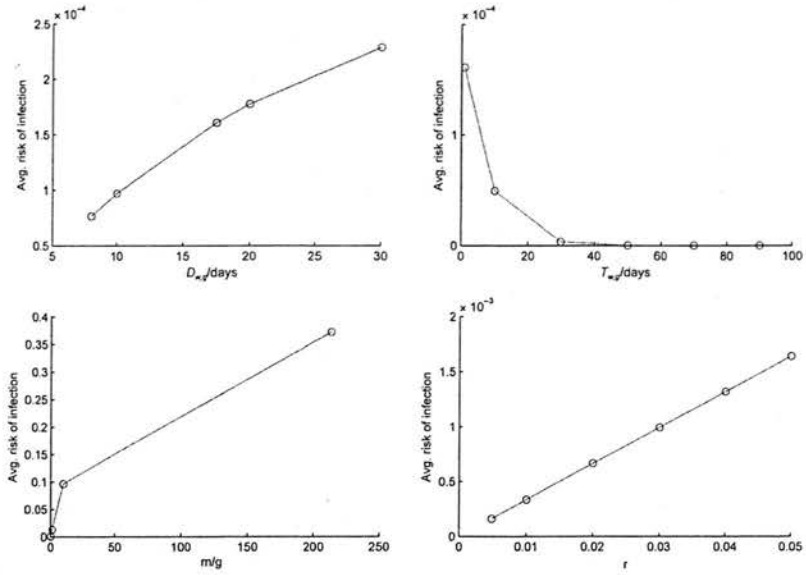

Fig. 3. Sensitivity analysis of parameters given point-value estimates in baseline model, where parameter estimates are varied over plausible values (shown in Table 1). $D_{w,g}$, and r had a significant positive effect on the risk estimate as the parameter estimates increased, and there was a marked effect in reducing the risk as the time between grazing and camping ($T_{w,g}$) increased.

infection in children has been reported after hand-washing when handling young calves for long periods of time (Evans and Gardner, 1996) and so the efficacy of this measure, especially hand-washing in non-running water, is uncertain and depends on the provision of proper hand-washing facilities. For both these control measures, more data are required on their efficacy before a more detailed quantitative assessment can take place.

Given the sensitivity analysis above (see Fig. 3), extending the period between the end of grazing and camping would reduce the risk considerably, and currently is the only control measure which can be quantified to any degree.

Discussion and Conclusions

Living within an area spread with animal manure has been shown to represent a risk of human *C. parvum* infection (Lake et al., 2007); however, the fraction of cases attributable to environmental contact (on land) in the UK is currently unknown, and given a lack of relevant data (farm prevalence of *C. parvum* infection, frequency of visiting farmland, behaviour of humans while on farmland, etc.) it is not possible to estimate the attributable fraction using risk assessment methods. However, using worst-case scenarios, such as camping on contaminated land, we can estimate an upper level of group risk, which can be used as a preliminary estimate of the magnitude of the risk (and hence whether control strategies are required). Using sensitivity analysis, we can also investigate those factors that are important in driving the risk, and hence identify (relevant) data gaps and assess control strategies.

We estimate that the (worst-case) average risk of infection for a group camping on land recently grazed by infected cattle is one case for every 6211 person visits. This level of risk meant that less than 2% of camping events (of 100 persons) on contaminated land would result in one or more infections. For the scenario of spreading contaminated slurry on land, the average risk of infection was similar. We chose camping as a relatively high-risk activity (where possibly immunologically naïve campers, e.g. children, city dwellers, would be exposed to soil/faeces over a long duration), and chose conservative (worst-case) parameter estimates where data were lacking, and hence the true risk of infection, from either oocysts deposited through faeces or slurry, is probably much lower than estimated. The national risk of infection would be very much lower if farm prevalence is not high.

Scenario analysis of the model showed that the natural decay of the oocysts in the environment dominates the magnitude of the risk: hence the most beneficial, and practical, control strategy (should one be needed) would be to maximise the time between the end of grazing, or the spreading of slurry, and the camping event. However, the decimal reduction times quoted in Hutchison et al. (2005a and 2005b) and used within the model are uncertain because of the wide variation in survival and transport of oocysts in different environmental conditions, and the results of Hutchison et al. (2005a and 2005b) could include the effect of transport away from the sampling area as well as die-off of oocysts. Oocysts appear to be relatively mobile in the soil, and heavy rain can transport oocysts metres from the original faecal material within hours (Davies et al., 2004). This may decrease the number

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of oocysts available for ingestion, if they can move below the surface out of reach of exposure, but alternatively exposure may be greater if oocysts remain near the surface in the soil (rather than the faeces), making ingestion more likely if persons do not think they have contaminated their hands with faecal material.

Of interest is the dose–response parameter used within the model: at present we have used the same model as other *C. parvum* risk assessments (e.g. Haas et al., 1996), which has two major assumptions of constant host response (to infection) and constant strain infectivity. However, a more recent model looking at the variation in dose-response to different strains of *C. parvum* suggests that for some strains the likelihood of infection is much higher than that quoted by Haas et al. (Teunis et al., 2002). The sensitivity analysis carried out shows that even small increases in r can result in substantial increases in risk (Fig. 3). Using the dose–response model developed by Teunis et al. (2002) for the strain with the highest infection rate (TAMU), the average rate of infection for the grazing model increases from one infection in every 6211 person-visits to one in every 51 person visits. There is currently not enough information (e.g. dose–response data) to quantify either the strain or host effect within the baseline model. We chose to use the Haas et al. (1996) model as a more representative dose response for all *C. parvum* strains, but given the results of this risk assessment further research in the area of dose response, for both host and strain variability, may be warranted.

Only the parameters relating to calf, rather than adult, shedding of oocysts appear to significantly affect the risk of infection. Although calves are expected to shed higher numbers of oocysts than adult cattle, the larger amount of faeces produced by adults mean that, per unit, calves do not shed significantly higher numbers of oocysts onto the land than adults. However, calves affect the risk more than adults because of the higher prevalence within very young calves. Although not modelled, calves may also pose a greater hazard than adult cattle because of the possibly higher prevalence of *Cryptosporidium* spp. pathogenic to humans in young calves relative to older cattle (Fayer et al., 2007).

The model contains many simplifying assumptions, made because of a lack of data (length of infection in young and adult cattle) and a need to simplify a complex exposure pathway, e.g. assuming cattle faeces are spread homogenously in the top 1 cm of soil. In reality, cattle faeces would be spread heterogeneously across the field, and dispersion of oocysts would occur, for example during rainfall. A similar heterogeneity would exist for slurry, as many farmers would not significantly agitate slurry before spreading (Smith et al., 2001). We have investigated a more realistic assumption by assuming a negative-binomially distributed clustering of oocysts in the soil and slurry for the direct and indirect exposure scenarios respectively. Although the individual dose a person might be exposed to is slightly more variable than in the baseline model, the average dose does not change significantly, hence the results of the model remain the same whether we assume homogenous or heterogeneous mixing of oocysts in the soil/slurry.

Where simplifying assumptions were necessary, they were made conservatively in order to maximise the risk of infection: in this way we provide an upper level of risk. Should this upper level of risk be judged acceptable, then further data collection or control strategies may well be judged unnecessary. Therefore, if this risk is unacceptable and further work is required, then sensitivity analysis has shown that several other factors require further data collection to increase the accuracy of the risk estimate: the within-herd prevalence and the mass of soil that might be ingested in this type of scenario. Given research into the transport of oocysts through soil (Davies et al., 2004), the assumption of homogenous mixing in the top 1 cm of soil may not be too far from reality. If a national risk estimate (such that the attributable fraction of *C. parvum* cases could be estimated) or rigorous validation of the model is required, then farm prevalence data are essential to extend the model pathway to incorporate the likelihood of a positive suckler herd. This model does not consider the effect of factors such as weather conditions (temperature, moisture, etc.) or husbandry on oocyst survival on the field. Recent work (Peng et al., 2008) has modelled the effect of temperature on oocyst survival in water, soils, and faeces and such work might be incorporated in any future version of the current model. Of additional interest would be to investigate the risk of infection attributable to the shedding of oocysts by sheep, which perhaps would be more likely graze land to be used by campers.

In conclusion, we have estimated that the risk of *C. parvum* infection for campers on land grazed by infected cattle is relatively low. However, this is a worst-case scenario: the true risk is likely to be much lower, but further data collection would be required in a number of areas to assess the risk more accurately, especially strain variation for human dose–response. In any event, it is likely that leaving a fallow period of 50 days between grazing and camping would minimise the risk of human infection considerably (by an order of two magnitudes).

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2.36

Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread

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We describe the investigation of an outbreak of Q fever in the town of Cheltenham, England. The outbreak was detected in June 2007, and prospective and retrospective case finding identified 30 confirmed or probable human cases. The investigation identified windborne spread of *Coxiella burnetii* from nearby sheep farms as the most likely source of infection. A telephone survey was conducted to identify risk practices at local farms. Subsequently the atmospheric dispersion model NAME was used to identify whether air from the identified farms with high risk practices had been carried into Cheltenham town centre during the risk period. Three high risk farms were identified and the modelling showed that air from all of these farms was carried over Cheltenham in the estimated risk period. The investigation resulted in an information campaign to farmers and production of improved advice for livestock farmers on reducing the risks of transmitting Q fever to humans.

Introduction

Q fever is caused by the bacterium *Coxiella burnetii*, which has major zoonotic potential and is found worldwide in many different animal species, including wildlife [1]. It is not a notifiable disease in animals or humans in the United Kingdom (UK). Infection in animals is mainly subclinical and inapparent, although it can occasionally cause abortion. However, specific laboratory examinations for *C. burnetii* are not undertaken routinely in animals in the UK, and the infection is only likely to be detected as part of in-depth investigations into major abortion outbreaks in domesticated ruminant species. Furthermore, the veterinary diagnostic tests that are currently available are of limited value. Hence accurate surveillance data on prevalence is lacking, although it is considered endemic in domestic animal populations [2-3]. Large numbers of bacteria are present in the placenta and birth products of infected animals and are released during delivery [1]. The bacterium persists in the environment in a resistant spore-like form which may become airborne and transported long distances by the wind [4-7].

Humans may contract disease by inhalation or, more rarely, by drinking unpasteurised milk or through tick bites. The incubation period varies from 10 to 14 days but may be as long as 39 days depending on the infectious dose [8]. Disease in humans ranges from asymptomatic to severe and can be fatal. It often presents with fever or influenza-like illness, but may cause pneumonia, hepatitis, meningoencephalitis or perimyocarditis. Rarely the disease becomes chronic and leads to endocarditis [9]. Infection, particularly early in pregnancy, may result in abortion, or later in pregnancy to premature labour [10]. Several human outbreaks of Q fever have occurred in the UK, some of which have been associated with windborne spread of contaminated material from infected animals and contaminated farmland [5,11-12].

On 29 June 2007, the Gloucestershire Health Protection Team was notified of five cases of Q fever in patients living in the town of Cheltenham, England with onset of illness between 1 May and 14 June. No cases had been reported in this area in the previous three years. An outbreak investigation team was summoned to investigate the outbreak, consisting of members of the Health Protection Agency South West (HPA SW), Gloucestershire Hospitals National Health System (NHS) Foundation Trust, Cheltenham Borough Council,

HPA Centre for Infections (CFI), Veterinary Laboratories Agency (VLA) and Defra. It was considered that airborne infection from infected animals was the most likely source of the outbreak and the investigations described here were subsequently initiated to pursue this hypothesis further.

Methods

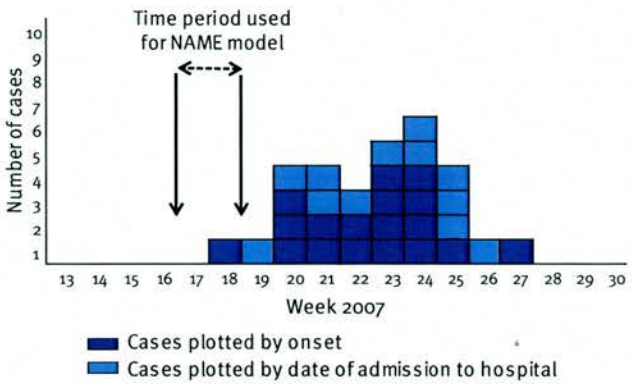
Epidemiological and laboratory investigations

Suspected cases were interviewed by telephone by staff of the HPA SW using a standard questionnaire for Q fever with additional questions relevant to the local area. The questionnaire included questions on: animal exposure, food history (including consumption of unpasteurised milk), risk activities, work details, tick exposure, distance of home to farmland and places visited. After a 6th case was reported, it was decided that active case finding should be undertaken. All General Practitioners (GPs) in the Cheltenham area were informed of the outbreak and encouraged to consider the diagnosis of Q fever in patients with relevant symptoms. Retrospective case finding was attempted by investigating hospital admission records for diagnoses of unspecified pneumonia. The purpose was to identify additional patients whose exposure history may have helped to identify a source of infection. No attempt was made to identify all possibly infected patients in the county as it was felt that self-limiting illnesses did not need to be identified. The most common presenting clinical feature that could be distinguished by clinical coding of admissions was searched for. Patients discharged with a diagnosis of unspecified pneumonia were contacted and asked to leave a blood sample for Q fever serology.

The standard laboratory method in use in the clinical diagnostic laboratory for screening for Q fever was the phase I and II complement fixation test (CFT) which, if positive at a titre of 1:16 or greater, prompted referral of the serum to the HPA reference laboratory for analysis by enzyme-linked immunoassay (EIA).

FIGURE 1

Epidemic curve for the outbreak of Q fever in Cheltenham 1 May-8 July 2007 (n=30)



Cases were plotted with date of onset if this information was known, otherwise the date of hospital admission was used.

The following case definition was used in the investigation: A confirmed case was defined as a person who lived in or visited Cheltenham between 1 April and 31 June 2007 who presented with pneumonia or clinical symptoms consistent with Q fever acquired during this time period. A confirmed case subsequently also needed to exhibit IgM antibody titres displaying assay positivity in serial dilutions of more than 1:80 or a four-fold increase of phase II CFT titres against *C. burnetii* with paired sera taken at least seven days apart. A probable case was defined as a person that lived in or visited Cheltenham between 1 April and 31 June 2007 who presented with pneumonia or clinical symptoms consistent with Q fever acquired during this time period. A probable case subsequently also needed to exhibit IgG antibody titres displaying assay positivity in serial dilutions of more than 1:80 or a single test with four-fold raised CFT titre against *C. burnetii* compared to baseline. The risk period for exposure was at the time of the ongoing outbreak investigation estimated to have been 23 April–7 May 2007. This period was chosen to cover the time distribution of cases from the earliest disease onset date minus maximum incubation period until onset of disease in the last detected case minus minimum incubation time. More cases were identified retrospectively. Testing for Q fever was initiated by GPs or hospital physicians on request of the Health Protection Agency South West on the basis of respiratory symptoms or symptoms of a influenza-like illness (such as malaise, lethargy, myalgia, arthralgia and headache). Additionally, blood samples received for testing for any respiratory pathogens were automatically included by the laboratory.

Veterinary investigation

The location of livestock farms in the vicinity of Cheltenham and the numbers of animals present were mapped using data obtained from the Animal Health Agency. The VLA was consulted regarding recent reports of Q fever affecting farm animals in the area. In order to further investigate the possibility that nearby livestock farms may have been the source of the outbreak, a semi-structured telephone questionnaire survey was carried out to investigate any potentially relevant disease history (i.e. abortions/reproductive failures) and husbandry practises on local farms that may have contributed to the risk. The selection of farms was based on their geographical location in relation to the distribution of cases, using meteorological information regarding the predominant wind directions at the likely time period of exposure. The likelihood of a farm being the source of the outbreak was categorised empirically using a qualitative risk assessment approach, where three categories of risk factors were assessed. Firstly, the risk of infection: human illness, reproductive problems in livestock and presence of ticks that are potential vectors for *C. burnetii*. Secondly, the risk of release: lambing/calving dates and place, manure handling and movement of animals. Lastly the risk of human contact: distance from town centre, distribution of manure

to public, transport of animals and manure in densely populated areas and public access to animals. The qualitative risk assessment was based on the proportion of risk practices present in relation to all possible risk practices. Since little is known about the relative importance of different risk factors for transmission of *C. burnetii* in farm animals, the practices were not weighted. The farms were categorised as low, medium or high risk, based exclusively on the risk assessment, without establishing whether *C. burnetii* was present in the animals.

