SEASONAL PREVALENCE AND DISTRIBUTION OF TICKS ON THE ACCRA PLAINS OF GHANA AND THEIR ASSOCIATION WITH DERMATOPHILOSIS

VOLUME I

by

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VOLUME I

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Enoch B.M. Koney
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ABSTRACT

The professional and scientific literature on the epidemiology, treatment and economic importance of dermatophilosis has been reviewed. Particular attention being paid to the role of ticks, especially *Amblyomma variegatum* in causing overt disease and tick associated losses. In addition chemical and alternate methods of tick control including the use of resistant breeds of cattle, modification of vegetation and the microclimate, the introduction of tick-resistant grasses and host vaccination against ticks are discussed.

An experiment herd of 26 animals and five local herds containing 605 animals on the Coastal Plains of Ghana were studied.

The seasonal abundance of ticks, their association with dermatophilosis and the systemic effects of the ticks on their hosts were investigated. Four genera, *Amblyomma, Boophilus, Rhipicephalus* and *Hyalomma* were identified on cattle, *A. variegatum* being the predominant tick species occurring throughout the year with peak infestations in the two rainy seasons. A highly significant positive correlation was revealed between *A. variegatum* and dermatophilosis in four of five local herds. Unexpectedly, a significant correlation was found between *Hyalomma rufipes* and dermatophilosis twice and between *Rhipicephalus senegalensis* and dermatophilosis once in the local herds.

Clinical dermatophilosis developed two months after severe tick infestation. The immune responsiveness of tick-infested cattle was suppressed as assessed by the lymphocyte transformation test (LTT) and confirmed by [^{3}H]-thymidine incorporation assays. Decreased lymphocyte responsiveness in the Friesian cattle coincided with peak tick levels and clinical dermatophilosis. *In vitro* lymphocyte responsiveness to Concanavalin A in culture medium containing foetal calf serum was suppressed by serum from cattle infested with ticks. Similarly, serum from cattle infested with ticks and infected with dermatophilosis suppressed lymphocytes derived from "clean" animals i.e. control animals treated with acaricides.

Repeated experimental infections in cattle healed spontaneously in spite of the fact that the immune responses of the animals were suppressed. Resolution occurred fast after the second and third infections but not after the fourth infection which healed at the same time as the first.

Cross-reaction was observed between *D. congolensis* and PPD. The practical significance of the observed association in relation to tuberculosis skin test is discussed.

It is suggested that indigenous breeds of cattle which are more resistant to ticks and dermatophilosis be kept on the Coastal Plains of Ghana. It is also recommended that acaricides with long residual activity backed-up with long-acting oxytetracycline injectables be used during periods of high tick activity to keep tick levels at low numbers. Selective hand-dressing may then be used to treat any individual animals with large tick burdens in the dry seasons.

ABBREVIATIONS

A	*Amblyomma*
AET	2-amino ethyl isothorium bromide
Ag	Antigen
Antigen M	*D. congolensis* medium derived antigen
Antigen S	*D. congolensis*-sonicated whole cell antigen
ARI	Animal Research Institute, Ghana
A-TF	Acaricide-treated Friesians
A-TZ	Acaricide-treated Zebu-type cattle
B	*Boophilus*
B/A	Blood agar
BCG	Vaccine strain of *Mycobacterium bovis*
BHI	Brain-heart infusion
Con A	Concanavalin A
Ci	Curies
Ci/mol	Curies per mole (amount of radioactivity)
°C	Degrees Celsius
cm^2	Square centimetres
CMI	Cell-mediated immunity
CPM	Counts per minute
CTVM	Centre for Tropical Veterinary Medicine
D	*Dermacentor*
D	*Dermatophilus*
D. congolensis	*Dermatophilus congolensis*
Dc_1	*D. congolensis* spore or whole cell antigen
Dc_2	*D. congolensis* medium-derived antigen
DNCB	1-chloro-2, 4-dinitrobenzene
DPM	Disintegrations per minute
DTH	Delayed-type hypersensitivity
E_{AET}	Sheep lymphocytes treated with AET
E-rosette	T-lymphocytes are referred to as E rosetting cells
F	Variance ratio
FCS	Foetal calf serum
g	acceleration due to gravity
H	*Hyalomma*
H	Kruskal-Wallis statistic
γIFN	Gamma interferon
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
k	Number of treatments
kg	kilogrammes
km	kilometres
kDa	Kilodalton
ILCA	International Livestock Centre for Africa
IL-2	Interleukin-2
IL 2R	Interleukin-2 receptors
LL	Lepromatous leprosy
LTT	Lymphocyte transformation test
LWG	Liveweight gain
M	Millions
M	Mycobacterium
M	Friedman's statistic

MC	Mononuclear cell
mCi	Millicurie
MHC	Major histocompatible complex
mg	Milligrammes
ml^{-1}	Per millilitre
mm	Millimetres
ml	Millilitres
MMIF	Macrophage migration inhibition factor
n	Number of observations/samples
NSP	Neutralized soya-peptone
P	Probability
%	Per cent
PBS	Phosphate buffered saline
PGE_2	Prostaglandin
PHA	Phytohaemagglutinin
PMN	Polymorphonuclear leucocyte
PPD	Purified protein derivative of *Mycobacterium tuberculosis*
PWM	Pokeweed mitogen
r	Pearson's correlation coefficient
R	*Rhipicephalus*
rpm	Revolutions per minute
RPMI	Rosewell Park Memorial Institute medium
$	Dollar (U.S.)
SDS-PAGE	Sodium dodecyl sulphate-polyacrimide gel electrophoresis
t	Students statistic
T	*Trypanosoma*
T	Wilcoxon's statistic
TB	Tuberculosis
T.b.	*Trypanosoma brucei*
T-cells	Thymus derived lymphocytes
T-lymphocyte	Thymus-derived lymphocytes
TL	Tuberculoid leprosy
Tris/Hcl	Tris (hydroxymethyl) amino methane
$T4^+$	Helper lymphocyte phenotype
$T8^+$	Suppressor T-lymphocyte phenotype
U	Mann-Whitney statistic
μCi	Microcurie
μg	Microgram
μl	Microlitre
UTF	Untreated Friesians
UTZ	Untreated Zebus
v	Degrees of freedom
WAMCO	West Africa Milk Company Ltd, Nigeria

INTRODUCTION

Dermatophilosis, a skin disease caused by the bacterium *Dermatophilus congolensis*, has a worldwide distribution and affects a wide range of hosts. The disease may be acute or chronic, resolve spontaneously or under certain conditions, prove fatal (Oppong, 1976). Recovered animals become re-infected in some cases. The disease has frustrated many attempts at using exotic breeds of cattle to improve bovine meat and milk production in the humid and subhumid tropics, including Ghana (Koney, 1984). Dermatophilosis causes severe economic losses through reduced rates of liveweight gain, damage to hides and outright deaths.

Dermatophilosis was first reported in Ghana in 1925 (Beal, 1929). Later Chodnik (1956) and Oppong (1970) investigated the epidemiological features of the disease on the Accra Plains of Ghana in more detail. More recently, Koney and Morrow (1990) studied the prevalence of the disease in animals under different management systems on the Accra Plains of Ghana.

Many epidemiological factors have been implicated in the cause, spread and resolution of the disease. Among these are rainfall, relative humidity, ticks, biting flies, thorns and tree branches, abrasions, contact and breed susceptibility. The skin protective layers provide an effective barrier against *D. congolensis* infection, and this skin barrier has to be damaged before infection can occur (Roberts, 1967; Stewart, 1972). However, there are some anomalies because it has not been possible to reproduce the natural lesions of the disease under experimental conditions in animals whose skin protective layers have been broken by, for example, scarification. In Ghanian cattle natural lesions are chronic whilst experimental lesions heal very fast.

Circumstantial evidence has implicated ticks, in particular *Amblyomma variegatum*, in the pathogenesis of dermatophilosis, but in natural infections distribution of lesions do not always coincide with tick predilection sites.

A survey was therefore conducted to investigate the seasonal abundance and tick population dynamics on the Accra Plains of Ghana and the systemic effects, if any, ticks have on the immune system of infested animals. The objectives of the study were as follows:-

To investigate the association between *A. variegatum* and dermatophilosis,

To investigate the systemic effects of ticks on their hosts,

To compare levels of tick infestations on the different breeds of cattle i.e. Zebu-types, N'damas and Friesians,

To determine periods of the year when tick numbers and burdens on cattle were highest with a view of planning an effective tick control programme to reduce tick numbers to acceptable levels and hence minimize the incidence of dermatophilosis in Ghana.

An experimental herd of 26 animals comprising 15 Zebu-type cattle, five N'damas and six Friesians was established. Five Zebu-type cattle and three Friesians were used as control groups and kept tick free by regular application of acaricides while the rest of the animals were tick infested. The experiment herd was the source of blood samples for studying the systemic effects of ticks on their hosts.

Tick population dynamics and seasonal distribution of ticks on cattle on the Accra Plains of Ghana were studied in the experiment herd and five farms with traditional management selected in the study area. Selection of the five farms was based on cooperation of stockowners and herdsmen, previous history of dermatophilosis on these farms, accessibility to the farms in the rainy season and similarity of animals and management practices.

The results are recorded in two volumes. Volume one comprises the text, discussions and references and volume two contains the text figures, text plates and text tables together with all the appendices.

CHAPTER ONE

REVIEW OF THE LITERATURE

CONTENTS

Dermatophilosis was originally described as being a streptothricosis in the mistaken belief that the aetiology was a streptothrix. It has a wide host range but it is most commonly found in cattle and sheep (Henderson, 1928; Roberts and Valley, 1962; Austwick and Davies, 1958). It also affects many other mammalian species including horses (MacAdam, 1964), zebras and donkeys (Green, 1960), white tailed deer (Salkin, Gordon and Stone, 1975), polar bear (Smith, 1973; Neuman, Cook, Appelhof and Kitchen, 1975), camels (Gitoa, Evans and Atkins, 1990), lizards (Simmons, Sullivan and Green, 1972) and racoons (Salkin, Gordon and Stone, 1976).

The disease also affects man (Stewart, 1972; Albrecht, Horowitz, Gilbert, Hong, Richard and Connor, 1974) but infection in man is not severe (Kaplan, 1976; Hyslop, 1980).

AETIOLOGY

The aetiological agent of the disease is *D. congolensis*, a branching, filamentous, gram positive, polypleomorphic bacterium, which in culture medium occurs as hyphae and cocci (Bida and Dennis, 1976). The organism was first named and described by Van Saceghem (1915). It invades living epidermal tissue but not keratinized cells.

Dermatophilus congolensis is classified according to the following scheme (Stewart, 1972).

Class	:	Schizomycetes
Order	:	Actinomycetales
Family	:	Dermatophilaceae
Genus	:	Dermatophilus
Species	:	Congolensis

It is the only member of the family Dermatophilaceae and it exhibits some unique features in its life cycle; filaments arise as extensions of germ tubes from cocci and grow for several micrometers before branching and forming of septa. Firstly transverse and then longitudinal septa form, giving rise to parallel rows of individual cells which are encapsulated by a gelatinous matrix arising from thickening of the cell walls. Motile cocci about 0.5 - 1.0 μm in size are liberated when the matrix dissolves (Gordon and Edwards, 1963). Richard, Ritchie and Pier (1967) reported that each of the cocci can possess up to 50 flagella, which form either before or after liberation.

CLINICAL SIGNS

In 1915 Van Saceghem described the disease in cattle as an exudative dermatitis followed by extensive scab formation. It starts as a serous exudation which dries to form a characteristic matting of the hair known as a paint brush-like lesion. The serous exudate increases, coagulates and encrusts embedding some hairs and enlarging the lesions. The resulting scab thickens and is tightly adherent (Oppong, 1976) (Plate 1.1). The signs in sheep are also those of a proliferative and exudative dermatitis with bundles of wool fibres matted together by exudate (Bull, 1929). In chronic forms, the exudates in cattle coalesce to form extensive yellowish-brown crusts which are hard and firmly adherent to the skin (matted form) (Bida and Dennis, 1976). In animals the disease may be acute or chronic, resolve spontaneously or, under certain conditions, prove fatal.

DIAGNOSIS

Gross lesions of dermatophilosis have a characteristic appearance and the organism can be identified from impression smears made from the underside of scabs and stained by Giemsa. Under the microscope the organism contains multiple rows of gram-positive coccoid elements, forming large numbers of branching filaments characteristic of *D. congolensis* (Plate 1.2). To confirm diagnosis, the organism can be isolated in culture (Roberts, 1967). On blood agar, the organism forms greyish white colonies 1 to 2 mm in diameter, surrounded by a 1 mm zone of hemolysis. The colonies are convex, rough, sunken into the medium and firmly attached to it. The centre of colonies are normally depressed (Gitoa *et al.*, 1990).

The direct fluorescent - antibody test for *D. congolensis* developed by Pier, Richard and Farrell (1964) and more recently the use of monoclonal antibody to *D. congolensis*, described by How and Lloyd (1988a), are useful tests in confirming diagnosis of *D. congolensis* infection, especially in very old lesions where there may be difficulties in identification.

Pulliam, Kelley and Coles (1967) mentioned various antibody-detecting techniques used in assessing *D. congolensis* infection including agar gel precipitation, indirect haemagglutination and particulate antigen tube agglutination tests. Oduye (1974) and Aghomo and Lloyd (1983) investigated different *D. congolensis* antigen preparations to determine which was the most reliable for use in circulating antibody tests and both found concentrated culture supernatants to be the most effective source of antigen. Sutherland and Robertson (1988) found that the filament antigen produced more circulating antibodies than cocci. However, Woodman (1989) pointed out that the limitations of such tests are that they can only show that an animal had at some time been exposed to *D. congolensis* and not necessarily that it has an on-going infection. Using an enzyme-linked immunosorbent assay, Lloyd (1981) demonstrated that Ayrshire cattle which had apparently never shown any clinical symptoms of dermatophilosis, nevertheless possessed antibodies to *D. congolensis*.

TRANSMISSION

Several modes of transmission of *D. congolensis* are possible and the question of how the infection is transmitted is so far unresolved. Ticks have been incriminated in the transmission and pathogenesis of dermatophilosis (Plowright, 1956; MacAdam, 1962, 1964). Transmission of *D. congolensis* from cattle to a rabbit (MacAdam, 1962) and from cattle to a goat (Beaton, 1932) by *Amblyomma variegatum* has been reported. MacAdam (1962) established dermatophilotic lesions which lasted for three months on the ears of cattle by simultaneously swabbing the ears with *D. congolensis* and infesting the same ears with *A. variegatum* ticks. He speculated that exotic saliva of ticks damaged the tissues and allowed the organisms time to establish persistent lesions. Later, Oppong (1976) isolated *D. congolensis* from *Amblyomma variegatum* ticks taken from naturally-infected cattle in Ghana. Tick transmission was considered to be of prime importance, following effective control of the disease by acaricide (Henderson, 1928; Plowright, 1956). However tick transmission is not readily reconciled with the frequent reports of lesions which occur predominantly away from tick predilection sites.

The role flies play in the transmission of infection has been discussed by many workers. Richard and Pier (1966) transmitted *D. congolensis* from donor to recipient rabbits via the stable fly, *Stomoxys calcitrans*, and the housefly *Musca domestica*. Philpott and Ezeh (1978) were unsuccessful in repeating these transmission results with cattle except on one occasion. Stewart (1972) described the association between the bites of *Haematopota* sp. and *Tabanus* sp. and the development of *D. congolensis* lesions.

Wilson (1958) reported that in Bornu State, Nigeria, a definite relationship was established between the prevalence of swarms of *Lyperosia* flies and the occurrence of dermatophilosis. Controlling the flies by gamma benzene hexachloride[1] sprays reduced the incidence of the disease. Coleman (1967) stated that moist skin in combination with the bites of infected insects created an ideal situation for the transmission of the disease. MacAdam (1962; 1964) and Oduye (1976) have expressed similar views. However, from work done in the West Indies, Hadrill, Morrow and Heron (1991) had some doubts about the role of flies in the transmission of the disease. Other potential sources of infection such as contaminated soil are not thought to play a major role in the aetiology of the disease (Roberts, 1963).

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[1]Gammatox, Wellcome

Contact transmission between animals is disputed by some workers (Plowright, 1956; Soltys, 1964; MacAdam, 1970). However, others believe contact transmission plays a significant role in disease transmission; in their opinion contact transmission occurs during suckling and mating, especially during the rainy season (Le Riche, 1968; Roberts, 1967; Oppong, 1973; Austwick, 1976; Bida and Dennis, 1976). Oppong (1973) observed in Ghana a close relationship between the parts that first get infected on calves and lesions on dams and attributed this to the husbandry practices adopted among the Fulani herdsmen during milking. He also speculated that transmission of dermatophilosis on the Accra Plains occurred by contact during the wet season, when motile zoospores are released from scabs and the animals rub against each other. Contact between animals when motile zoospores are being released and the protective barriers of the epidermis are partially broken down may enhance transmission of infection under wet conditions. Ample opportunity arises for this form of transmission when cattle are kept in crowded kraals.

Multiple predisposing and precipitating factors are however, required for the establishment and development of natural infections of dermatophilosis.

FACTORS CONTRIBUTING TO THE ESTABLISHMENT OF INFECTION

The factors which predispose animals of susceptible species to natural infection with the disease are not well understood. Many factors have been incriminated, in different roles in establishment of the disease. Multiple predisposing and precipitating factors with a single casual agent under widely differing environmental conditions have been implicated in the development of the disease (Hyslop, 1980). These factors include: rainfall, vegetation, overcrowding, ectoparasites, methods of husbandry, intercurrent diseases, malnutrition and stress.

Dermatophilosis in animals is superficial and confined to the epidermis (Zlotnik, 1955; Chodnik, 1956). Many workers have reported that intact skin provides a protective barrier against *D. congolensis* infection, and that this skin barrier has to be damaged before infection can occur (Roberts, 1967; Stewart, 1972). Three barriers, the hair coat, surface sebum and the stratum corneum of the skin are known to protect uncornified epidermis against infection in sheep and cattle (Roberts, 1963; 1967). However, Amakiri (1973) observed that in cattle, skin thickness appeared to have little significance in the development of dermatophilosis and he suggested that the thickness of the stratum corneum was probably not an important barrier to natural dermatophilosis in Nigeria.

The skin basement membrane together with the stratum basale have been reported to be important barriers against dermal invasion by *D. congolensis* in natural infections of bovine dermatophilosis, especially after its penetration of the other epidermal layers (Amakiri, 1974, 1976). However, histological sections of biopsies of chronic nodular lesions of dermatophilosis in man disclosed the presence of multiple granulomatous foci throughout the dermis and subcutaneous tissue with extension into the underlying muscle (Albrecht, Horowit, Gilbert, Hong, Richards and Connor, 1974).

Roberts (1967) is of the view that there is a pronounced interdependence between the barriers and that most natural infections probably commence during periods when they are damaged or compromised. In the field the intact skin surface is frequently breached, especially in pastures containing thorny bushes and where there is a high arthropod challenge (Woodman, 1989). Other factors such as skin damage caused by repeated mounting (Moule and Sutherland, 1947), and thorn tree branches (Schulz, 1955; Zlotnik, 1955), ox-pecker birds (Beaton, 1955; Oduye, 1975a), and ectoparasites (Henderson, 1928) have been incriminated in the development of the disease.

MacAdam (1964) stressed that keeping natural cases of dermatophilosis free from ectoparasites allowed marked improvement in the disease. There seemed to be variations between breeds in their susceptibility to infection. Obeid (1976) and Leroy and Marchot (1987) reported that indigenous breeds of cattle in Southern Sudan were resistant to infection. They observed that none of 59 Dinka pure bred cattle out of an infected herd of 181 animals were affected by the disease. Coleman (1967) and MacAdam (1970) reported that West African Shorthorn (Muturu) and N'dama cattle were resistant to infection. Breed resistance to dermatophilosis by the indigenous creole cattle in the Caribbean has been reported (Barré *et al.*, 1988; Martinez, 1991 and Matheron *et al.*, 1989). Bida and Dennis (1976) pointed out that all indigenous Zebu breeds of cattle in Northern Nigeria and their crosses are susceptible to the disease. Isitor, Kazeem, Njoku, Adegboye and Dellmann (1988) reported severe generalized dermatophilosis in 26 Santa Gertrudis cattle imported from Oklahoma State, USA into Nigeria. European breeds of cattle were particularly susceptible to *D. congolensis* infection (Mornet and Thiery, 1955; Kelley *et al.*, 1964; Njindam, 1980).

Nwufoh and Amakiri (1981) observed a variation in the bacterial flora of the normal skin between N'dama, Friesian and White Fulani breeds of cattle. Bacilli predominated on the skin of the N'dama cattle, whereas in susceptible breeds e.g. Friesian and White Fulani, staphylococci predominated. The high colonial population of bacilli on the N'dama skin probably inhibits the growth of *D. congolensis* because they are known antibiotic producers. For example, Ojo (1975) demonstrated *in vitro* that *Bacillus pumilis*, which is closely related to *B. subtilis* inhibited the growth of *D. congolensis* and other microorganisms. Kingali, Heron and Morrow (1990) studied the effect of inhibitory substances produced by bacteria present on the normal skin of sheep on the growth of *D. congolensis in vitro*. They found that substances inhibitory to the growth of *D. congolensis* in culture, were produced by some bacterial skin commensals. They also identified 17 out of 18 of the isolates producing substances which inhibited the growth of *D. congolensis* to be Bacillus species.

Dermatophilosis is predominantly a disease of the rainy season (Beal, 1929; Stewart, 1930; Plowright, 1956; Soltys, 1964; Coleman, 1967; Oppong, 1970, 1973;

Kelley and Bida, 1970). Davis and Philpott (1980) were uncertain whether heavy rain had a direct effect by damaging the skin surface, or whether the association was an indirect one, whereby it facilitated release and transfer of motile cocci, or mediated increases in the arthropod populations which then caused damage to the skin or increased the rate of transmission. However, Roberts (1963) and Roberts and Graham (1966) observed that intense or prolonged wetting of the skin resulted in emulsification and disruption of the sebaceous film, as well as maceration of the stratum corneum. Ainsworth and Austwick (1959) reiterated the effect of constant wetting of the skin by rain in establishing *D. congolensis* infection.

Water may serve as a medium for transmission from existing lesions to other areas on the same animal (Roberts, 1967; Davis and Philpott, 1980). Water is probably also important in the transmission of the disease when animals come in contact and by carrying bacteria with splashes from one animal to another. Kingali and others (1990) suggested that rain water might leach out or dilute inhibitory substances against *D. congolensis* produced by the normal skin microflora, thus facilitating establishment of the disease. High humidity alone as the sole factor responsible for the development and spread of lesions on individual animals has been reported by Chodnik (1956). MacAdam (1964) disagreed with this view and argued that humidity controlled the population of ectoparasites which appeared to be the main factors in the spread of the disease. Nobel and colleagues (1976) associated the occurrence of an outbreak of the disease with an abrasive concrete slatted floor together with daily spraying of Israeli Friesian cattle with water.

Moule and Sutherland (1947) recorded that seasonal conditions have no significant influence on the disease. Schulz (1955) and Braibant (1962) also found no correlation between the incidence of the disease in cattle and rainfall. Similarly, Ramachandran (1984) working in Southern Sudan found no seasonal influence on the incidence of the disease.