Environmental investigation

In addition to livestock farms, other premises with livestock such as abattoirs and livestock markets were considered as possible sources. Information on these was obtained via the Meat Hygiene Service and local Animal Health offices. Information on other events involving animals which had been held in the area and the location of allotments (potential manure risk) were also collected. Risk sites were visited and investigated.

Meteorological investigation

Meteorological observations of near surface (10 m above ground) wind speed and wind direction in the time period before the onset of disease in confirmed and probable cases were obtained from the Met Office's observation site in Pershore approximately 30 km north of Cheltenham to help identify the source of the outbreak. This information was used to assist the veterinary investigation to decide on which farms to interview as described above.

Later, Numerical Atmospheric-dispersion Modelling Environment (NAME) [13], an atmospheric dispersion model, was used to investigate potential airborne transport of the *C. burnetii* between a number of suspected sources (notably local farms identified by veterinary investigation) and the infected persons in Cheltenham. NAME has a wide range of applications including air quality forecasting, predicting the transport and spread of chemical, biological and nuclear material, producing volcanic ash forecasts, identifying source locations and strengths, investigating pollution episodes and airborne spread of diseases. The model can be run in forward mode, predicting the transport and spread of airborne material released from an identified source. Alternatively, it can be run in backward mode, predicting the transport backwards in time from an identified receptor point, thereby showing the air history of material arriving at the receptor point and identifying potential sources.

NAME was run for the estimated risk period 23 April to 7 May, both in forward mode to give the predicted area at risk from the suspected farms and in backward mode to give the air history for air arriving in Cheltenham. Input meteorological data used in this study to drive NAME was hourly three-dimensional meteorological data from the Met Office's numerical weather prediction model (the Unified Model [14]) with a horizontal spatial resolution of 12 km. The accuracy of the atmospheric dispersion modelling is directly related to the accuracy of the input meteorological data and, whilst the meteorological data is likely to represent the larger scale atmospheric motions, it is not expected to capture the small scale local flow within the urban conurbation of Cheltenham (e.g. channelling of the flow within street canyons).

Table

Patient demographics and presenting symptoms, Q fever in Cheltenham May-July 2007 (n=30)

Criteria	Number (%)
Age range	19-72 years
Male	21 (70%)
Female	9 (30%)
Hospital admission	24 (80%)
Identified retrospectively	15 (50%)
Smoker	11 current smokers (37%), 7 ex-smokers (23%)
Non-smoker	9 (30%)
Smoking status unknown	3 (10%)
Presenting symptoms:	
Fever	25 (83%)
Headache	17 (57%)
Myalgia/Arthralgia	18 (60%)
Chest pain	13 (43%)
Cough/Shortness of breath[1]	26 (87%), 13/15 non lookback (87%)
Nausea (N), vomiting (V), diarrhoea (D)	4 NVD, 3 NV, 2 N, 1 D
Other symptoms	2 Loin pain, 2 dizziness, 1 confusion, 1 skin rash

1 Because retrospective case finding was based on presentation with pneumonia, the proportion with cough that were not part of retrospective case finding is also shown.

Results

Descriptive epidemiology and laboratory findings

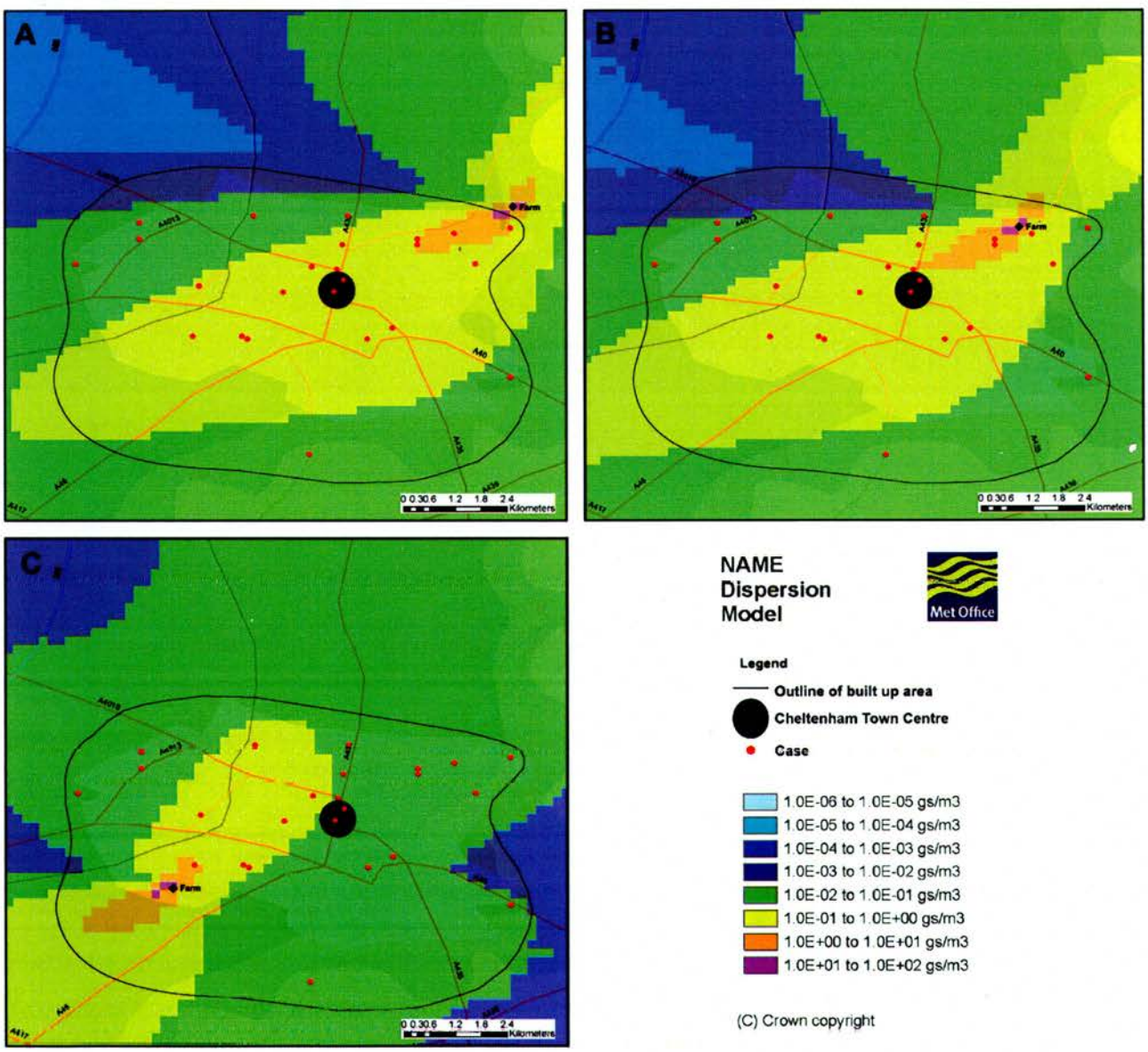
Interviews with suspected and confirmed cases did not reveal any common exposures other than living in or having visited Cheltenham town centre. The questionnaire did not identify any shared risk exposures or activities that could have resulted in transmission. The only factor the cases had in common was being a resident of or having visited central Cheltenham. A total of 30 cases all living in Gloucestershire were identified that met the case definition for a confirmed or probable case in the outbreak period. Fifteen had been identified through retrospective case finding among people hospitalised with pneumonia. Of the total of 30 cases, nine were female and 21 were male. The age range was 19-72 years and the median age was 48 years. The first onset of disease was on 1 May and the last on 8 July (Figure 1). Twenty-four cases were hospitalised. A summary of reported symptoms and demographics is given in the Table.

Veterinary investigation

There had been no recent reports of Q fever affecting farm animals in the area. We identified sixteen

FIGURE 2

NAME air dosage maps obtained by modelling a continuous release from the high risk farms (A, B, and C) for the time period 23 April-7 May 2007



NAME: Numerical Atmospheric-dispersion Modelling Environment.
The areas of highest dosage (pink, orange and yellow) covers Cheltenham town centre. A black diamond marks the location of the farm. Red dots mark the addresses of cases resident in Cheltenham. A black line illustrates the outer limit of the built up areas in Cheltenham. The filled black circle marks the town centre which all cases, including those not resident in Cheltenham, had visited at some time during the risk period for exposure.

farms that stocked farm animals in proximity to the Cheltenham town centre that were located along a south-west to north-easterly line to accommodate for the predominant wind directions for the period from 23 April to 7 May. Eleven farms completed the telephone survey. The remaining five farms either did no longer have livestock or could not be contacted. The risk assessment classified five low-risk farms, three medium-risk farms and three high-risk farms. The high risk farms A, B, and C were located 4.4 km, 2.7 km and 4.2 km from the town centre. All three farms stocked sheep and two farms also stocked cattle. Farm A calved and lambed during the risk period, transported a large batch of animals through Cheltenham town centre and sold manure to nearby allotments. Farm B reported outdoor lambing during the risk period and burning the fresh straw bedding and birth products outside every few days. This is an unusual management practice. Usually, abortion and birth products would be incinerated and bedding stacked up in a heap for a long time to kill pathogens before disposal. Farm C reported outdoor lambing and a few stillbirths and had several sheep movements close to the town centre.

Environmental investigation

The environmental investigation did not reveal any places or events in the town of Cheltenham that could have posed a risk during the estimated risk period from 23 April to 7 May.

Meteorological investigation

According to the meteorological observations from the Pershore observation site the predominant wind direction in the Cheltenham area during the estimated risk period was from the north-east and to a lesser extent from the south-west, which is the prevailing wind direction in the UK. Wind speeds were unexceptional, ranging from light winds to breezy conditions. Dispersion modelling using NAME showed that air from each of the suspected farms may have exposed the town to the bacterium at some point over the study period assuming there had been a continuous release (Figure 2). Therefore, none of the suspected farms could be ruled out as potential sources.

Discussion

At least 30 people were infected with *C. burnetii* in this outbreak. Further cases were not sought from household contacts or those with other possible presenting symptoms as the aim of the retrospective case finding was to aid the epidemiological investigation to identify the source of the outbreak. Previous outbreaks indicated that 2%-5% of those infected may be hospitalised [15]. Extrapolating from the 15 cases we identified retrospectively through hospital admission suggests that possibly up to 500 people may have been infected. The population of Cheltenham town is approximately 110,000 people and there are approximately 560,000 people in Gloucestershire who may visit Cheltenham as well as possible visitors from outside the county. National guidelines do not recommend the identification of all patients with Q fever and the seroprevalence among farmers, veterinarians, and people living in rural communities suggest that undiagnosed infection is common [15-16]. It was therefore not deemed appropriate to attempt mass screening.

The age and sex distribution of identified cases was similar to that of other outbreaks [1]. The epidemic curve shows that cases fell ill over a period of at least seven weeks, suggesting either that the release of bacterium was continuous or intermittent over a similar number of weeks or that the incubation period varied greatly. The incubation period may be prolonged when the infectious dose is small, which is likely in long-distance windborne transmission. No common risk factor was identified between cases other than living in, or having visited, Cheltenham and therefore we hypothesised that windborne spread of *C. burnetii* from nearby farms was the probable source of infection. Conditions were at times breezy, and strong winds have played a role in other outbreaks [5]. The predominant wind direction during the two-week period studied was from the north-east rather than the prevailing wind direction from the south-west which was the second most common wind direction. The telephone survey to the selected group of nearby farms revealed some high risk practices that could potentially have resulted in windborne spread. These were discussed with the farmers concerned and the practices ceased. Advisory information for farmers on Q fever control was also circulated via veterinary practices in England and Wales and put on VLA, HPA and HSE websites. Transportation of animals through populated areas has caused outbreaks previously [17], as have outdoor lambings [7]. One farm burnt the fresh straw bedding and birth products outside on several occasions, and this practice may facilitate windborne spread of *C. burnetii* by releasing incompletely burnt contaminated material into the air. The number of farms contacted was fairly small because of limited resources, but it included all the main livestock farms in the area. However, the possibility that we missed other farms with risk practices cannot be ruled out.

Laboratory investigation of the animals on the high-risk farms for evidence of *C.burnetii* infection was considered by the outbreak control team but was decided against, because the potential value of any results was perceived to be limited. The long time interval from the exposure date to sampling of the animals would complicate interpretation because, for example, farms may have sold infected animals that had aborted. Furthermore, interpretation of positive results would be complicated by the fact that little is known about the seroprevalence of infection in livestock in the UK generally and it would not be possible to put the serological results into perspective. The only scientifically viable option would have been to design a prevalence study combined with collection of risk factor information on all farms in the area. That was considered beyond the scope of this outbreak investigation.

To further support the outbreak investigation hypothesis that windborne spread from a local farm caused the cases in Cheltenham we employed the use of an atmospheric dispersion model. We chose NAME as this model has previously been used successfully to investigate airborne spread of diseases such as foot and mouth [18], bluetongue [19] and Legionnaires' disease. The model was run for the period from 23 April to 7 May. This time period was chosen as it would have explained the cases that were known at the time of the outbreak investigation but cannot explain all cases that were identified later. The modelling showed that air from all the farms was transported to Cheltenham town centre at some point during the period studied. Each of them could therefore have been the potential source of infection and none of the high-risk farms could be excluded. As we do not know the exact dates of transmission, we cannot say that one farm was more likely than the other, as the wind directions may have varied day by day within the studied period.

The modelling could, however, have been refined to potentially give more conclusive evidence, if further detailed information regarding the outbreak had been obtained such as more specific information on the potential time of release of *C. burnetii*, release rates of the bacterium, concentrations required for infection and exact time of infection. These parameters were not available due to uncertainties in the epidemiological investigation. Firstly, we know that all the cases lived in or visited Cheltenham town centre, but we do not know whether their presence coincided in time with windborne transport of contaminated air to Cheltenham town centre from a high risk farm. Secondly, the area of risk of exposure calculated by the atmospheric dispersion model may be an overestimate, if the release of *C. burnetii* was not continuous over the two-week transmission period identified. The release may not have been continuous as the time period between disease onsets suggests that people were infected intermittently over an extended period. Thirdly, although *C. burnetii* infection is considered endemic in UK farm animals, precise information about the infectious status of the investigated farms was unavailable. A well structured serological survey to measure the extent of *C. burnetii* infection in farmed livestock in the UK would answer questions relating to prevalence and relative geographical risk to the human population and greatly assist any further similar outbreak investigations.

Conclusion

Despite limitations, we believe that atmospheric dispersion models can be a valuable tool in similar outbreak investigations and this is supported by other disease outbreak studies using NAME [18-19]. In the Cheltenham outbreak it added support to our hypothesis of windborne spread of *C. burnetii* from a high risk farm, when an analytical study was not feasible. Furthermore, this investigation identified likely risk practices on local farms and engaged concerned stakeholders in the consideration of preventive measures leading to improved advice for farmers [20]. Enhanced local surveillance in the area in the following year (2008) did not reveal any cases of Q fever which suggests that high risk practices may have ceased.

Finally this investigation showed the strength and benefits of different agencies and authorities working closely together. In this investigation the close collaboration and information exchange between veterinary, human health, and meteorological agencies and the local authorities was perceived as beneficial by all involved. We believe that sharing and applying different techniques and information between different fields of research is of paramount importance for successful outbreak investigations.

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Q fever diagnosis in domestic ruminants: comparison between complement fixation and commercial enzyme-linked immunosorbent assays

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Short running title: Q fever ELISA

Abstract. Q fever is an important zoonotic disease caused by infection with the bacterium *Coxiella burnetii.* Veterinary diagnostic laboratories, including the Veterinary Laboratories Agency (VLA) in England and Wales, have traditionally relied on the complement fixation test (CFT) for serological diagnosis. However, Q fever has assumed greater significance in recent years following several large human outbreaks linked to exposure to infected ruminants and it is essential that more reliable tests are introduced to detect the presence of *C. burnetii* infection in animals. The objective of the current study was to evaluate the performance of 3 commercially available enzyme-linked immunosorbent assays (ELISAs) for detection of antibodies to *C. burnetii* and to compare the findings with the CFT using a sample panel of 548 sera from sheep, goats, and cattle, including animals of known disease status. The statistical analysis using TAGS (test accuracy in the absence of a gold standard) software and receiver operating characteristic techniques demonstrated that the 3 ELISAs all showed improved sensitivity over the CFT. The test based on ovine antigen demonstrated the best overall performance and therefore, the VLA has adopted this test for routine use.

Key words: Complement fixation test; comparison; *Coxiella burnetii*; enzyme-linked immunosorbent assay.

Introduction

Q fever is an important zoonosis with a worldwide distribution. The etiological agent, *Coxiella burnetii*, is a Gram-negative, obligate, intracellular bacterium classified within the order *Legionellales*.[2] *Coxiella burnetii* occurs in 2 antigenic forms: the pathogenic phase I, found in infected animals or human beings, and the avirulent phase II, obtained by repeated passages in embryonated eggs or in cell cultures. Additionally, *C. burnetii* may occur as a small-cell variant or large-cell variant. The small-cell variant undergoes sporogenic differentiation to produce resistant spore-like forms and can survive for long periods in the environment.

Coxiella burnetii is an Advisory Committee on Dangerous Pathogens (http://www.dh.gov.uk/ab/ACDP/index.htm) category 3 pathogen. Infection in animals is mainly subclinical, but sporadic abortions and occasionally large outbreaks are diagnosed in sheep, cattle, and goats. Infected animals shed large numbers of organisms at parturition, which presents a major zoonotic hazard for occupationally exposed groups, particularly since in its spore-like form, *C. burnetii* is very resistant to disinfection and desiccation.

In human beings, Q fever is often asymptomatic or causes only mild flu-like illness, but some cases develop chronic infection with potentially serious sequelae; pregnant women and people with pre-existing valvular heart disease are at particular risk.[6] In addition to occupational exposure, human beings can become infected (rarely) from tick bites or by consuming unpasteurized milk.[1] Large human outbreaks such as those in Cheltenham, England[20] and The Netherlands[6] are attributed to windborne aerosol infection from livestock farm premises, especially by contaminated dust, bedding, and biological secretions associated with parturition.

There has been increased interest in Q fever worldwide following a large outbreak in The Netherlands, in which there have been more than 3,300 human cases since the start of the

epidemic in mid-2007, with 2,236 of these being diagnosed in 2009.[11,17] This outbreak has encouraged many countries to reassess their surveillance and diagnostic methods for Q fever.[7,12]

In veterinary laboratories, including the VLA, the veterinary diagnosis of Q fever usually relies mainly on modified Ziehl–Neelsen (MZN)-stained smears of placental material from aborted fetuses, supplemented by immunohistochemistry (IHC) where appropriate, although polymerase chain reaction (PCR) is increasingly being used for disease confirmation.[9] Serology, usually the complement fixation test (CFT), is used to examine for evidence of exposure in groups of animals including aborted dams. The CFT has inherent problems, particularly anti-complementary (AC) activity in a significant proportion of samples, which makes it difficult to obtain meaningful results.[18] An AC reaction comprises breakdown of complement by a variety of factors including immunoglobulins and enzymes such as typsin[3] or bacterial contamination of the sample for analysis.[4] This reaction results in an inhibition of cell lysis in the CFT indicator system. The sensitivity and specificity of the CFT has also recently been questioned by the European Food Safety Authority (EFSA), who have recommended a standardized approach to diagnosis and case definitions for Q fever within the European Union through the adoption of ELISA and PCR technology.[7]

The current study presents the findings from testing a panel of 548 samples from cattle, sheep, and goats for the presence of antibodies to *C. burnetii* using the CFT and 3 commercially available ELISAs. Results were evaluated statistically to select the ELISA that gave the best overall test performance.

Materials and methods

Sample panel

Serum samples used in the present study (n = 548) originated from sheep, goats, and cattle taken from suspected clinical cases of Q fever submitted by private veterinary surgeons to VLA regional laboratories for diagnosis, and sera from herds and flocks of known disease status based on MZN, IHC, or PCR (Table 1). Apart from the freeze-dried ELISA standards supplied by the French Food Safety Agency (AFSSA) and Laboratoire Service International (Lissieu, France) that were stored at 4°C, all sera were stored at –20°C for up to 1 year prior to the study.

Serological tests and methods

Complement fixation test. The CFT was based on the protocol described in the World Organization for Animal Health (OIE) Manual of Standards[21] and had been in routine use by the VLA for the last 20–30 years. The test used human antigen (supplied by Dade Behring, Marburg, Germany) produced in monkey kidney tissue culture. The CFT detects both phase I and phase II antibody. Antibody concentrations were expressed as titers of between 1:10 and >1:640 using doubling dilutions. The OIE guidelines[21] state that a titer of ≥1:80 is significant and indicates recent infection, 1:10 to1:40 represent latent infection and <1:10 is negative.

Enzyme-linked immunosorbent assay. Three indirect ELISAs (1–3) were used for the validation and were performed strictly according to the manufacturers' instructions. ELISA 1 used an ovine-derived antigen; ELISA 2 used a tick-derived antigen (Nine Mile); and ELISA 3 was based on a bovine antigen. All used a conjugate that detected specific ruminant immunoglobulin G (IgG) antibody to provide evidence of exposure to *C. burnetii* infection. The results were based on optical density (OD) expressed as sample OD/positive control OD × 100 = sample-to-positive (S/P) ratio%. As detailed in the individual test kits, there were slight variations in the way S/P ratios were calculated between manufacturers to take into account the background OD of the sample or control (Table 2).

Analysis

All sera were tested by the CFT and ELISAs 1– 3. The results were analyzed statistically as detailed below.

TAGS and ROC. Receiver operating characteristic[5] (ROC) analyses are normally performed on test results from samples derived from animals of known disease status. These are regarded as the gold standard and are used to compare the diagnostic performances of other tests. A ROC curve is obtained by plotting the observed sensitivity against (1 – specificity) as the test cut-off is varied. A summary measure of the test performance is given by the area under the curve (AUC), which equals the probability that a randomly chosen positive sample will give a test result of a higher value than a randomly chosen negative sample. A perfect test will have the AUC equal to 1.

In the current study, however, there were limited gold standard individual samples available and therefore ROC analyses were performed using the CFT as the gold standard to determine the extent to which the ELISA results agreed with those obtained by the CFT. A fixed cut-off was used for the CFT, with titers of <1:80 including AC results regarded as negative and ≥1:80 as positive. The ELISAs used the manufacturers' cut-off criteria (Table 2) except that inconclusive results were omitted. The overall ELISA performance was measured by the AUC and the estimated sensitivity and specificity relative to the CFT.

Diagnostic test sensitivity and specificity of all 4 tests were also estimated statistically by maximum likelihood methods using TAGS (test accuracy in the absence of a gold standard) software[14] with the same cut-offs as described above. The estimation method assumes test independence conditional on the infection status of the individuals and constant test accuracy in each population. The validity of the TAGS estimate was assessed using a chi-square test that

compares the observed test results with those predicted from the fitted model. A lower chi-square value is indicative of good agreement between the observed and predicted results.

The results of the TAGS, ROC, and other test performance indicators were analyzed separately for individual species as well as treating all species combined as a single group. The best performing ELISA was selected based on the comparative results for diagnostic sensitivity and specificity from statistical analyses and also from agreement with CFT results. Estimates for analytical sensitivity, analytical and diagnostic specificity, repeatability, and reproducibility were then determined for this best performing ELISA.

Other test performance indicators

The ELISA diagnostic test specificity was also assessed by comparing results with negative CFT sera (75 sheep, 122 goats, and 173 cattle). Analytical sensitivity was assessed by performing doubling dilutions of representative positive samples from the sample panel. The representative positive samples were comprised of 1 cow and 1 sheep serum from the known positive status group and 1 cow, 1 sheep, and 1 goat serum from the unknown status group. The end-point titration (the last dilution that obtained a positive result) was determined for both ELISA and CFT. The analytical specificity for the sheep tests was assessed by testing sera from 3 sheep that had been naturally infected and shown to have high CFT antibody titers to *Chlamydophila abortus*, which is the main abortifacient pathogen likely to be involved in the differential diagnosis of Q fever in Britain. These samples were not included in the main sample panel. Similar samples that could be used to test for analytical specificity for the other animal species were unavailable.

Assay repeatability (intraplate variation) was assessed by testing multiple replicates of samples selected from the sample panel. Seven samples from all species were selected that gave

low, medium, and high S/P ratios when previously tested by ELISA. Repeatability data was assessed using the coefficients of variation (CV), expressed as percentages. Assay reproducibility was assessed by testing 6 sera in quadruplicate on 2 occasions by 2 operators at 2 different laboratories. The sera used comprised a single sheep confirmed negative, 3 sheep confirmed positives, and 1 goat and 2 cattle of unknown status.

The VLA also participated in the 2008 international Q fever ring trial using the CFT and the 2009 international ring trial, using only ELISA 1 (the VLA preferred test). In the 2009 ring trial, there were 74 participating laboratories (63 French, 8 other European laboratories, and 3 suppliers of ELISA test kits), 33 of which used ELISA 1 (AFSSA: 2009, InterLaboratory proficiency testing program for Q fever ELISA serology; EILA FQ: 2009, general report. AFSSA, Sophia Antipolis, France).

Results

TAGS and ROC

Treating sera from all species as a single population, the ROC analysis demonstrated agreement with the CFT for ELISAs 1 and 2 with similar AUC and sensitivity and specificity, (Tables 3, 4) but a much lower sensitivity for ELISA 3. Treating sheep, goats, and cattle separately gave marginally higher AUC values for ELISA 1 than for ELISA 2 in all 3 species, but those for ELISA 3 were consistently lower.

When all samples were treated as a single population (Table 5), the TAGS analysis gave lower sensitivities for the CFT (36.2%) and ELISA 3 (55.7%). ELISA 2 had the highest sensitivity (98.6%) but the lowest specificity (97.1%). The TAGS analysis for individual species showed that ELISA 2 had the highest sensitivity and specificity when testing sheep samples, whereas the CFT and ELISA 3 gave much lower sensitivities. For goat samples, sensitivity was

slightly higher for ELISA 2 (93.1%) than ELISA 1 (91.6%), but the specificity was lower at 91.2% compared to 98.9%; ELISA 3 and CFT again had much lower sensitivities. The model gave a poor fit (chi-square = 18.4, p = 0.005) to the cattle samples in the current study and therefore the sensitivity and specificity estimates may be unreliable.

Other test performance indicators

When testing samples from the negative and positive flocks (Table 1, disease status groups "Negative 1," "Positive 3," and "Positive 4"), the CFT results gave a similar distribution of titers for each flock (Table 6). It was not possible to make a clear distinction of disease status between the 2 flocks using the CFT. The CFT scored 2 out of 65 samples with a titer of ≥1:80 from the negative flock and 3 out of 59 samples with a titer of ≥1:80 from the positive flock. There were a total of 30 (24%) samples that gave AC results by CFT; 29 of these samples gave a negative result when tested by ELISA 1. All ELISAs demonstrated 100% specificity by correctly identifying all samples results as negative from the negative flock. ELISA 1 identified 8 positive samples from the positive flock, ELISA 2 identified 6, and ELISA 3 failed to detect any.

Further evaluation of ELISA 1

Based on the comparative results for diagnostic sensitivity and specificity of the statistical analyses, and agreement with CFT results, ELISA 1 had the best overall test performance, and therefore, it was evaluated further. When tested against sera with negative CFT titers (<1:10), ELISA 1 scored as negative 74 (98.7%) out of 75 sheep samples, 102 (83.6%) out of 122 goat samples, and 170 (98.26%) out of 173 cattle samples. When assessing analytical specificity, 100% correct results were obtained when testing sheep samples with no cross reactivity for samples containing antibodies to *C. abortus* (Table 7). Analytical sensitivity using samples from all 3 species demonstrated the final end-point titer of both ELISA 1 and CFT to be

similar (Fig. 1). Assay repeatability was assessed using intraplate variation, which gave a range of CV from 6.9% to 12%. This range was considered within acceptable limits (Table 8). Satisfactory reproducibility data was demonstrated overall: both operators in laboratory 1 scored all results correctly, and in laboratory 2, operator C scored 47 out of 48 and operator D scored 46 out of 48 results correctly (Table 9). Further evidence supporting ELISA 1 was found from the 2008 and 2009 Q fever serology international ring trials in which the VLA participated. The 2009 trial included 33 laboratories that used ELISA 1, which gave 100% correct results for both trials and, where the CFT was included (2008 trial), demonstrated improved sensitivity and specificity compared with that of the CFT.

Discussion

Despite its inherent problems, the CFT has been a mainstay of Q fever serological testing in veterinary and human diagnostic laboratories for many years. However, ELISA is increasingly being used for the testing of a wide range of animal species,[8,19] is shown to be more sensitive and specific than the CFT,[10,13,15] and has recently been recommended by EFSA for test harmonization.[7]

Poor agreement was evident between the ELISAs and CFT[10,16] using ROC analysis with a CFT cut-off titer of 1:10 but good agreement (0.8 AUC) when treating all species as 1 population using a CFT positive cut-off titer of $\geq$1:80. When test results of samples from individual species were analyzed separately, ELISAs 1 and 2 gave similar sensitivity and specificity, but ELISA 3 was less sensitive. When analyzing all species as 1 population, results from both the ROC and TAGS analyses confirmed that ELISAs 1 and 2 were the tests that performed best.

When testing sera from groups of known disease status (Table 1) by CFT, the distribution of titers was very similar for both the negative and positive flocks, and the CFT failed to discriminate between negative and positive sera or flocks. The CFT also produced a high proportion (24%) of sheep samples with an AC untestable sample result, which is a recognized problem with CFT.[13,18] This high proportion of test failures adversely impacts test efficiency and significantly contributes to the cost of testing, since repeat sampling is required. Test failures were not encountered with ELISA, which is also less labor intensive and particularly useful for testing a large number of samples such as for surveys.

The ELISA results from testing sheep samples from negative and positive disease status flocks (Table 1, disease status groups "Negative 1," "Positive 3," and "Positive 4") clearly demonstrated that ELISA 1 was the best performing test, correctly identifying all samples as negative from the negative flock and scoring the highest number of positive results (14%, 8/59) when testing samples from the positive flock. This rate of seroprevalence, which was also demonstrated by the TAGS analysis, is typical of that found in Q fever–positive flocks.[16] The results from testing individual animals from a group with a low seroprevalence of infection should be viewed with caution. It is suggested that serological tests should only be used for determining flock status, rather than individual animal status, which is the approach recommended by EFSA.[7] When focusing on all groups of sheep samples, the TAGS analysis showed that ELISA 2 was the best performing test. When treating all species as 1 population, the TAGS analysis gave ELISA 2 the overall highest sensitivity (98.6%) whereas ELISAs 1 and 3 gave the best specificity (99.1% and 99.3%, respectively). When test results of samples from individual species were analyzed separately, ELISA 2 gave the best sensitivity when testing sheep and goat sera, but ELISA 3 had better specificity than ELISA 2 for goat sera. The TAGS

model gave a poor fit of test performance when testing cattle samples and, therefore, estimates of sensitivity and specificity in this species should be treated with caution.