Malnutrition and poor body condition have been implicated in predisposing animals to dermatophilosis infection (Simpson, 1950; Egerton, 1964; Davis, 1984). Sanders, How, Lloyd and Hill (1990) showed that malnutrition greatly prolonged persistence of dermatophilosis lesions in sheep. However, Oduye (1976) was of a different opinion. He is of the view that poor body condition is an end-product of infection rather than a predisposing factor. MacAdam (1964) reported that high planes of nutrition did not protect against *D. congolensis* infection. Samui and Hugh-Jones (1990a) observed that animals suffered recurrent infections regardless of their nutritional status. However, malnutrition may cause a change in the resident skin flora, which then allowed opportunist pathogens, such as *D. congolensis*, to invade the skin (Jenkinson, 1976).

Experimental infections in animals resolve within weeks and remain localized to test sites whereas natural lesions are protracted and spread to other parts of the animal (Kelley, Huston, Imes and Weide, 1964; MacAdam, 1970; Abu-Samra and others, 1976; Davis and Philpott, 1980). It is possible that a combination of predisposing factors is required for the establishment and spread of dermatophilosis and that they may differ in different areas.

Woodman (1989) commented on the possible dual role ticks and flies may play in facilitating the establishment and development of generalized dermatophilosis through mechanical damage and immunosuppression. Suppression of the immune system has been attributed to concurrent infections and the occurrence of dermatophilosis has been associated with that of trypanosomiasis, rinderpest, mange, lympy skin disease and orf (Plowright, 1956; Stewart, 1972; Munz, 1976). Isitor, Delmann, Adegboye, Ezeokoli and Chineme (1984) postulated the existence of a synergistic relationship between *D. congolensis* and pox virions. Isitor and others (1988) also demonstrated virions in 77 per cent (40 out of 52) of lesions from natural cases of bovine dermatophilosis, using electron microscopy.

The role ticks play in suppressing the immune system has been reported by Wikel, Graham and Allen (1978) and Wikel and Osburn (1982). They reported a degree of immunosuppression resultant from tick infestation.

ECONOMIC EFFECTS OF DERMATOPHILOSIS

Dermatophilosis has a limiting effect on cattle productivity through reduced rates of liveweight gain, outright deaths (Soltys, 1964; Egerton, 1964; Coleman, 1967; Wilkinson, 1979), reduced milk yield (Nobel, Klopfer and Newman, 1976), low work performance (Oduye, 1975b; Lloyd, 1976) and decline in fertility (Ogwu, Osori and Kumi-Diaka, 1981).

Working with sheep in Australia, Edwards, Gardner, Salerian, Love, Spicer and Bryant (1986) reported that for 1984/85, dermatophilosis was associated with a loss of $20 million in production. The most important economic loss on *D. congolensis*-affected sheep, in the view of Gherardi, Sutherland, Monzu and Johnson (1981, 1982), is a consequence of blowfly strike on dermatophilosis lesions.

Kelley and Bida (1970) observed that the disease annually caused an estimated loss through hide damage, debility and death due to toxaemia of more than £1,000.000 in Nigeria. More recently, Ibu, Makinde and Nawathe (1987) were of the opinion that losses due to poor weight, decreased milk yield and spoilage of hides and skin in Nigerian cattle affected with the disease, exceeded US $300 million per year. However in their view the most serious affect, was the difficulty in maintaining highly susceptible exotic stock free of the disease while upgrading local cattle by cross-breeding programmes.

Wilkinson (1979) has shown that the disease caused up to a 6 per cent mortality in sheep in Australia. Lloyd (1976) and Abdullahi (1982) have reviewed the literature on the economic losses caused by dermatophilosis. Abdullahi's work concentrated on Nigeria where an incidence of 10.5 per cent, with a 2 per cent case mortality and a 4 per cent culling rate for cattle were noted. Oppong (1973) observed that cattle owners in Ghana got approximately one third the price of an otherwise unaffected animal when they sold *D. congolensis*-affected animals for slaughter at the local markets. Samui and Hugh-Jones (1990b) reported that in Zambia, the sale of a live animal affected with dermatophilosis represented a loss of about 33 per cent of its normal market value.

From a survey carried out in Zambia in 1985, Samui and Hugh-Jones (1990b) estimated the financial loss per case of treatment and/or premature disposal, slaughter or death to cattle owners in Zambia to be K.202 (US $91). The cost due to draught oxen being affected was estimated at K.478 (US $193) per affected ox. The cost of reduced milk production, replacing affected cows and calf deaths, directly or indirectly from bovine dermatophilosis, was estimated at K.132 (US $78) per affected milking cow. Samui and Hugh-Jones (1990b) also estimated the total annual national cost of bovine dermatophilosis in Zambia in 1985 to be K.6.9 million (US $3 million). They further suggested that the true financial cost of dermatophilosis in Zambia in 1985 may have been up to 1.8 times the estimated cost.

HIDES AND SKINS

The most obvious economic losses caused by dermatophilosis result from damage to hides and skin (Oduye, 1975a; Austwick and Davies, 1958) and downgrading of wool (Austwick and Davies, 1958). The hides industry in Nigeria has been singled out as suffering large financial losses due to the disease (Bida and Dennis, 1976). Abdullahi (1982) indicated that for hides and skins, estimates of losses due to dermatophilosis in 1976 ranged from N1.6 million to N10.33 million in Nigeria.

The damaging effect of the disease on processed hide and the deleterious effect it has on the expansion of the leather industry have been reported by many workers (Soltys, 1964; MacAdam and Haalstra, 1971; Oduye, 1975b; Bida and Dennis, 1976). Ainsworth and Austwick (1959) pointed out that affected leather is unsuitable for many purposes and the loss of value could be disastrous to countries in which processing hides and skins is a major trade. Bida and Dennis (1976) put the depreciation in value of hides affected by dermatophilosis in Nigeria at approximately 20 per cent.

EFFECT OF DERMATOPHILOSIS ON REPRODUCTIVE PERFORMANCE

Bovine dermatophilosis is one of the most important diseases limiting improved cattle production in the tropics (Ilemobade, 1984). Ogwu and co-workers (1981) reported that the disease affects the reproductive performance of cattle. They expressed the view that scrotal dermatophilosis would affect testicular function due to increased thickness of the affected scrotum. Sekoni (1983) reported a case of sterility in a Friesian bull from generalized scrotal infections and abnormal spermatogenesis. However, Kumi-Diaka, Njoku and Osori (1980) failed to find any correlation between the degree and extent of scrotal dermatophilosis and spermatogenesis. The authors found no significant difference in scrotal thickness between the normal and dermatophilosis-infected animals. They speculated that this explained the lack of correlation between scrotal dermatophilosis and spermatogenesis. Kumi-Diaka *et al.* (1980) however observed that although severe chronic scrotal dermatophilosis did not adversely affect semen quality, libido in affected bulls was depressed. This observation led to the conclusion that the reproductive performance of affected bulls would not be optimal.

Ogwu and others (1981) reported atrophy of the ovaries and subsequent anoestrus in cows with moderate to severe infection of dermatophilosis. They further reported that in cases of localized infection of the perineum, lesions often became so extensive that they mechanically obstructed the vulva thus interfering with natural mating.

Lesions of dermatophilosis on the udder often affect the teats which may become encased by thick bark-like scabs, making milking or suckling difficult. These udder lesions result in undernourishment of calves with a resultant increase in calf mortality. A decrease in milk yield of 20 per cent has been reported in dermatophilosis-affected cattle (Nobel *et al.*, 1976). The disease has restricted the introduction of potentially productive exotic cattle breeds to West and Central Africa due to their high susceptibility (Lloyd, 1976; Stewart, 1972; Hyslop, 1980; MacAdam, 1964).

Animals affected with dermatophilosis in Ghana are slaughtered before they reach maturity (Oppong, 1973). Culling of animals before they reach maturity could be interpreted as putting a constraint on the genetic pool. However, it may also be viewed as selection of animals more resistant to the disease.

COST OF TREATMENT AND CONTROL OF INFECTED CASES

Early lesions of dermatophilosis may respond to various treatments because the causative agent is highly sensitive to a wide range of therapeutic agents (Plowright, 1958; Roberts and Graham, 1966). Mild lesions may also undergo spontaneous regression or healing during the dry season. Blancou (1976) reported spontaneous recovery of up to 20 per cent of animals during the dry season whereas Samui and Hugh-Jones (1990a) reported that spontaneous recovery in cattle affected with dermatophilosis was infrequent. They claimed that many farmers maintained that all bovine dermatophilosis cases must eventually be culled.

Severe generalized lesions as well as chronic lesions appear to be intractable because of the inaccessibility of the organism at the site of infection where it is protected from external agents by a thick layer of scab, and to some extent, from parenteral agents by virtue of its relative remoteness from the blood supply (Woodman, 1989).

Topical application of medicaments such as sulphur in groundnut oil (Bida and Dennis, 1976), carbolic acid and aureomycin spray (Oduye, 1975a) have been reported to be successful in treating the disease. However, the method is of limited value because it is time consuming and impractical in severe cases especially when many animals in the same herd are affected.

Blancou (1969) claimed that a combination of penicillin and streptomycin in high doses was effective in the treatment of the disease. Ilemobade, Gyang, Bida and Addo (1979) achieved a 93 per cent (26 of 28) recovery rate in Zebu cattle affected with dermatophilosis in Nigeria, using a single intramuscular injection of long acting oxytetracycline. Sarradin, Akakpo, Bornard and Mohamadou (1985) also found long-acting oxytetracycline efficacious as they acheived cure rates of 89 per cent. In addition, they found a significant increase in the cure rate when the antibiotic therapy was combined with weekly dipping.

The reponse to long-acting oxytetracyclines in the treatment of dermatophilosis has been variable as other workers failed to achieve such spectacular results. Gbodi and Natife (1982) obtained 33 per cent cure rate in treatment of Friesian cattle during the wet season. Likewise, Marchot and Leroy (1987) obtained 35.9 per cent recovery rate after single treatment of infected cattle with long-acting oxytetracycline in Sudan. Lloyd, Hawkins and Pragnell (1990) treated *D. congolensis*-affected cattle in two ranches in St. Kitts using single intramuscular injection of long-acting oxytetracycline at the rate of 20 $mg.Kg^{-1}$ and complete recovery occurred in 21 per cent of the affected animals. They observed that the disease tended to recur in treated animals reared under low standards of husbandry. On the other hand, they associated improvements in tick control and feeding, with a continuing low level of infection. Lloyd and others (1990) suggested that long-acting oxytetracycline can be used to conveniently halt outbreaks of dermatophilosis and allow time for changes in management designed to reduce recurrence of the disease. Samui and co-workers (1990a) found that tick control and early proper treatment of infected cattle are the most important factors influencing bovine dermatophilosis patterns and rates of occurrence in Zambia.

Apart from the relative ineffectiveness of parenteral administration of antibiotics in treating cases of dermatophilosis it is very expensive and therefore systemic treatment of dermatophilosis may not be economically justified (Stewart, 1972; Oppong, 1973; Oduye, 1975a).

HUMORAL IMMUNE RESPONSE AND CONTROL BY VACCINATION

The role of antibodies in the host response to infection is controversial. Numerous studies of the humoral response to *D. congolensis* have identified specific antibody production, yet the protective role of these, if any, remains obscure (Woodman, 1989). Animals may possess 'natural' agglutinins which react in *in vitro* tests for *D. congolensis* antibodies. These non-specific antibodies are present only at low levels and are therefore easily distinguished from specific antibodies produced in response to infection (Lloyd, 1981).