Although ROC and TAGS analyses indicated similar sensitivity and specificity in ELISAs 1 and 2, the former correctly detected all known standards and positive control samples. However, it has been shown that the use of an ovine-derived antigen (ELISA 1) was more sensitive than that using tick-borne (Nine Mile)–derived antigen (ELISA 2; Rodolakis et al.: 2007, Is the *Coxiella burnetii* nine mile strain a suitable antigen for detection of CIF antibodies of ruminants by ELISA? *In*: Proceedings of the 13th International World Association of Veterinary Laboratory Diagnosticians Symposium, November 11–14, Melbourne, Australia), which supported the findings in the current study with sheep sera from known disease status flocks.

ELISA 1 was selected as having the best overall test performance and was subjected to further evaluation using a negative panel group of negative CFT sera with CFT titers of <1:10 to assess diagnostic specificity. ELISA 1 showed 98.7% agreement with sheep sera, 98.2% with cattle, and 83.6% with goats. Furthermore, ELISA 1 did not cross-react with sera from sheep that were infected with *C. abortus*, which in practice is likely to present the main differential diagnosis. One possible drawback is that ELISA 1 does not differentiate between field infection and vaccination (manufacturer's information sheet). However, Q fever vaccination is not currently practiced in the United Kingdom. Analytical sensitivity as measured by final end-point titers of known positive samples from all the animal species tested demonstrated that the detection limits were similar and comparable for both the ELISA 1 and CFT. Test reproducibility for ELISA 1 was also shown to be satisfactory. Final confirmation of the suitability of ELISA 1 came from the international ring trial results. In conclusion, the main advantages of ELISA over

the CFT include better sensitivity and specificity, elimination of AC results, and improved testing efficiency by use of automation methods. The VLA concluded that ELISA 1 had the best performance of the 3 ELISAs under trial.

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Declaration of conflicting interests

The authors declared that they had no conflicts of interests in their authorship and publication of this contribution.

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Table 1. Serum sample panel tested by serology, polymerase chain reaction, or immunohistochemistry for *Coxiella burnetii*.

Disease status of group*	No. of samples by species			Comments
	Sheep	Goats	Cattle	
Negative 1	65	0	0	A flock with no previous history of Q fever
Negative 2	1	0	0	AFSSA† serum standard
Negative 3	2	0	0	Ring trial samples
Positive 1	3	0	0	Ring trial samples
Positive 2	1	0	0	AFSSA serum standard
Positive 3	51	0	0	Seronegative animals from a Q fever–positive flock.* Same flock as Positive 4.
Positive 4	8	0	0	Seropositive sheep.* Same flock as Positive 3.
Positive 5	0	0	13	Cattle samples include 1 weak positive standard supplied by Laboratoire Service International, 10 samples from ring trial, and 2 Great Britain field samples
Unknown 1	18	153	233	Obtained from routine testing at the Veterinary Laboratories Agency
Total	149	153	246	

* Based on standard Veterinary Laboratories Agency case definition and clinical history/gross pathology and/or seropositive by complement fixation test (>1:80).

† French Food Safety Agency (http://www.anses.fr/index.htm).

Table 2. Manufacturers' enzyme-linked immunosorbent assay (ELISA) result interpretation (cut-off criteria) by sample-to-positive ratio.*

Kit	Negative	Inconclusive	Positive
ELISA 1	≤40%	Not applicable	>40%
ELISA 2	<30%	≥30% to <40%	≥40%
ELISA 3	≤40%	>40% to ≤50%	>50%

* Criteria apply to sheep, goats, and cattle.

Table 3. Receiver operating characteristic (ROC) analysis on all enzyme-linked immunosorbent assays (ELISAs) by all species as 1 group and individual species.*

	All species (n = 548)	Sheep (n = 149)	Cattle (n = 246)	Goats (n = 153)
ELISA 1				
AUC	0.833	0.755	0.903	0.867
SE	0.035	0.067	0.044	0.038
ELISA 2				
AUC	0.812	0.724	0.893	0.856
SE	0.038	0.076	0.049	0.042
ELISA 3				
AUC	0.788	0.632	0.861	0.802
SE	0.033	0.079	0.051	0.045
Chi square†	3.63	3.53	0.65	5.06
p value‡	0.163	0.171	0.721	0.080

* AUC = area under curve; SE = standard error.

† Test statistic indicating agreement between observed and predicted results.

‡ The probability of getting a value ≥ chi square with 2 degrees of freedom.

Table 4. Sensitivity and specificity by species as determined by receiver operating characteristic analysis using a fixed cut-off.*

Test/Species	n	Sensitivity (%)	Specificity (%)
ELISA 1			
Sheep	149	66.7 (30.0–92.5)	95.0 (90.0–98.0)
Goats	153	64.3 (35.1–87.2)	79.9 (72.2–86.2)
Cattle	246	81.3 (54.4–96.0)	93.9 (90.0–96.6)
Overall	548	71.8 (55.1–85.0)	90.4 (87.4–92.8)
ELISA 2			
Sheep	149	77.8 (40.0–97.2)	95.0 (90.0–98.0
Goats	153	71.4 (41.9–91.6)	71.9 (63.7–79.2)
Cattle	246	81.3 (54.4–96.0)	87.4 (82.4–91.4)
Overall	548	76.9 (60.7–88.9)	85.3 (81.9–88.2)
ELISA 3			
Sheep	149	22.2 (2.8–60.0)	97.9 (93.9–99.6)
Goats	153	7.1 (0.2–33.9)	89.9 (83.7–94.4)
Cattle	246	62.5 (35.4–84.8)	90.4 (85.9–93.9)
Overall	548	33.3 (19.1–50.2)	92.3 (89.7–94.5)

* ELISA = enzyme-linked immunosorbent assay. Figures shown in parentheses are 95% confidence intervals.

Table 5. Sensitivity and specificity results from all tests by all species as 1 group and individual species using TAGS (test accuracy in the absence of a gold standard) software analysis.*

	All species		Sheep		Cattle		Goats	
	Se (%)	**Sp (%)**	**Se (%)**	**Sp (%)**	**Se (%)**	**Sp (%)**	**Se (%)**	**Sp (%)**
ELISA 1	87.0	99.1	88.8	98.5	82.6	100	91.6	98.9
ELISA 2	98.6	97.1	100	99.6	100	98.9	93.1	91.2
ELISA 3	55.7	99.3	32.3	99.3	51.9	99.5	31.0	99.1
CFT	36.2	98.3	56.4	98.5	36.7	100	20.6	97.3
Chi square†	7.613		1.491		18.4		7.108	
p value‡	0.268		0.960		0.005		0.311	

* Se = sensitivity; Sp = specificity; ELISA = enzyme-linked immunosorbent assay; CFT = complement fixation test.

† Test statistic indicating agreement between observed and predicted results.

‡ The probability of getting a value ≥ chi square with 6 degrees of freedom.

Table 6. Distribution of complement fixation test (CFT) titers in sera from a negative and a positive sheep flock.

	CFT titers							
	<10	**10**	**20**	**40**	**80**	**160**	**320**	**AC***
Negative flock (n = 65)								
n	20	12	14	5	2	0	0	12
%	30.7	18.5	21.5	7.7	3.1	0	0	18.5
Positive flock (n = 59)								
n	12	15	9	2	1	1	1	18
%	20.3	25.4	15.3	3.4	1.7	1.7	1.7	30.5

* AC = anticomplementary reaction.

Table 7. Results of analytical specificity testing using Veterinary Laboratories Agency (VLA) standards.*

Chlamydophila abortus positive sample	CFT titer		ELISA 1 (ovine antigen)	
	C. abortus	Q fever	Q fever result	Sample-to-positive ratio (%)
VLA positive standard 1	1:1,024	Negative	Negative	0
VLA positive standard 2	1:512	Negative	Negative	6
VLA positive standard 3	1:512	Not available	Negative	0

* CFT = complement fixation test.

Table 8. Repeatability—intraplate variation.*

Species/Sample	No. of replicates	Mean sample-to-positive ratio (%)	Standard deviation	Coefficients of variation (%)
Sheep				
A	44	62	4.60	7.4
B	48	274	28.50	10.4
Goat				
A	38	106	9.81	9.2
B	38	111	10.49	9.41
C	30	63	4.39	6.9
D	30	105	9.95	9.47
Cow				
A	5	54	6.20	12.0

* Samples representing low, medium, and high test reactors were selected from the sample panel.

Table 9. Reproducibility—correct diagnoses obtained at 2 different laboratories.

Species/Sample no.	Mean sample-to-positive ratio (%)	Total no. of replicates	Laboratory 1* Operator		Laboratory 2* Operator	
			A	B	C	D
Sheep†						
1	5	8	100	100	100	100
2	99	8	100	100	100	100
3	61	8	100	100	100	88
Goat‡						
4	79	8	100	100	100	100
Cow‡						
5	65	8	100	100	100	100
6	76	8	100	100	75	100

* Results shown as a percent of correct results.

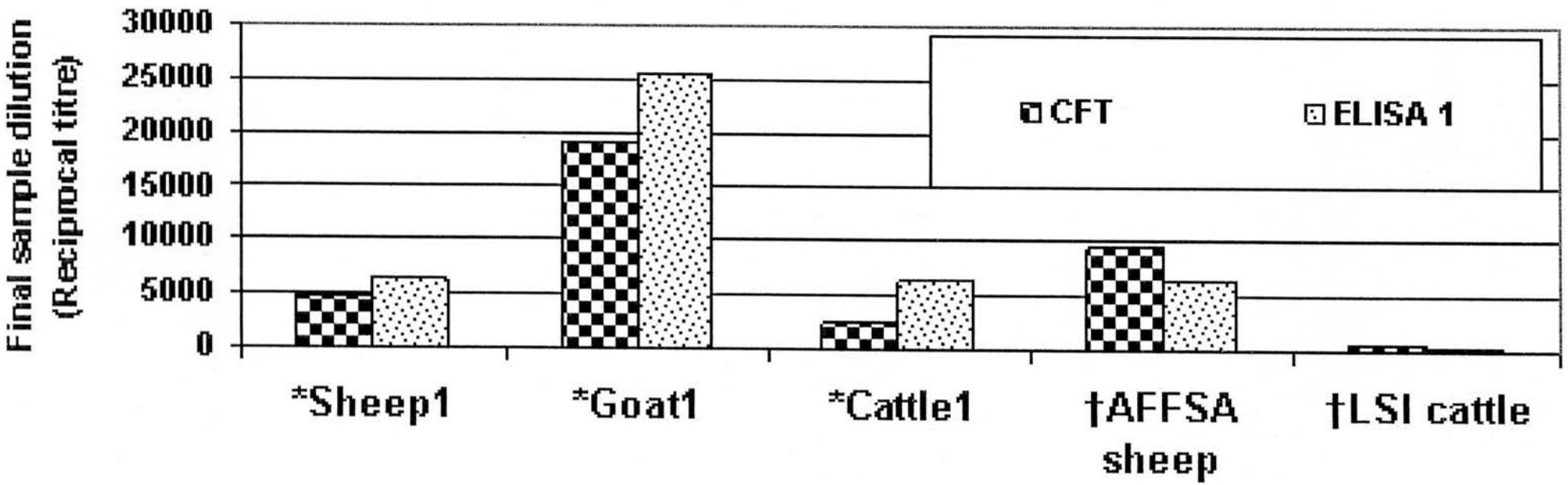
† Samples derived from known disease status groups in the sample panel.

‡ Samples derived from unknown disease status groups in the sample panel.

Figure legends

Figure 1. Graph to compare end-point titers of enzyme-linked immunosorbent assay (ELISA) 1 (ovine antigen) against complement fixation test (CFT) with representative field samples and recognized standards.

*Samples were derived from the unknown disease status group in the sample panel; the samples had previously been tested positive by ELISA 1 (ovine antigen) before being titrated to establish the end point titer (the last dilution that had a positive result). †Samples were derived from the known positive status group in the sample panel; the samples had previously tested positive by ELISA 1 (ovine antigen). AFFSA = French Food Safety Agency; LSI = Laboratoire Service International.


Final sample dilution (Reciprocal titre)
30000
25000
20000
15000
10000
5000
0
CFT
ELISA 1
*Sheep1
*Goat1
*Cattle1
†AFFSA sheep
†LSI cattle

2.38

World Association of Veterinary Laboratory Diagnosticians-14th International Symposium, Madrid, Spain, 17-20 June 2009

VALIDATION OF A REAL-TIME PCR TO DETECT *COXIELLA BURNETII*, THE CAUSATIVE AGENT OF Q FEVER

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Introduction

Coxiella burnetii, the causative agent of Q fever, is a gram-negative intracellular bacterium. *C. burnetii* can infect a wide range of animal species, including arthropods, birds and mammals. Infection is usually subclinical but associated abortions, stillbirths and weak offspring are occasionally observed. The bacterium can be shed intermittently by infected animals in birth products, milk, faeces and urine. Once excreted into the environment, *C. burnetii* can survive extracellularly in an infectious form for at least 150 days. Q fever can be spread between animals by the inhalation of contaminated aerosols and by the ingestion of contaminated material.

Q fever is zoonotic and infection in humans can either be acute or chronic. Chronic disease in humans may be fatal. In recent years, outbreaks of Q fever in humans have occurred both in the UK and across Europe, and the profile of this disease is rising. Outbreaks in humans are usually considered to be associated with airborne spread from infected ruminants, particularly during parturition. This makes it important to be able to diagnose the presence of infection accurately, particularly during outbreak infections.

We have therefore undertaken validation of a real-time PCR to detect *Coxiella burnetii* in ruminant (sheep, goats and cattle) abortion material (mainly cotyledons), which is considered to be an important potential source of human infection.

Material & Methods

Several different real-time PCRs described in the literature were compared to see if they were suitable for detecting the pathogen in the sample types of interest. The most suitable real-time PCR was then combined with an automated extraction to provide a rapid high-throughput assay. The characteristics of the assay such as analytical sensitivity and specificity were determined. The assay was then used to test a panel of 88 field samples of varying Q fever status. The real-time PCR results were compared to those obtained using other testing methods.

Results

The results of the validation demonstrated that the assay was highly suitable for detecting *Coxiella burnetii* in ruminant abortion material. The assay was shown to be highly sensitive and specific. The results from the testing of field samples will also be presented.

Discussions & Conclusions

We have validated a high-throughput sensitive assay which is suitable for detecting *Coxiella burnetii* in animal abortion material. This assay can be used both in the diagnosis of animal abortions and in the investigation of Q fever outbreaks.

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Papers

Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR

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A real-time PCR was developed to detect *Coxiella burnetii* (the cause of Q fever) in ruminant placentas and aborted fetuses. Primer and probe sets previously developed for human tissue studies were used to target the insertion sequence IS*1111* gene for *C burnetii*. The assay was highly sensitive, with a limit of detection of 10 copies of template, theoretically equating to a single bacterium, and did not cross-react with a panel of other bacteria. To determine sensitivity on field samples submitted for the diagnosis of abortion, results using the IS*1111* PCR assay were compared with a *com*1 PCR assay. When applied to ruminant abortion material, including placental cotyledons and fetal samples, the IS*1111* and *com*1 assays yielded positive results in 23 (25 per cent) of 93 and 19 (20 per cent) of 93 samples, respectively. One infected goat herd was monitored for 31 months: 57 (92 per cent) of 62 placental cotyledon samples from aborting and non-aborting goats, and 10 (30 per cent) of 33 fetal samples were positive by the IS*1111* PCR assay.