Infection with *D. congolensis*, either natural or experimental, results in the appearance of specific serum antibodies in the host which augment the 'natural' antibodies found in animals with no previous exposure to the organism (Roberts, 1967). However the presence of high titre serum antibody does not confer protective immunity to natural infection (Pulliam, Kelley and Coles, 1967; Merkel, Richard, Thurston and Ness, 1972). Roberts (1967) reported that both flagellar and somatic agglutinin were produced. The first antibodies to appear following experimental infection were haemagglutinants (IgM) (Richard, Thurston and Pier, 1976). Precipitating antibodies (IgG) were not produced after a single infection but were only present after repeated or chronic infection or vaccination (Roberts, 1967; Richard *et al.*, 1976).

Repeated infection or vaccination stimulated an anamnestic response (Roberts, 1964; Makinde and Ezeh, 1981). Accelerated healing of lesions occurred in conjunction with the anamnestic response and Makinde and Ezeh (1981) associated the high antibody titres with the accelerated healing.

Merkal, Richard, Thurston and Ness (1972) showed that lesion formation in hypersensitive rabbits was unaffected by treatment with methotrexate, which abolished the normal precipitin response to infection and decreased the haemagglutinating antibody titres. They concluded that *D. congolensis* - induced lesions were inflammatory reactions unrelated to the immune response.

Roberts (1966) has demonstrated that high titres of specific antibody greatly enhanced the destruction of zoospores in infected sheep and guinea pigs following neutrophil phagocytosis and considered that this resistance was only demonstrable when the epidermis was damaged by scarification but not when the intact epidermis was defatted. However, Jenkinson, Lloyd and Mabon (1979) observed that the procedure of using light petroleum for defatting the skin removed an emulsion from the skin surface that contained IgG and IgA.

Ellis and others (1987) suggested that humoral immunity particularly immunoglobulin IgA, at the skin surface played a role in lesion resolution in dermatophilosis in Merino sheep. Lloyd and Jenkinson (1981) have shown that following intradermal vaccination specific antibodies, IgA and IgM, to *D. congolensis* were secreted at the skin surface but it is not known whether these antibodies were transported to the skin from serum or were produced locally.

Pulliam and others (1967) demonstrated circulating antibodies in experimentally and naturally infected cattle by agglutination, agar gel precipitation and indirect haemagglutination tests. They, however, concluded that although *D. congolensis* is antigenic, the antibodies infected animals produce were not completely protective.

VACCINATION

Results from vaccination trials against dermatophilosis have been conflicting. Roberts (1967) reported that sheep which recovered spontaneously from dermatophilosis were subsequently resistant to reinfection, which implied the development of an immune response.

Roberts and Graham (1966) had limited success at vaccination, although other workers have reported more encouraging results. Chamoiseau and Lefevre (1973) vaccinated rabbits with live whole cell preparations of *D. congolensis* either intradermally or subcutaneously with Freund's complete adjuvant and claimed that protection was achieved against subsequent challenge. How and Lloyd (1988b) re-evaluated the work of Chamoiseau and colleagues (1973) and found significant but not complete protection against homologous *D. congolensis* challenge. Provost, Touade, Guillame, Peleton and Damsou (1974) vaccinated cattle in Southern Chad by intradermal inoculation of a young culture of *D. congolensis* (live crude filaments) and found it to produce significant protection.

Abu-Samra and others (1976) observed that their experimental animals were resistant to re-infection with *D. congolensis* and that infection confered some degree of transitory resistance. Thorold (1964) made similar observations in rabbits.

In a review article, Lloyd (1984) speculated that immunization might increase resistance to *D. congolensis* and accelerate the healing of lesions but its efficacy was dependent on the nature of the antigen used and the mode of vaccination. On the other hand, a study of 60 naturally infected cattle revealed that there was no correlation between the severity of infection and the antibody titre (Oduye, 1974). Recent studies have shown that during the different phases of its life cycle, *D. congolensis* can be fractionated into several antigenic fractions (Sutherland, Ellis, Robertson and Gregory, 1987) and that prevention of dermatophilosis by vaccination may be possible if the protective antigens can be defined (Sutherland and Robertson, 1988). Studies in sheep in Australia involving vaccination against dermatophilosis with crude washed antigen preparations of zoopsores, filaments and soluble components of the bacterium have shown that prior exposure particularly to filamentous components of the bacterium resulted in earlier resolution of lesions and less severe disease than in the unvaccinated sheep (Sutherland and Robertson, 1988). A similar observation was made by Blancou (1976) who concluded that whilst vaccination reduced the severity of the disease, it had little effect on the incidence of infection. Sutherland, Ellis and Edwards (1991) observed a difference in response to vaccination between pen trials and field studies with dermatophilosis in sheep. They found that under field conditions vaccination did not offer the same protection against dermatophilosis in sheep as was the case in animals housed in pens. They speculated that the differences in protection between pen and field studies with the *D. congolensis* vaccines they used was due to differences in the size of the challenge dose in the field combined with climatic effects and difference between *D. congolensis* strains in the field and the strain used for the pen studies. How and Lloyd (1990) found a ten-fold difference in the minimum infective dose of zoospores between two different isolates suggesting that one strain was more virulent than the other. How and Lloyd (1990), using rabbits as a model, demonstrated that recent vaccination increased (up to 10,000-fold) the dose of homologous organisms required to cause infection compared with unvaccinted animals. They concluded that the approximately 100-fold difference between strains in the extent of the vaccine-induced protection indicated that immunity was to some extent strain-specific. They suggested that vaccines designed for the prevention of dermatophilosis in the field need to include a range of local antigenic variants of *D. congolensis* if common protective antigens cannot be identified. Lloyd (1984) was optimistic that vaccination is the only method which appears to offer any hope of controlling the disease and speculated that immunoprophylaxis remained the most hopeful means for control of dermatophilosis in the tropics.

Several workers have reported that natural dermatophilosis does not stimulate development of a protective immunity (Bida and Dennis, 1976; Aghomo and Lloyd, 1983). MacAdam (1970) was able to reinfect calves, sheep, goats, horses and rabbits

6 weeks after initial infection. Pulliam and others (1967) noted that should there be any immunity, it should not depend on classic antibodies, because their existence does not prove the resistance of an animal to infection.

Since animals recovering from a natural infection do not acquire immunity to subsequent infection, current types of vaccines are unlikely to control the disease (Bida and Kelley, 1976; Hyslop, 1980).

IMMUNOSUPPRESSION

Dohms and Saif (1984) defined immunosuppression as "A state of temporary or permanent dysfunction of the immune system response resulting from damage to the immune system and leading to increased susceptibility to disease". Immunosuppression relates more to dysfunction of adaptive immunity rather than compromise of innate defences that serve as the first line of defence against broad classes of invading microorganisms. Muneer and others (1988) stated that numerous immunosuppressive agents affect avian and mammalian species including viruses, parasites, microbial toxins, chemicals, drugs, nutritional deficiencies and various environmental stressors. Tizard (1987) reported that pathogenic microbes have developed elaborate mechanisms of invasion, survival and dissemination in order to survive the varied cellular and humoral host responses. He pointed out that mimicry of host cell major histocompatible cell (MHC) antigens, masking by host antigen adsorption, antigen shifting of surfaces epitopes, intracellular survival, phagocytic evasion, and latency are ways that pathogens evade host responses. Dohms (1991) reported that immunosuppression provides a means of evading host defences by attacking cells of the immune system. He stressed that there are two categories of immunosuppression, antigen-specific and generalized unresponsiveness. Generalized immunosuppression involved overall compromise of the host adaptive responses. Because the immune system involves networks of interacting cells, damage to key cell populations may disrupt the entire system and lead to increases in opportunistic infections.

Immunosuppression caused by tick infestation has been reported by several workers. Wikel (1982a) infested guinea pigs with the tick *Dermacentor andersoni* and demonstrated that lymphocytes from these animals showed a reduction in their *in vitro* responsiveness to the T-lymphocyte mitogen Concanavalin A and phytohaemagglutinin (PHA), while stimulation by the B-lymphocyte mitogen *Escherichia coli* lipopolysaccharide was unimpaired. The author postulated that a reduced *in vitro* responsiveness of lymphocytes to Concanavalin A and phytohaemagglutinin, as a consequence of tick infestation, suggested that the tick-host interaction resulted in an alteration of reactivity apparently limited to T-lymphocytes. Peripheral blood lymphocytes obtained from animals unexposed to tick infestation were not stimulated to undergo blastogenesis by *in vitro* cultivation in the presence of salivary gland antigens (Wikel and Osburn, 1982; George, Osburn and Wikel, 1985). Wikel, Graham and Allen (1978) observed that lymphocytes from tick-infested guinea pigs were significantly less responsive to the mitogen PHA than similar cells from uninfested control animals. Wikel and colleagues (1978) and Wikel and Osburn (1982) indicated that the general lower responsiveness of lymphocytes derived from tick-resistant guinea pigs to PHA indicated that the process of tick infestation might be causing a degree of immunosuppression in the host. Stewart (1983) reported that the addition of tick saliva from *Rhipicephalus evertsi* and *Boophilus microplus* at varying dilutions to bovine mononuclear cells caused immunosuppression when compared with control tests. Fivaz (1989) observed strong immunosuppression in rabbits and cattle infested with *R. appendiculatus*.

Immunosuppression has also been implicated in other diseases and parasitic infections. For example, several lines of evidence indicate that patients with lepromatous leprosy have a depressed capacity to express or develop cell-mediated immunity and depressed lymphocyte transformation (Shepard, 1968; Turk, 1969). Delayed hypersensitivity in leprosy was evaluated by Waldorf, Trautman and Block (1966) by testing 34 patients with leprosy with 2, 4-dinitrochloro-benzene (DNCB) a chemical which sensitized 95 per cent of 43 controls. They found that half (17) of the 34 patients tested did not react to DNCB, a finding which indicated a generalized impairment in the ability to develop delayed hypersensitivity in patients with active uncomplicated lepromatous leprosy. More recently, Dohms (1991) stated that leprosy, caused by *Mycobacterium leprae*, is an example of a "pathogen mediated" suppression. He elaborated that tuberculoid leprosy (TL) is characterized by localized discrete lesions that contain low or undetectable numbers of bacteria. Patients show good reactivity to lepromin antigen both *in vivo* and in cultured lymphocytes *in vitro*. At the other end of the clinical spectrum, lepromatous leprosy (LL), lesions are more extensive and diffuse containing high numbers of bacteria; up to 10^{10} bacteria/g tissue have been found within LL lesions. Mononuclear cells taken from lepromatous patients show suppressor activities when cultured *in vitro*.

Mansfield and Wallace (1974) demonstrated that both *in vivo* and *in vitro* markers of cell-mediated immunity were depressed in rabbits chronically infected with *Trypanosoma congolense* as shown by marked suppression of peripheral blood lymphocyte responses *in vitro* to purified protein derivative and to phytohaemagglutinin. They also found that supernatant fluids of antigen-stimulated lymph node cell cultures from *T. congolensis*-infected rabbits failed to demonstrate migration inhibition factors. More recently Kierszenbaum and others (1991) reported depression of peripheral blood mononuclear cells by *Trypanosoma brucei rhodesiense*. They demonstrated that in the presence of *T.b. rhodesiense*, phytohaemagglutinin-activated human peripheral blood mononuclear cells displayed a markedly decreased capacity to express interleukin 2-receptors (IL-2R). *T.b. rhodesiense* also significantly reduced lymphoproliferation monitored by [^{3}H]-thymidine uptake.

Pelley, Ruffier and Warren (1976) reported that in mice with chronic schistosomiasis, continued stimulation of the immune system resulted in the development of profound unresponsiveness. Twenty weeks after infection, all parameters of cell-mediated immunity (granulomatous hypersensitivity) were affected. Delayed footpad swelling and migratory inhibitory factor production were suppressed and mitogen-incubated lymphocytes produced only 4 per cent of the DNA of normal cells.