Q FEVER is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular bacterium classified within the order Legionellales and the class Gammaproteobacteria (Angelakis and Raoult 2010). Ruminants (primarily cattle, sheep and goats) are the main reservoirs of infection, although other animals can also be infected (Angelakis and Raoult 2010). Infected animals excrete *C burnetii* in the placenta, vaginal mucus, faeces and milk, which contribute to environmental contamination as well as presenting a zoonotic risk through close animal contact (Berri and others 2000, Sánchez and others 2006, Rodolakis and others 2007).

Infection in animals is almost entirely subclinical, but it can occasionally be associated with abortion and reproductive failures (Arricau-Bouvery and Rodolakis 2005). Human infection occurs most commonly through inhalation of contaminated aerosols and can cause a wide spectrum of acute and chronic clinical manifestations (Arricau-Bouvery and Rodolakis 2005, Angelakis and Raoult 2010), although most infection is asymptomatic. Human cases mainly follow occupational exposure, but large outbreaks can occur from windborne aerosol infection from livestock premises (Delsing and Kullberg 2008, Wallensten and others 2010).

Diagnosing *C burnetii* infection quickly and accurately in animal abortion provides early warning of zoonotic risk and allows enhanced control measures to be implemented. Isolation of the organism is not routinely available: it is time-consuming, hazardous and restricted to specialised laboratories (Berri and others 2003). Serological techniques are useful for herd or flock surveys but do not accurately identify currently shedding animals (Rousset and others 2009). *C burnetii* can be demonstrated microscopically in modified Ziehl-Neelsen (MZN)-stained placental smears or by immunohistochemistry, but these techniques are limited by specificity and sensitivity (Arricau-Bouvery and Rodolakis 2005, Sánchez and others 2006).

The Veterinary Laboratories Agency (VLA) receives a large number of samples, submitted each year by private veterinary surgeons from farms in England and Wales, and the findings are used for scanning surveillance purposes. To enhance detection, a real-time PCR assay (targeting the insertion sequence IS*1111* gene for *C burnetii*) was developed to offer improved diagnostic sensitivity and specificity in placental and fetal tissues.

Materials and methods

Primer and probe sequences

Primer and probe sets targeting the insertion sequence IS*1111* and the outer membrane protein *com*1 gene of *C burnetii* were used in two real-time PCR assays. The following primer and probe sequences were used: IS*1111* forward TAACGGCGCTCTCGGTTT; IS*1111* reverse TGCCGGGAACGATGAAA; IS*1111* probe FAM-TGATGAATGTCACCCACGCTCGCA-TAMRA; *com*1 forward AATCGCAATACGCTGCCAAA; *com*1 reverse AGCAGCGCGTCGTGGAA; and *com*1 probe FAM-AGCAGCCGCTAAACA-TAMRA. These primer and probe sets have previously been used in studies on human tissues (Harris and others 2000, Marmion and others 2005).

Assessment of sensitivity of IS*1111* real-time PCR

The potential sensitivity of the IS*1111* real-time PCR assay was determined through the preparation of a standard curve when the assay was applied to serial dilutions of the target element contained

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samples that were positive by both assays (data not shown). For the small number of fetuses from which multiple sample types were positive, a higher quantity of DNA was detected in placenta than in other samples – Cq values obtained between 14 and 15 cycles when testing placental cotyledon samples were much higher than Cq values obtained between 21 and 27 cycles when testing fetal fluid and other fetal tissue samples.

Goat herd study

A total of 62 placental cotyledon samples were tested, including three samples originally included in the abortion study, and 57 (92 per cent) of them were found to be positive by the IS*1111* PCR assay (Table 2). Seven fetuses were also examined, and 10 (30 per cent) of 33 samples from these were positive, mainly from fetal fluid and liver.

Discussion

The insertion sequence IS*1111* was selected as the PCR target because it is present in multiple copies in *C burnetii* (Hoover and others 1992, Klee and others 2006) and potentially has increased sensitivity, particularly over single copy targets. It is therefore used as the target for PCR assays in both human and veterinary diagnostic samples from many countries, and a variety of primer sets have been described (Fournier and others 1998, Berri and others 2000, 2003, Tang 2009). When applied to serial dilutions of plasmid DNA containing the target region in this study the assay could theoretically detect a single organism (10 target copies), which is in agreement with other studies targeting the same gene (Harris and others 2000, Klee and others 2006).

When applied to field samples, sensitivity of the IS*1111* PCR assay was measured against the *com*1 assay. The latter was selected because the specificity of this gene as a target for *C burnetii* has already been determined (Marmion and others 2005). The IS*1111* assay showed good sensitivity and detected 100 per cent of samples that were determined positive by the *com*1 assay; it also identified *C burnetii* in four samples that were negative by the *com*1 assay. Twelve of the PCR-positive field samples were from herds where infection had also been demonstrated by immunohistochemistry or serology (data not shown), providing further support to the accurate diagnosis of *C burnetii* infection by PCR.

The IS*1111* PCR assay showed good specificity for *C burnetii* by not cross-reacting with other bacteria, which is particularly important because some of these may be present in ruminant abortion samples. This specificity was further demonstrated when negative PCR results were recorded from some of the field abortion samples that were known to be infected with other organisms, including *Campylobacter* species and *T gondii* (data not shown).

A high prevalence of infection was demonstrated in samples collected during the 31-month survey on a known infected goat herd, regardless of the individual animal abortion status, which is in agreement with other studies (Berri and others 2007, Rousset and others 2009). Although the results cannot be extrapolated to other herds, it adds to previous evidence suggesting that goat herds with abortions may have a higher prevalence of shedding *C burnetii* (Rodolakis and others 2007). Birth products from infected animals, regardless of abortion status, provide a potential source of environmental contamination and zoonotic exposure to *C burnetii* and should, therefore, be handled carefully.

There has been much recent interest in the use of PCR for diagnosis and screening purposes, especially with the ongoing Q fever outbreak in the Netherlands (Delsing and Kullberg 2008, Karagiannis and others 2009, Schimmer and others 2009). This study confirms that the IS*1111* PCR assay previously used in human tissue studies can successfully be applied to ruminants, including abortion samples and placenta from normal parturitions. It is important to note that samples used in this study were from a biased population and were not collected as part of a structured epidemiological survey.

Acknowledgements

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Q FEVER ABORTION OUTBREAK IN A BRITISH DAIRY GOAT HERD

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SUMMARY

An abortion outbreak was investigated in a large dairy goat herd. Q fever, a zoonotic disease caused by *Coxiella burnetii,* was diagnosed from placental samples. Further serological testing during the outbreak demonstrated widespread infection within the herd. Continuous shedding of *C. burnetii* was demonstrated in placental samples collected over a 31-month period after the initial diagnosis was made. The high abortion rate reduced the number of kids available for subsequent breeding, which required additional replacements to be purchased from another herd. This resulted in the introduction of *Chlamydophila abortus*, which caused further abortions. The outbreak also raised public health concerns with potential exposure of farm staff, potential risk from consumption of contaminated milk and potential local spread to people. The local Health Protection Unit of the Health Protection Agency was notified and a Veterinary Laboratories Agency advisory farm visit was carried out to assess zoonotic implications; however, no human cases were identified that could be linked to this goat herd.

INTRODUCTION

Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular bacterium classified within the order Legionellales and gamma group of Proteobacteria (Angelakis and Raoult 2010). Ruminants are the main reservoirs of infection, primarily cattle, sheep and goats, although other animals and ticks can also be infected (Angelakis and Raoult 2010). Infection in animals is usually sub-clinical but can be associated with placentitis, abortion, stillbirth and the delivery of weak neonates (Arricau-Bouvery and Rodolakis 2005). Abortion outbreaks with high morbidity have been described in goats (Waldhalm and others 1978, Counter 1999, Vellema and others 2009). Seroprevalence in goats may be high, as demonstrated by random herd surveys (Cekani and others 2008, Rodriguez and others 2010) and investigation within herds where Q fever abortion has been diagnosed

(Counter 1999, Berri and others 2007, Rousset and others 2007). Since 2007, Q fever has become a serious public health problem in the Netherlands, primarily affecting people living within 5 km of dairy goat farms (van der Hoek and others 2010). This paper describes an outbreak of Q fever in a British dairy goat herd.

CASE REPORT

Farm details

The herd consisted of approximately 1,000 home-bred does and had been established for over ten years. It had been experiencing a phase of rapid expansion in milking goat numbers when the outbreak started. Adult goats were permanently housed in large, clean, well bedded pens with good airflow and air space. Home-produced straw bedding was applied daily. Sheds were cleaned out every six weeks and manure was stacked for between one and nine months before being ploughed into ground used for producing conserved forage which was fed to goats. After cleaning out, the sheds were not washed and disinfected as they needed to be restocked immediately. Young replacement kids were moved to a separate site within one week of birth and re-introduced to the main adult herd shortly before kidding. Routine herd health procedures included louse control using a pyrethroid product and vaccination against Johne's disease, clostridial diseases, and pasteurellosis, but not any abortifacients.

Clinical history

An abortion outbreak started in February 2007, affecting mainly first parity goats. Most of the abortions occurred 4-6 weeks before the expected parturition date. Abortions also occurred in late 2008 and early 2009, following the introduction of new breeding stock from another herd in June 2008. The numbers of kiddings and abortions for 2005-2009 are summarised in table 1, indicating a rise in abortion rate in 2007-2009 over previous years. Goats were otherwise healthy and milk production was not adversely affected.

Table 1: Number of kiddings and abortions for 2005-2009.

Year	Total number of kiddings, including abortions	Number of abortions (% of all kiddings)
2005	1,165	36 (3)
2006	1,015	42 (4)
2007	1,159	116 (10)
2008	1,132	136 (12)
2009 (to end of March)	442	195 (44)

Laboratory investigation

Q fever was confirmed during the 2007 abortion outbreak. Tests were carried out on aborted fetuses and placental samples using standard methods

adopted by the Veterinary Laboratories Agency (VLA) (Harwood 2007). Three placental samples were grossly swollen with oedema, suppuration and gritty deposits. Acid-fast bacteria were demonstrated by modified Ziehl-Neelsen (MZN) staining of placental smears. On histopathology, necrotising placentitis in all three samples was characterised by trophoblast degeneration and necrosis with accumulation of fine cytoplasmic inclusions and perivascular neutrophilic cuffs. The inclusions were confirmed as *C. burnetii* by immunohistochemistry. Additionally, PCR tests for *C. burnetii* and *Chlamydophila* spp yielded positive and negative results, respectively. *Brucella* spp. were not isolated.

To determine if infection was likely to be widespread within the herd at that time, blood samples were collected from 33 adult goats during the abortion outbreak. These included animals that had and had not aborted. Samples were tested by CFT for *C. burnetii* antibodies. The results are summarised in table 2. The number tested was sufficient to demonstrate high prevalence within the herd. Titres of 1/80 or above are associated with infection.

Table 2: Serological results from adult goats during the Q fever outbreak in 2007 (n = 33).

Q fever CFT titre	<1/10	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,560
No. of animals with titre (No. that had aborted)	9 (2)	1 (1)	5 (4)	12 (5)	1 (1)	3 (1)	1 (0)	1 (1)

The outbreak resulted in loss of replacement kids, therefore more females were bought, mostly in June 2008, from a herd that had previously vaccinated against chlamydial abortion. After the purchase of new goats, a second wave of abortions occurred, starting in October 2008. Acid-fast bacteria were demonstrated in two placental samples from aborted goats and confirmatory PCR tests demonstrated both *C. burnetii* and *Chlamydophila* spp in one sample, and *C. burnetii* alone in the other. Serology of 14 aborted animals was used to assist diagnosis. The results are summarised in table 3. More animals were seropositive to *C. abortus* (minimum significant titre of 1/32) than to *C. burnetii*, suggesting that *C. abortus* was the likely cause of most of these abortions, probably introduced with the recent purchase of breeding females; however, the possibility of both pathogens causing abortions could not be ruled out. The definitive aetiological role of *C. abortus* was later confirmed by immunohistochemistry. A vaccination programme for *C. abortus* was started after infection was confirmed.

Table 3: Serological results from aborting goats in October 2008 (n = 14).

A: serological response to *C. burnetii*

Q fever CFT titre	<1/10	1/10	1/20	1/160	1/320			
No. of animals with titre	9	2	1	1	1			

B: serological response to *Chlamydophila* sp

Chlamydophila CFT titre	1/4	1/8	1/32	1/64	1/128	1/256	1/2,048	1/4,096
No. of animals with titre	2	2	1	1	1	1	1	5

Abortions continued into early 2009. Acid-fast bacteria were demonstrated by MZN staining of a placental smear from an aborted goat and PCR tests demonstrated *Chlamydophila* spp but not *C. burnetii*. Further blood sampling of 23 aborted animals identified that none had antibody to *C. burnetii* but all were seropositive to *C. abortus* (Table 4).

Table 4: Serological results from aborting goats in 2009 (n = 23).

Chlamydophila CFT titre	1/32	1/128	1/256	1/512	1/1,024
No. of animals with titre	2	2	8	8	3

Abortions declined significantly from April 2009, at which point all kiddings were from *Chlamydophila*-vaccinated goats.

Further epidemiological studies

Two studies were carried out to assist the understanding of the epidemiology of Q fever in this herd. Firstly, placental samples were collected from goats, following abortions and normal kiddings, and screened by PCR assay (Jones and others in press) to determine if continuous shedding of *C. burnetii* occurred after the abortion outbreak started in 2007. Secondly, replacement goat kids were serially blood sampled and tested by CFT to determine the risk period for infection (i.e. the point at which animals seroconverted).

i) Screening of placental samples

Sixty-two placental cotyledon samples were collected after the initial Q fever diagnosis, from aborting and non-aborting goats between April 2007 and October 2009, 57 of which were PCR-positive (Jones and others in press), demonstrating continuous shedding of *C. burnetii* over the 31 months that samples were collected.

ii) Serology of replacement goats

As replacement kids were reared at a separate site, and because mostly primiparous goats were affected by Q fever, it was considered possible that immunologically naïve pregnant goats were exposed to *C. burnetii* for the first time on returning to the milking herd. To investigate this possibility, it was intended to collect blood samples every month from a group of 12 replacement kids from one week of age until after they joined the milking herd, and to screen these for serological evidence of infection to determine when exposure might have occurred. Unfortunately, some of the original group of 12 were lost to follow up, so a larger group of 42 goats was included in the blood test at nine months of age immediately before moving to the main herd. Most of these were retested at either one month (29 goats) or three months

(11 goats) post-movement and 25 goats were also sampled ten months post-movement.

One kid was seropositive at one week of age with a titre greater than 1/10,240, which subsequently decreased and was undetectable by four months of age. It is unclear if this represented colostral antibody transfer or early infection. Two other kids, which previously had undetectable antibody titres, were seropositive at four months of age both with titres of 1/80. No significant antibody rises were detected in any goat following movement to the main herd. No conclusive observations could be made from this study, but the identification of seropositive kids during rearing raised the possibility that infection might have been circulating at this phase of production.

ZOONOTIC ASPECTS

This outbreak raised public health concerns. Potential risks included close contact of farm staff with infectious material, human consumption of contaminated milk and local spread, possibly aerosol transmission, to people outside of the farm. When Q fever was first confirmed, the local Health Protection Unit of the Health Protection Agency was notified and a VLA advisory farm visit was undertaken to assess zoonotic implications. The zoonotic aspects of Q fever in goats have been discussed recently at the Goat Veterinary Society (Pritchard 2009). Sources of infection include the placenta, birth fluids, vaginal mucus, milk, faeces and contaminated bedding. Prevention of human disease involves avoiding exposure to these, following the guidance published on the HPA and Defra/VLA websites (www.hpa.org.uk, www.defra.gov.uk/vla), and recommendations were made to adopt these hygienic procedures into the daily routine on the goat farm. Since this investigation, advice has also been published on the European Food Safety Authority (EFSA) website, which was issued following recent increases in animal and human Q fever, particularly in the Netherlands (www.efsa.europa.eu/en/scdocs/scdoc/1595.htm).