Dohms (1991) stated that for most immunosuppressed diseases of man and animals, little is known of the complete interactive mechanisms that produce down regulation of the immune responses. Mechanisms of immunosuppression by microbial pathogens, toxins, dietary components, or environmental stressors are incompletely understood. However, the exact immunological defects often cannot be conclusively described.

Blood and Mehra (1988) attributed mechanisms of immunosuppression in lepromatous leprosy patients to suppressor activities of T8+ bearing lymphocytes. Blood and Mehra (1984) postulated that non-adherent suppressor cells of T8+ phenotype were responsible for the specific suppression by blocking expansion of potential reactive T4+ helper lymphocytes. They developed an assay that measured the ability of non-adherent cells taken from patients and depressed polyclonal mitogen stimulation of T4-helper lymphocytes by the mitogen Concanavalin A. They used lepromin in their assay system and stimulated specific suppressor cells that blocked mitogen stimulation. Blood and Mehra (1988) showed that T8+ and T4+ lymphocyte populations modulated immunosuppression in leprosy. High T8 to T4 ratios showed poor responses in assay while depletion of T8+ cells restored responsiveness.

The ability of microorganisms and parasites to modulate *in vivo* and *in vitro* immunological responsiveness is an established phenomenon (Shenker *et al.*, 1991). However, the exact contribution of these exogenous regulatory processes to establishment of disease is not fully understood. Shenker and others (1991) pointed out that such inhibitory factors could lead to a state of immunological hyporesponsiveness that favours the establishment of disease.

Circumstantial evidence has led many workers to incriminate ticks in the course of dermatophilosis. However, distribution of lesions do not always reconcile with tick attachment sites. More recently some workers have suggested that ticks have a systemic effect on their hosts by immunomodulation (Barré, 1989; Martinez, 1991). Chapter 3 of this study investigates the effects of ticks on the immune system of their hosts.

IMMUNE RESPONSE TO *DERMATOPHILUS CONGOLENSIS*

Most studies of the immune responses to *D. congolensis* have been centred on experiment model infections. Roberts (1966) assessed the response of various animals to infection by measuring the diameter of erythema induced by intradermal injection of *D. congolensis* antigen, taking this as an index of hypersensitivity. He reported delayed-type hypersensitivity (DTH) responses to primary and secondary experimental infections in sheep, rabbits and guinea pigs. An immediate hypersensitivity reaction also occurred but only after more than one infection. The DTH response appeared to be of the tuberculin type and would therefore have been mediated by mononuclear cells. It first appeared four days after commencement of a daily infection schedule which was also the time at which the lesions began to resolve. From the above and other studies, Roberts (1967) concluded that the resolution of lesions was associated with DTH, whereas resistance to new infection was associated with antibody to somatic antigens of *D. congolensis.*

Ellis and colleagues (1987) found several aspects of the cellular responses observed in the skin of Merino sheep following inoculation with *D. congolensis* different from those reported by Roberts (1966). In their study, Ellis and others (1987) found invasion of the epidermis of Merino sheep by *D. congolensis* filaments, accompanied by intense neutrophil infiltration in the upper dermis and epidermis at 4 days post-inoculation. In Robert's study (1966), marked invasion of the ovine epidermis by *D. congolensis* filaments with intense neutrophil accumulation in the area occurred by 26 h post-inoculation. Oduye (1975c) found histological changes consisting of sub-epidermal oedema, and accumulation of neutrophils beneath the epidermis especially around the hair follicles as early as 4 hours post-inoculation. By 12 hours post-inoculation he observed a dense layer of neutrophils that had separated the epidermis in a group of cattle he studied.

Both Roberts (1966) and Oduye (1975c) used light petroleum and xylene respectively for defatting the skin before inoculation of *D. congolensis*. Ellis and colleagues (1987) attributed the use of light petroleum for defatting the skin by Roberts (1966) to the more rapid invasion of the skin in his study. The defatting procedure would have removed an emulsion from the skin surface that contained IgG and IgA (Jenkinson *et al.*, 1979). Macrophages and lymphocytes were never present in appreciable numbers in Robert's (1966) study, in contrast to the findings of Oduye (1975c) and Abu-Samra and others (1976) that from 60 and 72 hours post-infection the dermal reaction was predominantly a mononuclear cell accumulation. Ellis and others (1987) reported that at the time lesions were healing, the inflammatory cell infiltrate consisted mostly of lymphocytes, macrophages and plasma cells with few or no neutrophils. Roberts (1966) showed that infected sheep developed a delayed-type hypersensitivity (DTH) reaction after primary infection, which resulted in accelerated infiltration of neutrophils, decreased filament penetration of follicle sheaths and earlier healing in subsequent infections. However, sheep with chronic dermatophilosis also showed DTH reactivity, but their lesions persisted and although a second infection resolved rapidly in some, others developed chronic infections at the site of a second infection (Roberts and Graham, 1966).

Ellis and colleagues (1987) were of the opinion that lesion resolution in sheep was not solely a function of DTH reactivity. They pointed out that in the primary inoculation of their study, resolution was not correlated with the dense aggregation of lymphocytes and macrophages in the superficial dermis. These cells are usually considered as the morphological component of the DTH reaction (Roitt, 1974). In contrast they detected correlation between lesion resolution with the presence of increased numbers of plasma cells around wool follicles and skin glands in the dermis below dermatophilosis lesions.

Merkal and co-workers (1972) found that the lesions on experimentally-infected rabbits developed and resolved in similar fashion whether or not the animal had been treated with methotrexate, an inhibitor of cell division which suppressed both DTH and immunoglobulin production. The authors concluded that recovery from infection depended on non-specific inflammatory responses which are not affected by methotrexate. In contrast to the work of Merkal and others (1972) administration of another immunosuppressive agent, cyclophosphamide, was found to greatly prolong the duration of experimentally-induced *D. congolensis* lesions in rats (Woodman, 1989).

Abu-Samra, Imbabi and Mahgoub (1976) noted that the histopathological changes seen in naturally infected cattle were suggestive of a progressive disease of the tuberculin delayed hypersensitivity type. Abu-Samra (1980) stressed that the reaction in experimental infection may be different to that in natural infections. He pointed out that the massive doses given to elicit an experimental infection were unrepresentative of the repeated small doses received through damaged skin barriers in the field. He speculated that these repeated small doses give rise to a state of hypersensitivity with the release of mediators such as macrophage migration inhibition factor, chemotactic and lymphotoxic factors. Woodman (1989) pointed out that in the field, DTH may also develop to agents other than *D. congolensis*, such as biting-arthropods, reactions which may interfere with the normal hypersensitivity responses to *D. congolensis*.

Davis and Philpott (1980) mimicked the effect of biting-arthropods by inducing a DTH response in goats to 1-chloro-2,4-dinitrobenzene (DNCB) and then inoculated the site with *D. congolensis*. They demonstrated that the lesions they induced were pathologically similar to those of natural chronic infections and persisted for as long as the DNCB was applied. They speculated that a DTH reaction to an arthropod bite in the field was followed by a period of immune suppression at the site due to feedback mechanisms operating. Thus, no immune response developed if the site became invaded by *D. congolensis* and the infection therefore progressed to a chronic state.

Later, Davis (1984) used the number of *D. congolensis* cocci recovered from infected guinea pigs as an index of degree of infection. He found that animals which were sensitized to DNCB yielded higher numbers of cocci, and for longer period than DNCB-naive animals. On the other hand, fewer cocci were harvested from guinea pigs which had developed tolerance to DNCB than those sensitized to it, a finding suggesting that *D. congolensis* established itself where hypersensitivity reactions to other agents were present. Thus, it might be thought that where an animal was undergoing a DTH response to an unrelated agent, the cell-mediated response to *D. congolensis* at that site would be inhibited. However, Higgins (1983) showed that rats sensitized to DNCB showed the same course of macrophage migration inhibition activity as controls.

It must be appreciated that the immune response to *D. congolensis* is complicated, more so when in natural situations, concurrent immune responses to other agents add a further dimension of complexity.

IMMUNE RESPONSE TO TICK INFESTATION

Resistance of cattle to ixodid ticks was first reported by Johnson and Bancroft (1918). Resistance to ticks is not well understood; it is complex and has many components to it (Willadsen, 1980; Wikel, 1982c; Brown, 1988).

A number of immune response mechanisms and mediators have been reported to be stimulated in mammalian hosts during ixodid infestation. These include precipitating antibodies, immediate hypersensitivity and cell-mediated immunity (Willadsen, 1980; Wikel, 1982c). Acquired resistance to ixodid infestation is expressed in a number of ways such as the reduced number of ticks feeding to repletion, reduced engorgement weights, reduced oviposition and viability of ova, prolonged time to obtain a blood meal and death of the tick on the resistant host (Riek, 1956; Strother *et al.*, 1974). George and others (1985) infested purebred and crossbred *Bos indicus* calves with *A. americanum* and showed that resistance was acquired by both the purebred and the crossbred calves after first infestation and resulted in statistically significant decreases in the percentages of females that engorged, the mean weights of engorged females and the mean weights of egg masses. Intradermal injection of salivary gland antigen into sensitized animals showed both immediate and strong delayed reactions to *A. americanum*, confirming earlier observations of Wikel and Osburn (1982). Brown, Barker and Askense (1984) studied bovine resistance to *A. americanum* ticks in Friesian cattle. The animals acquired immunity to *A. americanum* ticks resulting in significant decreases in feeding and ovipositional success associated with a local cutaneous basophilia response and peripheral basophilia. They speculated that basophils were involved in bovine immunity to ticks as had been established in the guinea pig model (Allen, 1973; Allen, Doube and Kemp, 1977; Gordon and Allen, 1979; Brown and Askenase, 1981).

Walker and Fletcher (1986, 1987) studied the histology of attachment sites of *R. appendiculatus* ticks on cattle. They found that the basic lesion that forms is a sterile inflammatory abscess in the dermis dominated by infiltration of neutrophils and mononuclear cells. When the cattle became resistant, delayed hypersensitive reactions of two types developed. Intra-epidermal pustulation reduced attachment of the ticks. Infiltration of the dermis by extra numbers of cells of all types, both with higher proportions of granulocytes than in the basic inflammatory abscess, reduced engorgement of the ticks (Walker and Fletcher, 1987).

Immediate hypersensitivity is responsible, in part, for the resistance of cattle to ticks. A major expression of this resistance is the rejection of larvae in the 24 hours of the life cycle; within this period they make shorter, repeated attachments on host of high resistance compared with less resistant ones (Riek, 1956).

Snowball (1956), Riek (1962) and Bennett (1969) reported that host grooming activity was an important factor in reduction of tick burdens, and animals restricted from grooming yielded increased numbers of engorged ticks. George and co-workers (1985) postulated that cutaneous immune reactivity directed toward the attached tick served as a source of irritation which stimulated host grooming.

Pharmacologic mediators in the vicinity of tick attachment sites were implicated in the resistance response. Willadsen and colleagues (1979) observed that the amount of histamine in the skin of cattle resistant to *B. microplus* correlated directly with the degree of resistance and the intensity of immediate hypersensitivity reactions.

Based on earlier reports by Allen and colleagues (1979) that Langerhans cells bind tick salivary gland secretions and by Ptak and others (1980) that Langerhans cells acted as antigen-presenting cells in the induction of contact hypersensitivity, Brown and Askenase (1985), and Brown (1988) postulated that Langerhans cells take up and present tick antigens to sensitized T-cells which then secrete chemotactic factors that recruit circulating basophils bound with anti-tick antibody, to infiltrate the local site and effect the immune resistance response.

TICK-ASSOCIATED LOSSES

Losses due to tick infestation are considerable. McCosker (1979) stated that of all external parasites, ticks cause the greatest economic losses in livestock production worldwide. He observed that ticks and the diseases they transmit to livestock are a major animal health problem in all developing countries.