The owner was advised of the clinical signs associated with Q fever and to report any suspicious human disease to a medical practitioner. Six people had regular contact with goats and none developed any suspicious clinical illness. In relation to transmission from milk, the risk associated with this route is minimal compared with occupational or environmental aerosol exposures, but it was reassuring that all milk from this herd was pasteurised before retailing for human consumption and none was consumed unpasteurised on farm. The risk of aerosol transmission to the local human population was also considered; however there were no reports of increased numbers of human cases of Q fever in the same geographic area, and the only British human outbreak reported during 2007 occurred in Cheltenham, at least 100 km from this goat herd (Wallensten and others 2010).

ADVICE ON Q FEVER CONTROL IN GOATS

Advice on the control of Q fever in goats was centred on the guidelines for preventing zoonotic disease discussed above, especially safe disposal of

potentially contaminated material after abortions and normal kiddings. Isolation of animals likely to be shedding the organism was recommended, for example those that had recently aborted, but this was not possible due to practical restrictions for housing and milking; however, given the likelihood of widespread infection and the retrospective knowledge that many placental samples were PCR-positive for *C. burnetii*, isolation of aborted goats might not have had a significant effect in this case. More frequent cleaning out of sheds to remove potentially contaminated bedding material was also recommended, but this was difficult to implement due to practical limitations on housing space. After the diagnosis of Q fever had been made, prophylactic oxytetracycline injections were administered to pregnant goats in an attempt to reduce the abortion rate. The benefit of these injections could not be measured as no untreated control animals were used; however, recent reports suggest that treatment of goats and other small ruminants with oxytetracycline has little beneficial effect for controlling *C. burnetii* (Wouda and Dercksen 2007, Astobiza and others 2010). The limited knowledge of effective control measures is a concern that is highlighted in the EFSA report mentioned above.

DISCUSSION

This investigation highlights the ability of *C. burnetii* to cause a severe abortion outbreak in goats, assuming that it was the main cause of abortions in 2007. When Q fever was first confirmed in this herd, there was serological evidence of widespread infection, which is consistent with other reports of high seroprevalence in herds where Q fever abortion has occurred (Counter 1999, Berri and others 2007, Rousset and others 2007). The original source of infection was not determined, but following initial confirmation it persisted in the herd for at least 31 months, as determined by PCR testing of placental samples. It is possible that chronic endemic infection with *C. burnetii* was present before the 2007 outbreak. This is supported by the observation that mostly primiparous goats were affected, and the outbreak possibly reflected the increased number of younger, immunologically naïve goats retained for breeding in this expanding herd. Although the infection risk for replacement goats entering the main herd could not be demonstrated by serial serology, this possibility cannot be discounted solely on the basis of the results from the single cohort tested.

Chlamydial abortion was an unfortunate consequence of purchasing replacement stock, to compensate those lost through Q fever abortions, and added to the overall financial impact of the outbreak. The diagnosis of this second abortifacient demonstrated the value of continuous monitoring of abortion samples to identify newly introduced infections. The simultaneous occurrence of multiple abortifacients should always be considered when interpreting MZN-stained placental smears: *C. burnetii*, *Chlamydophila abortus* and *Brucella* spp are all acid-fast abortifacients (Arricau-Bouvery and Rodolakis 2005) and definitive confirmation requires further tests, such as immunohistochemistry, PCR and culture.

Although serology was a useful ancillary test for diagnosing *C. burnetii* infection, the presence of antibody was not always associated with clinical disease and infection of the placenta was not always associated with seroconversion (data not shown). The latter observation probably reflects the CFT used, which has lower sensitivity than some other serological tests (Rousset and others 2007, Kittelberger and others 2009) and, to improve accuracy, it will soon be replaced at VLA by a recently validated ELISA.

Although this outbreak was not associated with human disease, the potential zoonotic risk should always be considered when Q fever is diagnosed in animals. The public health profile of this disease, including the role of goats in zoonotic transmission, has recently been elevated by a serious ongoing human epidemic in the Netherlands, primarily affecting people living within 5 km of dairy goat farms (van der Hoek and others 2010). Surveillance systems for Q fever should therefore be maintained both in animal and human populations.

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2.41

SHORT COMMUNICATIONS

Possible zoonotic transmission of toxigenic *Corynebacterium ulcerans* from companion animals in a human case of fatal diphtheria

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Corynebacterium ulcerans is a bacterium with a worldwide distribution and broad host range. The organism has been recovered from clinically affected and healthy wild and domesticated animal species, as reviewed by Tiwari and others (2008). In the UK, De Zoysa and others (2005) reported the isolation of C *ulcerans* from cats with a nasal discharge. It is a potential, although infrequent, cause of mastitis in cattle (Wilson and Richards 1980); udder infection may be prolonged and accompanied by an increased milk somatic cell count (Hedlund and Pohjanvirta 1989).

Human beings can harbour C *ulcerans* in the pharynx without developing symptoms. Many strains of the organism produce a diphtheria toxin that is immunologically identical to that of *Corynebacterium diphtheriae* (Lipsky and others 1982), and it has been associated with cases of classical diphtheria, pharyngitis, sinusitis, and extrapharyngeal disease; in some cases, disease has been fatal (Tiwari and others 2008). The consumption of raw milk products is a recognised risk factor for human infection (Galbraith and Barrett 1986). However, most recent cases in the UK have had no identifiable source, suggesting that there may be other routes of infection (De Zoysa and others 2005). This short communication reports the possible zoonotic transmission of toxigenic C *ulcerans*, identified during the course of a joint medical/veterinary investigation of a fatal human case of diphtheria.

Signs of diphtheria were detected in an elderly woman, who became ill and was hospitalised. She died despite being given diphtheria antitoxin and antibiotics. A bacterial isolate from the woman's pharynx was submitted to the Health Protection Agency (HPA) Streptococcus and Diphtheria Reference Unit (SDRU), Colindale, and was confirmed as toxigenic C *ulcerans* using standard methods; the isolate was biotyped using the API Coryne system (bioMérieux) and tested for toxin production by the conventional Elek immunoprecipitation test (Efstratiou and George 1999).

Person-to-person transmission of C *ulcerans* is thought to occur only rarely, but laboratory investigations and the public health response to diphtheria caused by toxigenic C *ulcerans* should be similar to those for C *diphtheriae*, as it is important to determine the source of infection (Bonnet and Begg 1999). In this case, an Outbreak Control Team (OCT), led by a consultant in communicable disease control at the HPA, was formed. The OCT identified a number of possible sources of infection, including animals on a 106-cow dairy farm where the patient had stayed with family shortly before becoming ill. Staff from the Veterinary Laboratories Agency (VLA) visited the farm at the request of the OCT, and pharyngeal swabs were taken from four dogs, two cats and two guinea pigs; samples were also obtained from the bulk tank milk and bulk tank milk filter. In addition, individual quarter milk samples were taken aseptically from eight cows that had elevated milk somatic cell counts consistent with subclinical mastitis. A pharyngeal swab was also collected from a neighbour's dog, with which the patient had had contact in the seven days between leaving the farm and becoming ill.

The milk samples were all cultured on blood agar, incubated aerobically, and on Hoyle's tellurite agar, incubated both aerobically and anaerobically. C *ulcerans* was not isolated from any of the milk samples. The bulk tank milk filter swab and the pharyngeal swabs from the nine pet animals were examined by the HPA SDRU, using their standard methods (HPA 2005). Toxigenic C *ulcerans* was isolated from two of the farm dogs (siblings identified as D1 and D2), but not from any of the other samples. Molecular typing (ribotyping) confirmed that the isolates from the patient and the two dogs had indistinguishable ribotype patterns, all U1 (De Zoysa and others 1995).

In view of these findings, the owner was advised to isolate dogs D1 and D2, which had previously had direct or indirect contact with other cats and dogs and people on the farm. Although the owner was protected by previous diphtheria vaccination, other members of his family were not immunised, and it was thought appropriate to attempt to eliminate carriage of the organism in the animals by antibiotic treatment. The C *ulcerans* isolates from dogs D1 and D2 were found to be sensitive in vitro to penicillin, erythromycin, tetracycline, ciprofloxacin, clarithromycin, moxifloxacin and levofloxacin in tests carried out at the HPA SDRU (at breakpoint concentrations judged by the British Society of Antimicrobial Chemotherapy to be sensitive for human beings). Penicillin has previously been reported to be ineffective in eliminating C *ulcerans* infection in a dog in France (Lartigue and others 2005). Following discussions between the OCT and the local veterinary practitioner, metaphylactic oral treatment of all of the cats and dogs on the farm with 5 mg/kg bodyweight enrofloxacin (Baytril; Bayer), administered daily for 14 days, was instigated. Enrofloxacin is metabolised to ciprofloxacin in vivo (Cester and Toutain 1997).

Two weeks after the end of the treatment period, all the cats and dogs on the farm were re-sampled by pharyngeal swabbing. Toxigenic C *ulcerans* was again isolated from dog D1, and from one of the cats (identified as C1) that lived apart from the other animals in the house; the isolate from the cat was also shown to have the ribotype pattern U1. All the cats and dogs on the farm were swabbed again one week later in an attempt to clarify whether they were excreting the organism. On this occasion, toxigenic C *ulcerans* was recovered only from the pharynx and nostril of dog D1, which had developed a unilateral serosanguineous nasal discharge and ulceration of the mucocutaneous junction of the same nostril.

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All the cats and dogs were given a second course of oral antibiotic treatment, consisting of 23·4 mg/kg bodyweight of a combination of spiramycin and metronidazole (Stomorgyl; Merial Animal Health), administered daily for 10 days. Spiramycin is similar to erythromycin and is the only macrolide licensed for oral use in cats and dogs in the UK. Cat C1 developed diarrhoea after five days and the treatment was discontinued. Dogs D1 and D2 were re-swabbed two weeks after the end of the treatment period and C *ulcerans* was not isolated; the nasal discharge and ulceration in dog D1 had resolved. At the request of the owners, the other animals were not resampled.

Cases of diphtheria are rare in the UK, and human infection with toxigenic C *ulcerans* strains has become more common than infection with toxigenic C *diphtheriae* strains (De Zoysa and others 2005). Vaccination in childhood should prevent disease caused by both organisms (Tiwari and others 2008) but may not convey lifetime protection (Kjeldsen and others 1985), and elderly people may be more susceptible (Edmunds and others 2000). Recent reports suggest that C *ulcerans* may be an emerging human pathogen, although its epidemiology is not well understood; in 22 of 28 case reports of diphtheria-like illness around the world, the source of human infection was not identified, and only three of these cases were attributed to the consumption of milk (Tiwari and others 2008). Raw milk has not been reported as a source of human C *ulcerans* infection in the UK for more than 20 years, and the organism is very rarely isolated from diagnostic mastitic milk samples submitted to the VLA laboratories. In this case, no evidence was found of C *ulcerans* infection in the dairy herd on the farm. Inquiries confirmed that the human patient had not consumed any raw milk from the herd during her visit to the farm, and raw milk was not fed to the cats and dogs on the farm.

Animals have recently been identified as possible sources of human C *ulcerans* infection. De Zoysa and others (2005) reported that toxigenic isolates from cats with a nasal discharge were found to have the predominant ribotype patterns observed among human clinical isolates (including U1, a common human type), suggesting that cats could be a potential reservoir for human infection in the UK. Lartigue and others (2005) and Aaron and others (2006) reported suspected transmission of toxigenic strains of C *ulcerans* from dogs to human beings in France. In the case described by Lartigue and others (2005), a dog showed evidence of chronic labial ulceration, sneezing and rhinorrhea, and the isolates obtained from the dog and the patient had indistinguishable ribotype patterns. C *ulcerans* ribotype pattern U1 has also been found in otters in the UK (Engler and others 2001). In the case reported here, the recovery of indistinguishable ribotype strains of toxigenic C *ulcerans* from the patient and the companion animals in the household suggests interspecies transmission. No other sources or associated cases of human infection were detected.

In investigations of this type, the number of potential contacts between human patients and animals may be large. The practical and financial implications of sampling animals for testing, and subsequent treatment, must be considered. Pharyngeal swabs, especially those from asymptomatic carriers, may yield a mixed bacterial flora, requiring the use of selective culture media such as Hoyle's tellurite (Efstratiou and George 1999). Few veterinary laboratories have experience of using such media, and animal samples may need to be sent to public health laboratories for investigation. In addition, national guidelines recommend that laboratory workers who may be exposed to diphtheria should be protected by immunisation, and that cultures of toxigenic C *ulcerans* should be handled in a Class 1 safety cabinet (Efstratiou and George 1999).

Treatment of infected companion animals, with the aim of eliminating subclinical carriage of toxigenic C *ulcerans*, may be appropriate in situations such as this case. The Zoonoses (Monitoring) (England) Regulations (2007) provide for powers of entry to all premises, including private dwellings, to determine whether any zoonotic agents are present, but there are no legal powers for restraint or treatment of companion animals. This is particularly relevant if an animal known to be carrying a zoonotic pathogen is not showing clinical signs. Treating such animals with antibiotics at the request of the public health authorities potentially raises a number of ethical issues, especially if it is likely to cause unnecessary stress to the animal. Voluntary compliance with relevant control measures should be discussed with the animal's owners and their veterinary surgeon. As prolonged courses of antibiotics can be costly, the matter of who pays for animal treatments should also be considered, as owners are under no obligation to treat asymptomatic animals.

In the UK, veterinary surgeons supplying antibiotics must follow the prescribing cascade and be guided by sensitivity tests. However, most antibiotics used by public health microbiology laboratories in sensitivity tests are not licensed for veterinary use, and the drug concentrations used may be inappropriate for use in animals. As well as penicillin, the macrolide erythromycin is the preferred antimicrobial medicine for treating diphtheria in human beings caused by C *ulcerans* (Tiwari and others 2008); however, there is no licensed erythromycin product for use in cats and dogs in the UK, and gastrointestinal disturbances may occur as a side effect of treatment, as with some other macrolides. In the present case, enrofloxacin failed to eliminate infection in all the treated animals, but treatment with spiramycin (in combination with metronidazole) appeared to be effective in eliminating persistent C *ulcerans* infection in dog D1.

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2.42

Toxigenic *Corynebacterium ulcerans* infection in a veterinary student in London, United Kingdom, May 2010

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We report on a case of toxigenic *Corynebacterium ulcerans* infection in a fully immunised veterinary student, investigated in London, United Kingdom, in May 2010. There was no ongoing transmission in human contacts. Possible animal sources were identified.

Introduction

Diphtheria can result in an acute infection of the upper respiratory tract or a cutaneous lesion. It is caused by three toxin-producing *Corynebacterium* species: *C. diphtheriae* responsible for epidemic disease, *C. ulcerans* and, more rarely, C. pseudotuberculosis, the last two being zoonotic infectious agents [1,2].

In the United Kingdom (UK) between 2000 and 2009, 43 isolates of toxigenic corynebacteria were identified in England and Wales, 27 of which were *C. ulcerans*. Three unvaccinated individuals presented with clinical symptoms typical of classic diphtheria, but most had milder respiratory infections (sore throat or tonsillitis) and had been either partially or fully immunised [3,4].

Cattle are a documented reservoir for *C. ulcerans* and risk factors for human infection with this species include contact with these and other farm animals [5]. Less commonly, *C. ulcerans* can also be spread through consumption of unpasteurised milk and unpasteurised milk products [6]. More recently, contact with dogs and other companion animals has also been proposed as a source of infection [7-9]. Despite limited evidence of person-to-person spread of *C. ulcerans*, this is a potential route of transmission [6,10]. It is therefore recommended that the public health response following isolation of toxigenic *C. ulcerans* from a human case be the same as that for toxigenic *C. diphtheriae* [6].

A primary course of diphtheria-containing vaccine is included in the UK's vaccination schedule and is given at the age of two, three and four months, plus a preschool booster between the age of three years four months and five years. This is followed by a booster between the age of 13 and 18 years [11].

Case report

A 20-year-old veterinary student in London, United Kingdom, with no significant medical history, experienced four to five weeks of recurrent sore throat and tonsillitis unresponsive to a full course of penicillin towards the end of March 2010. As symptoms remained, the case was reviewed by the family doctor and a throat swab was taken from white tonsillar lesions on 10 May 2010.

The local Health Protection Unit (HPU) was notified by a hospital microbiology registrar on 17 May 2010 that the throat swab had grown *C. ulcerans*. A second throat swab, taken on 18 May, when the case was still symptomatic was then sent to the Streptococcus and Diphtheria Reference Unit for primary culture and toxigenicity testing. The case was clinically assessed by the family doctor and, in consultation with the HPU and microbiology registrar, a course of erythromycin was started on 18 May 2010. The sample was confirmed as toxigenic on 21 May 2010.

The case had received a full recommended UK course of immunisation with diphtheria toxoid, plus a booster at 14 years. The family doctor was advised by the HPU to offer a convalescent diphtheria booster vaccination as part of the routine management of the case.

In-depth discussions with the case confirmed that there was no exposure to unpasteurised milk and no recent overseas travel.

As part of a veterinary medicine undergraduate course, the case had been in recent contact with animals on two separate farming placements. The date of symptom onset was determined to be during a lambing placement at a farm in the last two weeks of March 2010.

Risk assessment of human contacts

All close contacts were followed up as indicated by national guidelines [6].

The risk assessment identified four household contacts sharing college accommodation, two close contacts and a partner. One household contact had mild symptoms (sore throat). Four of these seven contacts had records of a full course of immunisation for diphtheria, while the others had no record of past immunisations against diphtheria (including the contact with mild symptoms).

Three family members were also identified as contacts, as the case had stayed with family during the farm placements. One family member had developed a sore throat after the case's onset of symptoms. A complete immunisation record was found for two of these family contacts, including the one who developed a sore throat.

Throat swabs were taken from all of these contacts, who were also provided with prophylaxis and advised on signs and symptoms of the infection by their family doctor. Family doctors were advised by the HPU to offer a booster vaccination if none had been received in the previous 12 months. All swabs were found to be negative for *C. ulcerans*.

One further asymptomatic contact who accompanied the case during the lambing placement (and was therefore subject to the same potential exposure) was also identified. As all samples of contacts had been negative for *C. ulcerans*, a throat swab was taken, but no prophylaxis or vaccination given. This throat swab was also negative.

Risk assessment of animal contacts

A detailed risk assessment of the two farming placements was undertaken by the local HPU, in close collaboration with the Veterinary Laboratories Agency, to determine if a likely source of infection could be identified and if there was any ongoing risk to the public or farm owners and workers.

The second placement commenced after symptom onset and was therefore disregarded as a possible source of infection. The first was a lambing placement on a farm with 800 sheep. However, enquiries to the farm's veterinarian established that the usual standard of animal health and hygiene on the farm was high, there were no reported health problems in the sheep flock during lambing and there were no dogs (working or pets) on the farm. The farm owners and workers were all reported to be fit and well and their contact with the sheep and lambs was determined to be minimal outside lambing season, which had ended some weeks before the case was notified to the HPU. Moreover, this farm is not open to the public. Consequently, the ongoing risk to human health was assessed to be very low and no further investigations were deemed to be necessary.

The case had been in close contact with a number of domestic animals (dogs, cat, rabbit and chickens) during the same period as the lambing placement. However, none of these were reported to have exhibited any clinical signs suggestive of *C. ulcerans* infection and, in view of the time lapse and large number of potential exposures, it was not feasible to undertake any animal sampling [7].

Discussion

Infection with a toxigenic strain of *C. diphtheriae* or *C. ulcerans* can cause serious illness in unvaccinated individuals: five deaths from diphtheria have been recorded in the UK since 1986, three of them due to *C. ulcerans* infection [10]. The number of clinical cases is small and there have been no previous toxigenic corynebacterial isolates from veterinarians. There is no booster vaccination for the general adult population, and the severity of the infection can be especially serious in elderly people in contact with pets [12,13].

While the source of infection for this case was not proven, the lambing farm or domestic animals were considered to be the most likely sources. Domesticated animals have been implicated as potential reservoirs of *C. ulcerans* and the organism is a recognised commensal in several animal species, usually without overt clinical signs, although it has been associated with nasal discharge in cats [5-8]. Furthermore, the antibiotics licensed to eliminate the pathogen in humans are often not licensed for animals. As discussed previously by other authors, a number of difficult issues arise in attempting to eliminate subclinical zoonotic infections from healthy animals for perceived public health reasons [7]. It may be more appropriate to ensure that occupationally exposed groups, such as veterinary students, are appropriately immunised.

The case presented here had been fully immunised against diphtheria. This probably explains the relatively mild presentation of symptoms, as the vaccine provides protection against the effects of toxin produced by the bacteria. Most UK laboratories will only screen for corynebacteria if there is a clinical indication of diphtheria and/or contact with a known case, which suggests that other mild diphtheria cases may be missed. Indeed, the delay in initial diagnosis of this case was due to the low level of clinical suspicion.

Conclusions

Toxigenic *C. ulcerans* infection remains rare, but it can be fatal, especially among those who are not

immunised. As animal sources can be difficult to identify and control, maintenance of high vaccination coverage among the child and adult populations is essential.

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2.43

A case of human leptospirosis linked to pet rats

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BACKGROUND

- Leptospirosis is a relatively uncommon zoonotic infection in England and Wales with an average of less than 40 laboratory confirmed human cases annually (Source: HPA).
- *Leptospira interrogans* is subdivided into numerous serovars and further classification is possible with molecular typing.
- In the UK, clinical disease in animals is mainly seen in dogs (kidney and liver disease), cattle (milk drop, reproductive failure) and pigs (reproductive failure).
- *L. icterohaemorrhagiae* infection can cause liver disease in a wide range of species, including man (Weil's disease).
- Wild animals, especially rodents, are common carriers and reservoirs of infection but are clinically unaffected.
- Although wild rats are known to be frequently infected[1], pet (fancy) rats are considered to be free of infection.
- Infection is acquired from direct or indirect contact with infected animal urine; incubation period in man varies from 3 to 30 days.
- Serosurveys in the USA have demonstrated evidence of past infection in up to one third of people tested[2].
- It is usually a mild, self-limiting flu-like illness in man although Weil's disease results in haemorrhages and hepato-renal failure.
- There are 1 or 2 deaths per year from leptospirosis in the UK.

CASE REPORT AND INVESTIGATION

- In July 2006, the Veterinary Laboratories Agency (VLA) diagnosed leptospira infection in 2 pet rats and reported it to HPA.
- The owner of the rats (index case) had been in hospital with hepato-renal failure. Weil's disease was suspected.
- The 2 rats were from a group of 4 (from litter of 18) brought in by a "Saturday girl" and distributed through a pet shop earlier in year.
- The pet shop did not usually sell rats (just hamsters, gerbils, mice, rabbits) but shop owner kept 2 pet rats.
- The animals offered for sale were healthy; pet shop met Health and Safety requirements (enforced by Local Authority).

Figure 1: Timeline of key events

CONTROL MEASURES ADOPTED

- Pet shop visited by a local animal warden, EHO and CCDC.
- Shop owner advised to remove all (14) small rodents from sale and from public access; subsequently agreed to test these, plus his own 2 rats. This required culling to undertake PCR on kidney tissue.
- Litter mates of infected rats were traced and owners given advice on leptospirosis control, hygiene measures and testing.
- The 2 other sibling rats (from the original litter of 18) distributed from the pet shop were also culled for laboratory testing.
- Of the remaining 14 sibling rats: one had died, 6 were untraceable and 7 were traced but the owners declined testing. The mother of the index rats was traced but testing was also declined.
- The owners of potentially infected rats were advised that they should not be handled by other people, not used for breeding and not mixed with other rats.

LABORATORY TESTING

- *L icterohaemorrhagiae* infection was confirmed in the index case following serological testing at the hospital where being treated.
- Initial screening of the rats used LightCycler Real Time PCR which detects a 370bp amplicon of the 16S rRNA gene of pathogenic leptospires[3].
- Re-amplified purified DNA template was sequenced to confirm the presence of pathogenic leptospires.
- Speciation was undertaken using Denaturing High Pressure Liquid Chromatography (DHPLC)[4].
- Indistinguishable molecular profiles consistent with one of five serovars including *L icterohaemorrhagiae* found in PCR positive rats.
- Two rats were also tested by serology using a microscopic agglutination test (MAT)[5]. Very high titres (1/1600 and 1/400) for *L. icterohaemorrhagiae* were detected.

Table 1: Summary of results of laboratory testing of animals

Specimen	PCR/DHPLC (Kidney)	MAT (Sera)
Index rat 1	POSITIVE	Not done
Index rat 2	POSITIVE	Not done
Sibling rat 1	POSITIVE	POSITIVE
Sibling rat 2	Negative	POSITIVE
Resident rat 1	POSITIVE	Not done
Resident rat 2	Negative	Not done
Assorted small rodents (14)	Negative	Not done

Figure 2: Rodent contact tracing and testing

CONCLUSIONS AND RECOMMENDATIONS

- The source of infection was identified as contact with pet rats. This conclusion was based on the most comprehensive range of animal tests currently available (VLA are also developing multi locus variant analysis (MVLA) to further improve serovar identification).
- We were unable to determine whether the primary source of infection was the 4 litter mates brought into the shop, the resident rats or whether the two groups were independently infected.
- It seems possible that infection of fancy rats may be more common than previously thought and further research is indicated in this field.
- Serological surveys are needed to compare the level of past exposure in rat fanciers compared with the general human population.
- Pet rats should be considered a potential risk for leptospiral infection and the importance of hygiene emphasised to owners.

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Human *Leptospira interrogans* serogroup icterohaemorrhagiae infection (Weil's disease) acquired from pet rats

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LEPTOSPIROSIS is an important zoonotic disease caused by a helical, motile bacterium belonging to the genus *Leptospira*. Leptospires are widespread among feral and domesticated mammals, which are the maintenance hosts for over 250 known serovars (Higgins 2004); each serovar has a preferred animal host. Organisms in the genus *Leptospira* are traditionally classified by conventional serology, although more recently a system based on genomospecies using molecular typing methods has been introduced. Leptospirosis is a major cause of economic loss to intensive cattle and pig industries of the developed world (Ellis 1998).

Human beings are susceptible to a large number of *Leptospira* serovars and are always an accidental host; infection is almost invariably acquired through direct or environmental contact with infected urine (Acha and Szyfres 2001). Known risk factors include occupational exposure among farm, abattoir and sewerage workers, water-associated recreational activities, household pets and contact with domesticated animals. In 2006, there were 44 reported cases of human leptospirosis in England and Wales, and three deaths have been recorded since 2002 (DEFRA 2007).

Wild rats (*Rattus norvegicus*) are a well documented source of human infection with *Leptospira interrogans* serogroup icterohaemorrhagiae (Carter and Cordes 1980, Jansen and others 2005). Approximately 50 to 70 per cent of the UK's wild rat population is thought to be infected, although Webster and others (1995) considered that there was a lower prevalence in rural areas. Information about leptospirosis in pet rats is lacking in the literature, but outbreaks of infection have been observed in laboratory rats (Natarajaseenivasan and Ratnam 1996) and laboratory rats have been used to model infections with serogroup icterohaemorrhagiae (Tucunduva de Faria and others 2007).

This short communication describes a confirmed human case of Weil's disease linked to contact with pet rats and describes the ensuing collaborative veterinary, medical and environmental health efforts to prevent the occurrence of further associated cases.

In June 2006, a 25-year-old man developed a 'flu-like' illness, which progressed to hepatorenal failure over a 48-hour period. The patient subsequently recovered. The illness was confirmed serologically as Weil's disease by the Health Protection Agency's Leptospira Reference Unit (LRU), using an in-house IgM ELISA (positive at 1:320) and the microscopic agglutination test (MAT) for IgG and IgM (positive at 1:640); the agent was serogrouped as *L* icterohaemorrhagiae.

Because of concerns about a possible association with Weil's disease, the two pet rats belonging to the patient were euthanased at his request and examined postmortem. Pathogenic leptospiral DNA was subsequently detected in kidney tissue from both rats using a LightCycler (F. Hoffmann La Roche) real-time PCR hybridisation assay (Fearnley and others 2008). Although it was not possible to confirm definitively by PCR, denaturing high-performance liquid chromatography (HPLC) performed at 57°C, 58°C and 60°C indicated that the pathogen fell within a small group of serovars that included Icterohaemorrhagiae. Denaturing HPLC can be used to detect mutations (Hurtle and others 2002, Randall and others 2005) and to identify regions of single nucleotide polymorphism in the 16S rDNA sequence from the three pathogenic species (*L interrogans, Leptospira borgpetersenii* and *Leptospira kirschneri*) found in Britain (J. S. Fenner, J. Errington, C. G. Dalley, L. P. Randall, M. J. Woodward, M. F. Anjum, unpublished observations). Leptospiral DNA was amplified using primers designed to the anterior region of the 16S rDNA gene, and the 180 base pair (bp) amplicons were hybridised with 180 bp amplicons from a known gold standard (Copenhageni strain AM050565; Veterinary Laboratories Agency). Denaturing HPLC was then used to detect heteroduplexes formed as a result of sequence differences.

In Great Britain, the relevant legislation concerning pet shops is enforced by local authorities. Under the Pet Animals Act 1951, pet shops must be licensed and operated to a sufficient standard to secure the welfare of animals and prevent the spread of infectious diseases. The Health and Safety at Work etc Act 1974 and the Control of Substances Hazardous to Health Regulations 2002 place further duties on pet shop owners to conduct their business such that members of the public and employees are not placed at risk. In view of the likely association between the present case of Weil's disease and the patient's pet rats, environmental health officers from the local authority made extensive inquiries to determine the source of the rats and trace other possible human contacts.

Investigations indicated that both rats had been purchased from a small pet shop three months previously and were part of a litter of 18 that had been bred privately. The original breeding pair was obtained from a breeder who owned approximately 70 rats, which were housed in a barn and exhibited at county shows. This raised concerns that the entire litter and the breeding pair may have been infected. Horizontal transmission from other rodents in the pet shop – either pets being offered for sale or feral animals – was also considered as a possible source of infection. The pet shop, which operated to good standards of hygiene and management, had an effective contract with a pest control company to minimise the risks from vermin, and there was no evidence of wild rodent activity when the premises was inspected by the local authority officers.

Six of the litter of 18 rats had been sold through the pet shop: the two infected animals described and four others, which had been sold to two separate customers. One of the owners was traced and agreed to have the rats euthanased and examined postmortem because of the concerns surrounding zoonotic transmission. Blood taken at euthanasia from both rats was strongly seropositive (1:400 and 1:1600) for *L* Icterohaemorrhagiae by MAT, and pathogenic leptospiral DNA was subsequently detected by PCR in kidney tissue from one rat. Sequencing and denaturing HPLC results were again consistent with serovar Icterohaemorrhagiae. The owner, who was clinically well, was seronegative (IgG and IgM) for *L* icterohaemorrhagiae by ELISA and MAT undertaken at the LRU. A further eight rats (including the breeding female) were traced but not examined; the remaining four rats could not be traced. Owners of potentially infected pet rats were advised about the need for hygiene precautions and preventing contact with feral rats. Instructions were given to not allow other people to handle the rats, not to use them for breeding or mixing with other pet rats, and to dispose of their carcases securely when they died. Prophylactic antibiotic medication of the pet rats was also discussed, but declined by the owners.