There have been very few detailed studies on the economic effects of ticks on livestock and these dealt mainly with the one-host tick, *Boophilus* species. McCosker (1979) pointed out that in 1906, indirect and direct losses caused by *B. annulatus* in the United States of America (USA) was estimated at US $130.5 million. Later in 1976, estimates of the economic importance of ticks other than *Boophilus* species in the USA indicated an annual loss of US $62 million. The author estimated the total annual world losses due to ticks, tick-borne diseases and tick control to be US $7.0 billion or US $7.8/head of cattle based on world cattle population of 1,214 million. Bram (1983) stated that studies in Australia indicated that the total annual loss caused by the cattle tick, *B. microplus*, amounted to A $5 per head of cattle, or 4 per cent of the gross value of cattle slaughtered in 1972/73.

Cattle infested with ticks loose bodyweight. Williams, Hair and NcNew (1978) showed that 30 engorged *A. maculatum* Koch females caused a loss in liveweight gain (LWG) of 33 g per tick in *Bos taurus* cattle. Barnard (1985) indicated that at an infestation level of 20 *A. americanum* females, the loss in LWG in infested cattle was 27.6 g per engorged female. More recently, Pegram and colleagues (1989b) confirmed these earlier findings when they demonstrated significant losses in LWG in indigenous cattle in Zambia associated with adult *A. variegatum* infestations. Differences in mean LWG between treated and untreated cattle and the cumulative total estimate of *A. variegatum* females indicated losses of about 45-60 g per engorged female. Ticks are responsible for the transmission of a large variety of pathogens that affect livestock (McCosker, 1979; Bram, 1983; Drummond, 1983; Springell, 1983; Norval *et al.*, 1989). In addition, tick infestations cause physical damage to livestock manifested by paralysis or toxicosis, "tick worry" irritation, restlessness and anaemia. For example, Bram (1983) estimated each ox lost 1-3 ml of blood for every *B. microplus* which successfully completed its life cycle on an animal. Pegram and others (1989b) noted that larger ticks such as *Amblyomma* species proportionally engorge more blood than *Boophilus* species. Tick infested animals are readily invaded by bacteria, fungi and are predisposed to infestation by other arthropods and pathogens. Norval and others (1989) reported secondary screw-worm infestations in wounds associated with *Amblyomma hebraeum* bites. They commented that as long as the fly was present, frequent inspections and treatment to prevent a build-up of clusters of *A. hebraeum* on cattle was essential. The direct injury to hides caused by tick bites is economically significant. Ticks are therefore controlled for two reasons; first to eliminate the adverse effects of ticks *per se* on livestock production i.e. meat production, and second to reduce the economic losses, either death or chronic wasting, caused by major killing diseases such as East Coast fever, heartwater and babesiosis.

CONTROL OF TICKS

CHEMICAL CONTROL

The most common method of killing ticks is by the use of chemical acaricides (Brander and Pugh, 1971; Wharton, 1976; Drummond, 1983). Drummond (1983) stated that acaricides used to control ticks on livestock or in the environment should be applied in such a manner that the ticks will be killed, the treatments will not harm livestock or applicators, the tissues of treated animals will not contain illegal residues, and the environment will not be adversely affected. Barnett (1961) and Shaw and others (1970) have reviewed the control of ticks with acaricides. The most popular method of controlling ticks on livestock is the application of acaricides directly to the animal host either by dipping in dipping vats, spraying or hand dressing.

Several general groups of chemical compounds effectively kill ticks on livestock. These include arsenicals, chlorinated hydrocarbons, organophosphorus compounds, carbamates (Wharton, 1976; Drummond, 1983), amidines (Nolan, 1979) and pyrethroids (Hamel and Duncan, 1986; Hamel, 1987; Heller-Haupt and Varma, 1982). Efficacy of the synthetic pyrethroids permethrin, cypermethrin and deltametrin against *R. appendiculatus*, *A. hebraeum* and *A. variegatum* was investigated and confirmed by Heller-Haupt, Varma, Crook and Radalovicz (1979).

The development of acaricide-resistant tick strains has with time rendered one chemical agent after another ineffective (Brander and Pugh, 1971; Wharton, 1976; Drummond, 1983). Hamel and Duncan (1986) reported that there were no drug residue and tick-resistance, as yet, with the flumethrin-based synthetic pyrethroid, Bayticol.

Because of cost of developing, licensing and production of new acaricides (Fischer, 1983) and the emergence of tick-resistance to acaricides (Bigalke, 1980; Springell, 1983), alternate means of tick control have been advocated. Wharton (1983) stressed that the most logical method of alleviating tick depredations would be to capitalize on host-parasite relationships that evolved in nature. He observed that cattle survived in Asia and Africa despite babesiosis and theileriosis and their *Boophilus* and *Rhipicephalus* vectors. Host resistance, expressed by an animal's ability to prevent the maturing of large numbers of ticks and disease immunity, are host survival mechanisms for external and internal parasites.

Bram (1983) asserted that although chemical control of ticks will, for the foreseeable future, form the basis of any control programme, alternate methods of control should also be incorporated whenever feasible.

Some breeds of cattle are more resistant to tick infestation than others, as discussed earlier, in this chapter. Spickett (1987) reviewed breed susceptibility to ticks. Resistance to tick infestation has been shown to be heritable (Heweston, 1972; Seifert, 1971). Moreover, Smith and others (1989) demonstrated that homogenates from unfed ticks can be used reliably as antigen in delayed-type hypersensitivity tests for assessing the immune status of cattle. They showed that the delayed-type hypersensitivity test can be used in the field for identifying tick-resistant and tick-susceptible animals. Alternate methods of tick control in tick endemic areas should incorporate raising tick-resistant animals.

Spickett (1987) and Petney and others (1987) elaborated on alternate methods of tick control including modification of vegetation and the microclimate in which the ticks live i.e. veld burning, bush clearing and the introduction of tick-resistant grasses such as *Melinis minutiflora* (molasses grass) and plants with hooked trichomes forming a barrier to ticks such as *Desmodium*.

HOST VACCINATION AGAINST TICKS

Willadsen (1980), Wikel (1982c) and Spickett (1987) reviewed the literature on artificial immunization against ticks. Wikel (1982c) stated that a limited number of investigators have attempted to induce resistance to ectoparasite infestation by artificial immunization. Results were mixed, but the indications were that such a control approach might be practical for selected species, particularly long-term blood feeders such as ticks. He asserted that host vaccination would be more feasible in an integrated pest management scheme. Gregson (1941) infested two guinea pigs with *D. andersoni* nymphs, and one of the animals was subsequently given four weekly subcutaneous injections with a nymph extract. Upon re-infestation, the injected animal allowed fewer nymphs to engorge than did the animal infested only once. Allen and Humphreys (1979) used midgut, reproductive, and other internal organs of female *D. andersoni* to induce significant resistance to re-infestation. Wikel (1981) used a salivary gland antigen from a partially engorged female *D. andersoni* to induce a significant degree of resistance to tick challenge. Wikel (1982c) was convinced that artificial immunization can be used to partially protect against tick infestation.

ROLE OF PARASITES, PATHOGENS AND PREDATORS IN TICK CONTROL

Petney and others (1987) and Spickett (1987) reported on the effect of pathogens, predators, and interactions with other species on ticks but Petney and colleagues (1987) reported that very little information was available on the role of these factors in reducing the number of ticks present at a given time. Nevertheless, they suggested involvement of the bacterium *Bacillus thuringiensis*, the red and yellow billed ox-peckers, *Buphagus erythrorhynchus* and *Buphagus africanus* and ants in predation of free-living stages of ticks and their consequence on population levels.

ECOLOGY OF *AMBLYOMMA VARIEGATUM*

Petney and colleagues (1987) reviewed the ecology of the African vectors of heartwater, with particular reference to *A. hebraeum* and *A. variegatum*. In the present study general information relating to host attachment sites, climatic requirements, habitat preferences and populations dynamics of *A. variegatum* have been discussed in Chapter 2.

CHAPTER TWO

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS AND THE ASSOCIATION OF *AMBLYOMMA VARIEGATUM* WITH DERMATOPHILOSIS ON THE COASTAL PLAINS OF GHANA

Page No.

CHAPTER TWO

INTRODUCTION

Ticks cause a significant annual loss of revenue worldwide due to direct withdrawal of blood from the host when feeding, pathogen transmission and secondary bacterial infections at attachment sites (Steelman, 1976). Tick-associated losses to the livestock industry have been discussed under "Control of Ticks" in Chapter One. Ticks have been implicated in the establishment of dermatophilosis by many workers. In cattle, the role of ticks in the pathogenesis of the disease is reported to be important in some areas and, under these conditions, tick control with acaricides reduces the incidence (Henderson, 1928; Hobday, 1952; Plowright, 1956; Macadam, 1962).

DISTRIBUTION OF *A. VARIEGATUM*

Amblyomma variegatum is one of the major tick parasites of cattle over a wide area of Africa (Plate 2.1). They are normally distributed in tropical areas within latitudes 12° of the equator and where it occurs, it is often the most common tick on cattle (Hoogstraal, 1956; Walker, 1987). *Amblyomma variegatum* has also established itself from infested cattle imported from Senegal to Guadeloupe about 150 years ago thence to other islands in the Caribbean (Uilenberg *et al.*, 1984).

RAINFALL

The single common factor in their ecology is relatively high rainfall. *Amblyomma variegatum* occurs in areas where the minimal rainfall is between 400-750 mm per annum (Hoogstraal, 1956; Walker, 1974; Pegram, 1976; Pegram *et al.*, 1981). At low rainfall (400 to between 750-1000 mm in Tanzania), *A. variegatum* becomes less common (Yeoman and Walker, 1967). In areas with one rainy season annually, *A. variegatum* tick has a single generation a year as was observed by Wilson (1950) in Malawi and Hoogstraal (1956) in the Sudan.

In Kenya and Uganda with two rainy periods each year, multiplication is faster and two or three generations may breed during a 12 month period (Wilson, 1953). Petney *et al.* (1987) made a similar observation.

In general, adult abundance of *A. variegatum* is greatest during the rainy season. Thus in Tanzania (Yeoman and Walker, 1967; Tatchell and Easton, 1986), Zambia (Pegram *et al.*, 1986) and Malawi (Wilson, 1946, 1950) peak abundance occurs from November/December-January. However there are substantial variations in the maximum abundance and the exact timing of peak occurrence between years. Pegram and others (1986) and Tatchell and Easton (1986) observed substantial differences in maximal abundance between regions with different climatic characteristics in Zambia and Tanzania, respectively. In Ethiopia, adults occur from March-July, during the spring rains (Pegram *et al.*, 1981). In Nigeria adults peak in May-June rainy season (Bayer and Maina, 1984). Norval (1983) reported that in Zimbabwe, adult *A. variegatum* occurs throughout the year, but heavier loads are present on cattle in the warmer, rainy season from September-May. *Amblyomma variegatum* is normally found throughout a wide altitude range - from sea level to 2590 m (Hoogstraal, 1956; Petney *et al.*, 1987). Among the *Amblyomma* genera, it is only *A. variegatum* which occurs above 2000 m above sea level.

HOSTS

Hoogstraal (1956) pointed out that *A. variegatum* is a major tick parasite of cattle whereas sheep and goats are infested to a lesser degree (Plate 2.2). *Amblyomma variegatum* adults have also been found in horses, donkeys, camels as well as pigs, dogs and cats (Hoogstraal, 1956; Walker, 1974; Yeoman, 1968; Pegram *et al.*, 1981). However, *A. variegatum* adults are most prevalent on medium and large herbivores (Hoogstraal, 1956). Macleod and others (1977) reported the occurrence of *A. variegatum* on wild hosts but explained that it is usual for adult *A. variegatum* to be less common on wild hosts than on cattle.

Petney and others (1987) stressed that because visual search methods for ticks on large hosts are unreliable, the small larvae and nymphal stages may be easily overlooked by these methods.

Hoogstraal (1956) listed medium and large mammals as well as birds as being frequent hosts of nymphs while larvae are recorded predominantly from birds and small mammals. Sheep and goats were reported to be better hosts for nymphs than for adults (Yeoman, 1968).

ATTACHMENT SITES

Adult and nymph *A. variegatum* ticks occur commonly on the ventral surface of the host including the dewlap, brisket, abdomen, axillae and genitalia (Hoogstraal, 1956; Yeoman and Walker, 1967; Walker, 1974; Mohammed, 1978). Walker (1974) found nymphae on the heels whereas, they were found at the elbows, hocks, and elsewhere on the legs where few adults were present by Yeoman and Walker (1967) and Mohammed (1978). Larvae have been recorded from the head including the ears and other parts of the body (MacLeod *et al.*, 1977; Mohammed, 1978). Walker (1974) recorded immatures from the head and around the eyes and ears of birds.

HABITATS

Amblyomma variegatum is absent from drier, arid areas but can be found in semi-arid and humid areas (Walker, 1974) where it occupies a wide variety of different habitat types, mainly with tree and/or bush cover (Yeoman and Walker, 1967; Walker, 1974). It also occurs in grassland with a high grass cover but not where the grass cover is low (Yeoman, 1968). Pegram (1976) recorded *A. variegatum* in wooded highlands in Northern Somalia.

AMBLYOMMA VARIEGATUM AND DERMATOPHILOSIS

Amblyomma variegatum ticks increase in numbers at the beginning of the rainy season when the incidence and severity of dermatophilosis is highest. This has led to the conclusion that this tick plays a major role in the disease (Hornby, 1920; Chodnik, 1956; Plowright, 1956; Oduye and Lloyd, 1971; Oppong; 1973, 1976; Morrow *et al.*, 1989; Matheron *et al.*, 1989; Morrow and Compton, 1991). The fact that some dermatophilotic lesions are at the predilection sites of the tick from which the organism *D. congolensis* has invariably been identified or isolated has provided strong circumstantial evidence for its possible role in dermatophilosis (Oppong, 1991). Moreover *D. congolensis*, the causal agent of the disease, has been isolated from the tick *Hyalomma asiaticum* by Kusel 'Tan (1967) in Asia and *A. variegatum* by Oppong (1976) and Martinez (1989) in Ghana and the Caribbean respectively.

The association between *A. variegatum* and chronic dermatophilosis has been attributed to their similar geographical distribution (Butler, 1975; Burridge *et al.*, 1984; Garris and Scotland, 1985). In particular, Norval (1986) reported that in Zimbabwe, the disease occurs only in a relatively small area that is infested with *A. variegatum* and is absent from a much larger area that is infested with *A. hebraeum.* In Zimbabwe, *A. variegatum* occurs in highveld areas of higher rainfall and *A. hebraeum* occurs in lowveld areas of lower rainfall. The difference in climate complicates the importance of ticks in establishment of the disease.

Nevertheless, the geographical relationship between *A. variegatum* and dermatophilosis is not absolute because cases of dermatophilosis occur in sheep in Australia, for instance, in the absence of *A. variegatum.*

BREED SUSCEPTIBILITY TO TICKS

Breed susceptibility in relation to resistance of cattle to ticks has been reported by several workers. Mbah (1982) and Tanya *et al.* (1985), found that in the Cameroon, animals with Gudali (*Bos indicus*) blood tended to be more resistant to tick infestation than Holsteins (*Bos taurus*). In general, it is believed that Zebu (*Bos indicus*) cattle i.e. Africander, Sahiwal, Sindi and Brahman are more resistant to tick infestation than Friesians (Brown, 1988). Studies carried out in Australia have showed that European breeds of cattle are normally infested with larger *B. microplus* burdens than Zebu cattle or crosses between the two types (Wharton *et al.*, 1969; Seifert, 1971). Utech and others (1978) observed that pure Zebus normally carried the lowest numbers of *B. microplus*. Bayer and Maina (1984) made a similar observation in Nigeria. Working in the subhumid zone of Nigeria, they found that Friesian-Bunaji crossbred cattle were more heavily infested with ticks than indigenous Bunaji cattle. From their findings, they suggested that Bunaji cattle have a higher degree of resistance than crossbred cattle. Oppong (1973), and Koney and Morrow (1990) noted that N'dama and West Africa Shorthorn in the Accra Plains of Ghana carried comparatively fewer ticks. Working in South Africa, Spickett (1987) reported that indigenous Nguni cattle had less ticks on them than Hereford or Bonsmara and suggested that host resistance contributed to the variation in the level of tick infestation in the different breeds.

Workers in the Caribbean acknowledge that the indigenous Creole cattle are resistant to ticks (Barré *et al.*, 1988; Barré, 1989; Martinez, 1989, 1991; Matheron *et al.*, 1989).

TICK CONTROL

The use of chemical acaricides is the most common method of de-ticking livestock (Wharton, 1983; Drummond, 1983).

Tick control in traditional herds of cattle on the Coastal Plains of Ghana is by hand-dressing, the herdsmen applying diluted acaricide solutions with pieces of cloth to tick-infested sites. De-ticking is planned to coincide with milking time and therefore treatment is normally restricted to milking cows and calves. Tick control on institutional, government-owned and large private farms is by weekly and fortnightly dipping or passing cattle through motorized spray-races.

Generally acaricides used are those available in local markets. The range of acaricides in the country includes organophosphates such as Supona[1] and Delnav DFF[2]; organochlorinated compounds such as Gammatox[3] and more recently the flumethrin-based synthetic pyrethroid, Bayticol[4]. Regular, year-round tick control with acaricide is not practised in West Africa and cannot be considered a permanent solution for controlling ticks on traditional managed herds. Cost and irregular availability of acaricides, lack of water in some areas, lack of infrastructure i.e. dip tanks, spray races, the development of resistance in ticks to a particular acaricide and the interference of enzootic stability to tick-borne protozoal and rickettsial diseases are some of the many factors to be considered in the use of acaricides in tick control. Studies in Zambia by Pegram and others (1989b) revealed that intensive tick control was not economically justified despite significant improvements in liveweight gain in tick-free cattle.

Using acaricides to control ticks at periods of the year when their numbers are high is preferable. This will entail using acaricides more often during parts of the year.

Knowledge of the seasonal pattern and relative abundance of ticks on livestock is a prerequisite for planning such a strategy.

The present study was designed to investigate tick population dynamics, their seasonal occurrence and their association with dermatophilosis with a view to embarking on a planned tick-control programme to control dermatophilosis.

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[1]chlorfenvinphos (Supona)
[2]dioxathion (Delnav)
[3]lindane (Gammatox)
[4]flumethrin (Bayticol)

MATERIALS AND METHODS

FIELD STUDIES

Experiment Herd - Project Site

Ghana is approximately 880 km long and 560 km wide and lies near the centre of the West African seaboard and across the Greenwich meridian between the parallels of 4° and 12° north latitude. The study site was in the coastal savannah zone i.e. Accra Plains of Ghana at Abladzei village 16 km north-east of Accra in the Ga District of the Greater Accra Region (Appendices 1 and 2). The average rainfall is between 750-1000 mm per annum with a relative humidity of 90 per cent as a normal daily maximum, the normal minimum being 70 per cent. Temperatures vary little during the year with an average maximum of 28°C and minimum of 22°C. Vegetation comprises tree savannah with clumps of thicket. Common grasses include *Andropogon gayanus* (Gamba), *Panicum maximum* (Guinea), *Pennisetum purpureum* (Elephant). These grasses are associated with a thorny bush steppe largely of acacia species. The soils are tropical sandy soils with a shallow impermeable claypan with extreme seasonal moisture variations (Innes, 1977). The area of the study drained freely and therefore was not waterlogged.

The farm was established as a fattening station for young Friesian bulls from a government-owned Friesian dairy herd about 10 km away. It was enclosed by perimeter fencing, had three cattle pens and a surface dug dam as the water source for the cattle. The cattle pens were partially roofed in addition to tree cover near the kraals which provided shade. The floors of the kraals were made of concrete.

The fattening project was discontinued in 1981 because of two severe outbreaks of dermatophilosis in the fattening animals.

CATTLE

The experiment herd of 20 cattle, all female and of a similar age (approximately 1 year) and comprising 15 Zebu-type and five N'dama cattle was established in June 1989. The animals were divided at random into two groups: one having five Zebu-type cattle that were treated with a flumethrin-based, synthetic pyrethroid pour-on acaricide (Bayticol[1]) on a regular basis. Initially, the acaricide was applied every three weeks but this schedule was changed later (April 1990) because a few *Amblyomma* ticks were found on the animals in January, February and March 1990 with the three week treatment intervals. After April 1990, acaricide was applied every two weeks. The five acaricide-treated Zebu-type cattle were used as control groups and were kept apart from the remainder of the herd so that acaricide does not get onto the untreated group which became tick infested, through direct contact.

The remaining ten Zebus were divided into two groups - untreated group 1 and untreated group 2 for logistic reasons relating to the lymphocyte proliferation studies which were carried out.

In addition, in April 1990, six Friesian cattle originating from a government farm where once weekly dipping to control ticks was practiced were added to the experiment herd, bringing the total number to 26 animals: 15 Zebu-type cattle, five N'damas and six Friesians. The Friesians were considered to be susceptible initially to ticks; they were added to the acaricide-treated i.e. control group, and the flumethrin based pour-on acaricide was used on them until the end of July 1990 when three of the Friesians were transferred from the acaricide-treated group to the untreated group. The three Friesian animals which were transferred were given prophylactic therapy with 2.5 mg kg^{-1} bodyweight of Imizol[2] and 20 ml of 200 mg^{-1} long acting oxytetracycline[3] injection prior to introduction into the untreated group. The treatment was repeated twice at two week intervals. This prophylactic therapy was given to protect the susceptible Friesians against tick-borne diseases such as babesiosis, anaplasmosis and heartwater.

The animals were managed according to local husbandry practices and the two groups i.e. acaricide-treated and untreated animals, were herded separately by two

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[1]flumethrin (Bayticol)
[2]imidocarb (Imizol)
[3]terramycin (Pfizer)

herdsmen to graze natural unimproved grasses and watered twice a day all year round. After grazing, the animals were penned separately at night so that acaricides from the treated animals did not get to the untreated ones through direct contact (Plate 2.3).

One of the untreated Zebu cattle was removed from the herd in October 1990 because it developed besnoitiosis resulting in extensive skin lesions. Another of the untreated Friesian cattle which had already developed severe clinical dermatophilosis died suddenly in mid January 1991.

Cattle in the experiment herd were cast and closely examined monthly over the period the survey covered (26 months) for the presence of ticks and the numbers of the adults of the various species present recorded. Ticks on the whole body of an animal was identified and counted *in situ*. The presence of larvae and nymphs was noted but only adult ticks were counted.

The presence of skin lesions on animals in the herd were also noted by close visual inspection and confirmed to be associated with *D. congolensis* in the initial stages of the study. Animals which had lesions were recorded as positive.

TRADITIONAL OR LOCAL HERD

Traditional or local herds were defined as groups of animals kept under management with minimum inputs in the form of deworming, tick control, animals grazed unimproved natural forage without supplementation and were exposed to the direct effects of rains and solar radiation because their kraals were not roofed and they were not provided with shade.

The criteria for selection of local herds were based on year-round accessible roads to the kraals, similarity of animals and management practices, travelling distances to be covered and continuous cooperation of the herdsmen over the study period.

Five local herds were selected in the Accra area for monitoring, on a continuous basis, the level of tick challenge and the prevalence of skin lesions under natural conditions. Five animals, all females, and of a similar age (6-9 months) from each of the selected herds, were ear-tagged and examined closely each month.