The pet shop mainly kept a variety of other small animals, including rabbits, guinea pigs, hamsters and gerbils, and did not normally sell rats. However, two aged rats, which had been acquired when the pet shop was first opened, were resident on the premises. They were not being offered for sale but shared a holding cage with other small rodents while the cages were being cleaned. The shop owner agreed to the euthanasia of these two rats and the 11 hamsters and three gerbils that were in close contact with them. Leptospiral DNA consistent with *L.* Icterohaemorrhagiae was detected in one of the rats but not in any of the other animals tested. A number of common pet species can be infected by pathogenic leptospires, including guinea pigs and hamsters, and it was notable that there was no apparent spread to the hamsters in this pet shop.

Since rats are clinically silent carriers of leptospirosis (Gratz 2006), it was not possible to determine precisely where and when the affected pet rats acquired the infection and which was the index case. There was no history of contact with wild rodents at either the pet shop or the two households with infected rats. This incident raises concerns about pet rats as a possible source of human infection with *L.* icterohaemorrhagiae. It also highlights the benefits of using the latest available diagnostic technology and of close collaboration between veterinary, medical and environmental health workers to reduce zoonotic risks to the public.

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2.45

■■ PUBLIC HEALTH

Weil's disease associated with the adoption of a feral rat

We wish to highlight a recent case of Weil's disease (*Leptospira interrogans* serogroup icterohaemorrhagiae infection) in a young woman, associated with the adoption of a semi-wild rat. The incident was investigated collaboratively by the West Yorkshire Health Protection Unit (WYHPU), the Veterinary Laboratories Agency (VLA) – Thirsk, and the Department of Infection and Travel Medicine, St James's University Hospital, Leeds. It follows the publication by Gaudie and others (2008) of a similar case of Weil's disease acquired from a pet rat.

The woman had owned two pet rats, one of which was a 'fancy' rat (A), which had been found feral in her garden about two years before this incident. The other rat (B) had been purchased from a pet shop about 12 months previously. Rat A developed a chronic intractable respiratory disease and was euthanased in late October 2008; the same condition was controlled in rat B with intermittent oral enrofloxacin (Baytril oral solution 10%; Bayer). In early November 2008, a third rat (C), thought to be an escaped pet rat, was adopted from the wild, having been brought in by a neighbour's cat. This semi-feral rat was examined by a local veterinary practice and subsequently kept as a pet, although it was kept strictly apart from rat B. Both rats were regularly handled by the woman, and less frequently by her partner, family and friends. Rat C was noticed to be urinary incontinent and all handlers aimed to wash their hands after contact with it.

In late November 2008, the owner was admitted to Leeds General Infirmary with a week-long history of lethargy, generalised myalgia, mild abdominal pain, a non-productive cough, abnormally heavy menstrual bleeding and episodes of epistaxis. She was pale, icteric, tachycardic and mildly pyrexic. Blood tests showed pancytopenia, acute renal failure and moderate hepatic disease. Her condition deteriorated and she was transferred to the intensive care unit at St James's University Hospital. *Leptospira* serology showed a positive IgM titre of 1:320 and a positive complement fixation test titre of 1:160, consistent with acute leptospirosis. The patient improved steadily with treatment and made a complete recovery. Serological testing of human in-contacts gave negative results for leptospirosis. A decision to euthanase the pet rats was made by the patient's partner shortly after her admission, when the possibility of leptospirosis was first mentioned. The carcases were submitted to VLA – Thirsk for postmortem examination, and pathogenic leptospiral DNA was subsequently detected by real-time PCR (Fearnley and others 2008) from the kidney of rat C, while tissue from rat B was negative. This technique is not specific for *L* icterohaemorrhagiae but, as with the incident described by Gaudie and others (2008), denaturing high-performance liquid chromatography subsequently showed it to be a member of the interrogans group, and one of five strains including icterohaemorrhagiae.

As with the case reported by Gaudie and others (2008), good inter-agency collaboration established a strong causal link between the rats and the occurrence of Weil's disease. Wild rats are a well-documented source of human infection with *L interrogans* serogroup icterohaemorrhagiae and other diseases (Carter and Cordes 1980), and the risks of acquiring zoonotic diseases, such as leptospirosis or salmonellosis, directly from feral rats or via pet rats that have contact with wild rats should not be underestimated. The practice of adopting feral rats should be actively discouraged. It is likely that veterinary practitioners may be among the first professionals from whom advice is sought in such circumstances, and awareness of the potential risks is essential.

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there is any evidence that TGE is still present after the main period of losses, discuss the problem with your veterinary surgeon before any breeding stock are moved off. Movements off to the abattoir are acceptable, but only if pigs do not go through a market. Otherwise this may lead to further spread of the disease.

After the outbreak

The biggest problem, particularly on larger breeding units, is that there may be a recurrence of TGE. Usually this is milder, with scour perhaps occurring only in purchased gilts or their litters. Sometimes there is a post-weaning scour. In the farrowing house the milder TGE that sometimes follows the initial outbreak can take the form of a scour in the older sucking piglets. Occasionally, however, there can be a more serious flare-up of disease. This usually affects only a few litters – often litters on gilts or on younger sows that were not fully exposed to infection during the first stage of the outbreak.

Clinical recurrences suggest that infection is still present on the farm. Veterinary advice should be sought. However, diagnosis and control in these endemic situations can be difficult.

Disease prevention

Normal disease security precautions

These are important because TGE can be introduced by people, vehicles or pigs.

On many farms the layout makes it impossible to achieve the ideal situation. However, simple inexpensive precautions, such as warning notices to keep off unwanted visitors or directing them to the house or office, or a means of attracting attention such as a bell or a hooter, can be very helpful. The name of the farm should be clearly displayed to avoid unnecessary visitors. Ideally, overalls and boots should be available for essential visitors such as veterinary surgeons.

Use a loading bay or similar area, well away from the pig buildings, to prevent hauliers and vehicles coming into contact with your animals and spreading infection. As far as possible, keep feedstuff vehicles away from the main farm yard too.

Starlings

Evidence suggests that wild birds, particularly starlings, have been responsible for much spread of TGE, particularly within a locality. Outbreaks typically occur during the winter when there is snow on the ground and pigs and starlings have close contact while foraging. Pigs outdoors or in open-fronted pens and yards are particularly vulnerable, and outbreaks often start in such places. Outside feeders are especially likely to be contaminated.

Try to make your farm less attractive to starlings. This can be done, for example, by avoiding spillage of food, keeping lids on hoppers, and by bird-proofing buildings as far as possible.

Conclusion

TGE is a recurrent problem and it seems that we can expect to see outbreaks in future winters. So far there is no specific vaccine available and no specific treatment can be recommended. However, as we have tried to show in this leaflet, much can be done to avoid the disease or to minimise its effects.

It must be pointed out, however, that certain aspects of TGE are not clearly understood yet, and that some of the suggestions given in this leaflet may need to be revised in the light of further information and experience.

Additional reading

Other ADAS publications which are relevant are:

B2410 Pig environment
L648 A simple disposal pit for foetal material and small carcases
L774 Protecting your livestock

For further advice, consult your veterinary surgeon.

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Transmissible gastroenteritis of pigs

Piglets on the left are affected and dying. Within 48 hours, the healthy piglets on the right were also affected, and died.

The disease

Transmissible gastroenteritis (TGE) occurs in many parts of the world. It was first seen in Britain in 1956. Since then epidemics have occurred at intervals of about five to seven years, mainly during the winter months and usually for two or three consecutive winters, followed by a quiet period. During recent years these outbreaks have been concentrated in the Eastern Counties, particularly Norfolk, Suffolk and Cambridgeshire.

The disease is caused by a virus (a coronavirus) which infects the small intestine, causing diarrhoea, vomiting and loss of appetite. All ages of pigs are affected and there is a high mortality amongst very young piglets.

Once an outbreak has started on a breeding unit, piglet losses usually continue for at least two or three weeks. However, the duration of an outbreak depends on many factors, particularly the size of herd and the speed with which immunity develops.

The virus is readily inactivated by disinfectants, heat and light, but survives well when frozen.

The clinical signs

Pigs of all ages are affected, though the

severity of the clinical signs varies between individual animals. Breeding stock and growing pigs usually show a watery diarrhoea and are off their food for a day or more. Some animals vomit. Lactating sows may lose their milk, whilst others show high temperatures for a short time and appear very ill, but this is relatively uncommon. A few sows may abort, probably due to the general effects of the disease.

Some finishing pigs will die if deprived of water whilst scouring, particularly on wet feeding systems. In these circumstances the provision of extra water is essential. Growth rate and feed conversion efficiency of finishers can be significantly retarded. However pigs infected at an early stage of their growing cycle may be expected to recover and can even show compensatory growth.

Sows that lose their litters from TGE, and are therefore dried off very early, normally come into oestrus within the usual time. However, the conception rate to the first service is often reduced or, if the sow holds, the litter size may be smaller than usual. The precise cause of these reproductive failures is not clear but may be associated with the very early weaning and also perhaps the inadvertent spread of, for example, parvovirus during the control of TGE. The effects on reproduction usually last for only a few weeks and relate to services carried out during the TGE outbreak.

The disease in younger weaned pigs is usually mild. However they may scour and show some unthriftiness.

The main effect of the disease is seen in sucking piglets, particularly those under a week old. These show a very severe foul-smelling, greenish-yellow watery diarrhoea, often containing flecks of undigested milk particles. Some piglets may also vomit. Affected piglets rapidly become dehydrated and emaciated prior to death. At the beginning and during the height of the outbreak all affected baby piglets may die, usually within 48 hours of showing clinical signs.

As the outbreak continues, the mortality rate in the baby piglets decreases until some litters are totally unaffected whilst in others only a few deaths occur. Eventually, after a period of at least two or three weeks but sometimes as long as two or three months, the outbreak comes to an end. The expected total mortality is about 1.3 piglets per sow on the unit (ie about 5% to 6% of the annual pig crop) but it may be half or twice this figure depending on the success of control and nursing measures and other factors.

Diagnosis

This can often be made from the clinical signs alone. Occasionally there may be confusion with Porcine Epidemic Diarrhoea (PED). This sometimes affects all ages of pigs giving a picture similar to TGE, but is usually confined to the breeding stock and finishers. In finishing units it is virtually impossible to distinguish between TGE and PED on clinical signs alone.

When an outbreak of TGE is suspected, the veterinary surgeon should be informed immediately. This will enable him to give prompt advice for reducing piglet losses and getting the outbreak under control. Confirmation is usually based on the examination of affected baby piglets submitted live to the local Veterinary Investigation Centre. Retrospective confirmation can be obtained from blood samples taken from older pigs at least ten days after infection.

Coping with an outbreak

The effects of an outbreak will very much depend on individual circumstances such as layout of buildings, husbandry system and particularly the

farrowing programme. A very important factor is the number of susceptible piglets on the farm at the commencement of the outbreak or born during the ensuing two or three weeks.

Other important points in determining the overall number of piglet losses include the speed with which the disease is diagnosed, the class of stock initially affected and the speed and extent of spread. Some outbreaks appear to start very slowly; others have run through the unit before a diagnosis has been made.

Control of an outbreak usually calls for a combination of measures. The most important is to build up the herd immunity so as to bring the outbreak to an end as quickly as possible. In addition, attempts can be made to reduce piglet losses until this immunity is achieved. It may be possible to isolate baby piglets to prevent infection reaching them until they are at least two or three weeks old. Affected piglets will require nursing.

These points will now be discussed in greater detail. These suggestions are based on experiences in the 1980/82 epidemic in East Anglia and in previous epidemics. It is most important, however, to discuss specific points with your own veterinary surgeon.

Isolation

As soon as TGE is suspected, try to isolate any unaffected sows and their litters. Effective isolation requires separate staff to look after the unaffected portion of the herd. They must have no contact with the scouring pigs. The isolated farrowing houses should as far as possible be bird-proof and rodent-proof to prevent spread from the affected part of the farm.

Unaffected sows due to farrow within two weeks should also be isolated and farrowed in isolation. It is better to spread the risk by farrowing them in small numbers in several different, even makeshift, buildings. Scouring sows should not be taken into farrowing houses where there are unaffected sows and litters.

Building up the herd immunity

Vaccines are used in some countries but without much success. Vaccines are not available in Britain. Because of this, the development of immunity depends on natural infection. With the exception of the isolated animals referred to above, the disease should be spread quickly to other stock on the farm. Piglet losses will only cease when all the pregnant sows and gilts have been infected and have produced antibodies in their milk and colostrum to protect their litters. It usually takes about two weeks for this immunity to develop. This is the reason for trying to isolate sows and gilts within two weeks of farrowing.

It is often better to deliberately infect growing pigs and all other pigs on the unit, as well as pregnant sows and gilts at the same time. Otherwise they may go down with the disease after it has gone through the breeding stock, thus prolonging the outbreak. It is probably unwise, however, to deliberately infect finishing pigs which are due to be marketed shortly.

It is usually possible to spread the virus around the farm by means of infected faeces. This can be given individually to pigs, smeared on troughs or mixed with feed or water. The virus can also be spread in a herd by mixing scouring or recently recovered animals with unaffected pigs, for example, in straw-yards. However, these or other steps should not be taken without full discussion with your veterinary surgeon. This is particularly important because of the risk of spreading other diseases such as parvovirus and swine dysentery around the unit.

It is worth identifying and recording those dry sows and gilts which scour after infection. Development of immunity is, to some extent, dependent on the extent to which they scour. Spreading infection should be continued amongst all dry stock which have not scoured until, as far as possible, all pigs on the farm have shown some clinical signs of infection. This should ensure that the herd immunity is as high as can be achieved.

Protecting future litters

Sows which have been isolated should be deliberately exposed to infection when their piglets have been weaned or are at least three weeks old. This is important in order to develop immunity in these sows to protect their future litters.

Treatment and nursing

If suitable nursery accommodation can be provided, piglet losses can often be reduced by weaning as early as six days of age, provided they are given an adequate supply of fluids and are kept as warm as possible. Piglets which are scouring on entry into suitable accommodation often recover very quickly.

Usually very little can be done to save three- or four-day-old piglets if they are affected before or at the height of the outbreak. No specific treatment is available. Glucose/electrolyte mixtures, either home-made or proprietary, can be given. However, although these are helpful in older sucking piglets (ie about a week of age or over) they do not often save many very young piglets. One particular problem is that the glucose/electrolyte mixture quickly becomes fouled and needs changing frequently. Also piglets can immerse themselves and become chilled or even drown. Cube drinkers are probably the most useful.

Provision of a good quality milk replacer or creep pellets can also be beneficial, particularly if the sows are showing agalactia (lack of milk).

It is important to keep affected piglets as clean, warm and dry as possible. If bedding is not normally used it should be provided in the form of shavings or straw.

Antibiotics are of limited value. However, they can be helpful in the slightly older age group of piglets, particularly where TGE is complicated by *Escherichia coli* (*E.coli*) infection, as is often the case during the recurrences that sometimes occur.

During the later stages of the outbreak, and during any recurrences, it is often possible to save piglets by cross suckling or by fostering ailing piglets onto immune sows, preferably after their own litters have been weaned.

General comments

Although the disease is not notifiable, owners are urged to report outbreaks to their veterinary surgeon who, in turn, will inform the local Veterinary Investigation Centre (MAFF). The voluntary early warning and notification system can then be put into operation to help limit the spread of the disease. These warnings do not give the farmer's name and address. Instead they usually state the affected parish, although a more general description can be used at the owner's request. The information is treated in confidence.

When an outbreak occurs on a unit please put a notice at the farm entrance advising people to keep away. This obviously reduces the spread of infection. Neighbouring farms, hauliers and feedstuff suppliers should also be informed. Only essential visitors should be allowed, and then only after taking full hygiene precautions to minimise the risk of spread.

Dispose of dead piglets carefully in order to prevent spread of the virus by dogs, foxes, cats or rats etc.

Do not sell or move off breeding stock to other farms for at least a month after the cessation of clinical signs. If