Vegetation type and management practices at these local kraals were similar to those described for the experiment group of animals except that animals in the local herds were milked.

The ticks present on each tagged animal were identified and the adults counted *in situ*. Ticks on the whole body of an animal were counted. Monthly monitoring of the numbers of ticks of the various species and the presence of skin lesions were continued for the duration of the survey period (26 months). A total of 25 animals were examined for the presence of ticks in the local herds. Tagged animals which were too difficult to handle during the survey period were replaced.

Approximate numbers of animals examined in each selected kraal over the survey period ranged from 75-160 per kraal (Table 2.1).

Samples of ticks on animals in both the experiment herd and the local herds were collected and identified at the veterinary laboratory in Accra, Ghana by Dr. A.R. Walker. The identification of the ticks was later confirmed by Dr. Walker in his laboratory at the C.T.V.M., University of Edinburgh, Scotland.

Acaricides were used in all five selected local herds at various times during the survey period and this modified patterns of tick infestation in these herds as described in the results.

Meteorological data such as mean monthly rainfall (mm), temperature (°C) and relative humidity (%) were collected over three years (1989-1991). At the start of the experiment, all the kraals under study were located within 15 km radius of one another. In January 1990, one kraal (local herd 2) was relocated at a new site 30 km from the rest of the kraals. The rainfall, temperature and relative humidity data were collected at two different locations. Data were collected at the Accra Airport which was close to local herd 1 and other data were collected at a meteorological station at

Mpoasem, near Legon University which was nearer to the experiment herd and local herds 3, 4 and 5.

RAW DATA

Raw data pertaining to the results in this chapter are listed in Appendix Tables 79-92 and Figures 2.1-2.40.

RESULTS

TICKS OBSERVED

Four genera comprising five species of ticks were found present on animals in the experiment as well as the selected local herds. They included: *A. variegatum, H. rufipes, R. senegalensis, B. decoloratus* and *B. annulatus.* Because differentiation was difficult and tedious, the two *Boophilus* species were counted together.

DISTRIBUTION OF TICKS ON CATTLE

Data collected from the experiment herd were used to assess the distribution of ticks on cattle. Ticks were recorded from pre-defined areas of the cattle as in Table 2.2 when they were collected during November 1989 and 1990. This time was selected for the anticipated high prevalence of most ticks.

Amblyomma variegatum was found mainly in groins and axillae but occasionally on other parts of the body. Out of 575 *A. variegatum* ticks counted during two months, 88 per cent were found in the axillae and groins.

More than 90 per cent of *R. senegalensis* ticks were found attached to the ears.

All the *H. rufipes* found during the two month period were under the tail and perineum.

Boophilus decoloratus and *B. annulatus* were generally distributed. A total of 66 *Boophilus* ticks were counted, 27, 22, 5 and 8 were attached in the axillae, groins, ventral and the limbs, respectively (Table 2.2).

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS ON THE FIRST GROUP OF FIVE UNTREATED ZEBU-TYPE CATTLE

A total of 6,285 ticks were counted on this group of cattle. The ticks comprised four genera, *Amblyomma, Hyalomma, Boophilus* and *Rhipicephalus. Amblyomma variegatum* was the commonest and formed 76 per cent of the total ticks counted. *Boophilus* was next in abundance being 12 per cent. The remainder was made up of *H. rufipes*, 7 per cent and *R. senegalensis*, 6 per cent, respectively (Table 2.3).

Amblyomma variegatum was found throughout the year with fluctuating numbers which reached high levels in April-May and December-January (Figure 2.1). The other genera of ticks were found in much lower levels and were nearly absent during some periods of the year (Figure 2.1). *Amblyomma variegatum* was by far the most abundant tick species infesting this group of cattle. The numbers of *A. variegatum* found on this group of cattle decreased during June through August and then started to increase again after October (Figure 2.1). The level of infestation with *A. variegatum* was higher in both 1990 and 1991 than it was in 1989.

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS ON THE SECOND GROUP OF FIVE UNTREATED ZEBU-TYPE CATTLE

Out of a total of 3,225 ticks counted over 26 months on this group of cattle, 74 per cent were *A. variegatum*, 16 per cent were *Boophilus* and the remainder 6 per cent and 4 per cent were *H. rufipes and R. senegalensis*, respectively (Table 2.3).

Amblyomma variegatum occurred throughout the year with their numbers increasing to high levels in November-December and April (Figure 2.2). The levels of *A. variegatum* declined in June through August and started increasing again in September. The other genera of ticks were found in much lower levels which declined to negligible levels during parts of the year (Figure 2.2). Again *A. variegatum* was the predominant species of tick that infested the Zebu-type cattle in experiment herd, group 2. Fewer *A. variegatum* ticks were found in this herd compared to the first group of untreated Zebu-types.

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS ON UNTREATED N'DAMA CATTLE

Ticks counted on the five untreated N'dama cattle over the survey period were 1,395. They included *Amblyomma, Boophilus, Hyalomma* and *Rhipicephalus*. *Amblyomma variegatum* were prominent and accounted for 67 per cent of the total number counted. *Boophilus* were next in number making up 19 per cent of the total. *Rhipicephalus senegalensis* and *H. rufipes* were 9 per cent and 5 per cent respectively (Table 2.3).

Amblyomma variegatum infested animals throughout the year attaining high levels in November-December and May-June in the first year i.e. 1989 (Figure 2.3). The height of *A. variegatum* infestation in the second year i.e. 1990, decreased, with a smaller peak in November (Figure 2.3). The numbers of adult *A. variegatum* ticks on the N'dama cattle were much lower than that observed on the untreated Zebu-type cattle (Table 2.3). *Boophilus* species were found in the second half of the first year and early part of the second year with a peak in October of the first year (Figure 2.3). However, *Boophilus* species were almost absent from the second half of the second year until the end of the survey in July 1991. There were considerably fewer *Rhipicephalus, Hyalomma* and *Boophilus* ticks on the untreated N'dama compared to the numbers of *Amblyomma* ticks present (Figure 2.3).

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS ON UNTREATED FRIESIAN CATTLE

Three Friesian cattle were exposed to tick infestation under natural field conditions for eight months. The level of infestation of the Friesian cattle with *A. variegatum* ticks increased very rapidly once acaricide treatment was terminated. By December 1990, four months after exposure to ticks, the number of *A. variegatum* ticks on the Friesians were over three times the average number observed on the Zebu-type animals (Figure 2.4). These high *A. variegatum* numbers were sustained until the Friesians were removed from the survey in March 1991. *Boophilus* ticks were the next in abundance with increased numbers in November and February. Levels of *Boophilus* ticks drastically declined in December and January and also in March (Figure 2.4). *Hyalomma rufipes* and *R. senegalensis* were found in low numbers on the Friesians during the eight months they were observed.

A total of 2,299 ticks were counted on the Friesians during eight months. Out of this number, *A. variegatum* was predominant with 70 per cent. *Boophilus* was next in abundance with 16 per cent. *Rhipicephalus senegalensis* and *H. rufipes* follow in that order, accounting for 10 per cent and 4 per cent (Table 2.3). As with the other groups of experiment animals examined, *A. variegatum* ticks have been more plentiful than other species of ticks found during the eight months observation period.

PREVALENCE AND DISTRIBUTION OF TICKS ON ACARICIDE-TREATED ZEBU-TYPE AND FRIESIAN CATTLE

The Zebu-type cattle treated with acaricide were basically tick-free for most of the survey. There were a few *A. variegatum* and *Hyalomma* ticks on the Zebu-type acaricide-treated cattle at the initial stages of the survey, averaging four *A. variegatum* ticks on these animals with the highest number of six ticks on an individual animal (Figure 2.5). After the initial application of acaricide at three week intervals, no ticks were observed on these animals until January-March 1990 when a few *A. variegatum* ticks were observed (Figure 2.5). The frequency of application of acaricide was increased from every three weeks to every two weeks and after that the animals were free of ticks.

The Friesian cattle had a few *A. variegatum* ticks on them when they were added to the herd in April 1990 (Table 2.3). Application of acaricide rendered them tick-free from June-September. From October 1990, the Friesians were infested with ticks again inspite of the fortnightly acaricide application (Figure 2.6). In December-January an average of four *A. variegatum* ticks were found on the three Friesians, the highest number of five ticks being found on an individual animal in January 1991. In May 1991, an average number of ten *A. variegatum* ticks were found on the acaricide-treated Friesians, with the highest number of 12 *A. variegatum* ticks on an individual animal (Figure 2.6).

COMPARISON OF *A. VARIEGATUM* INFESTATION BETWEEN THE DIFFERENT EXPERIMENT CATTLE GROUPS

In view of the fact that *A. variegatum* was the predominant tick species found on cattle in the experiment herd, the levels of infestation with *A. variegatum* was investigated on the different treatment groups of cattle. Statistical analysis by the Wilcoxon's test revealed very significant differences in tick infestation levels between the cattle ($P<0.01$) (Table 2.4).

The medians of the mean monthly counts of *A. variegatum* on the different treatment groups of cattle are given in Table 2.5. As expected, the acaricide-treated groups i.e. Zebu-types and Friesians had negligible numbers of ticks on them. In contrast, clear cut differences in innate response to tick infestation was seen between the experiment groups of cattle, the N'damas being more resistant, followed by untreated group 2 and untreated group 1 Zebu-type cattle respectively. The untreated Friesians were at the end of the spectrum being more susceptible to ticks as demonstrated by levels of tick infestation (Tables 2.4 and 2.5).

PREVALENCE AND SEASONAL DISTRIBUTION OF TICKS IN THE SELECTED LOCAL HERDS

Acaricide was used in all the five selected local herds at various times during the survey period and this was assumed to have modified patterns of infestations. However, at kraals where acaricides were used very infrequently, the level of tick infestation, especially *A. variegatum* followed a similar pattern to that seen in the experiment herd which was not treated with acaricide during the survey period. Five species of ticks were observed on cattle at all the five selected local herds. These were the same species found in cattle in the experiment herd.

LOCAL HERD 1

The level of infestation with *A. variegatum* at local herd 1 was highest in April, with a smaller peak in November 1989 (Figure 2.7). *Amblyomma variegatum* was found throughout the year but the level of infestation was much lower from October 1990-January 1991 than was observed at the same kraal over the same period the previous year. There was a slight increase in the level of infestation with *A. variegatum* in February 1991 but this decreased by March and remained at a low level through August 1991 (Figure 2.7). The number of *R. senegalensis* ticks present on animals in local herd 1, showed drastic fluctuations with fewer present between November 1989 and April 1990. The level of infestation with *Rhipicephalus* was highest in May 1990 with a small peak in September 1989 (Figure 2.7).

The level of infestation with *Rhipicephalus* fell off in November 1990 and April-August 1991. The numbers of *H. rufipes* and *Boophilus* ticks present on animals in this kraal remained low throughout the 26 month period, with *Boophilus* almost absent from June 1990 to August 1991 (Figure 2.7).

A total of 7,315 ticks were counted on animals in this herd. *Amblyomma variegatum* made up 48 per cent, followed by *R. senegalensis* with 31 per cent, *H. rufipes*, 16 per cent and *Boophilus*, 5 per cent (Table 2.6).

LOCAL HERD 2

Animals in this kraal were moved at the end of January 1990 to a new location where tick challenge appeared to be much higher because tick levels picked up as soon as the animals got to the new site (Figure 2.8).

Amblyomma variegatum ticks were found on the animals throughout the year with rising numbers and peak levels in April and November-December (Figure 2.8). *Amblyomma variegatum* infestation levels were decreased in January-March and July-September. *Boophilus* and *H. rufipes* levels were low and these dropped from January 1991 - July 1991 when neither *Boophilus* nor *Hyalomma* ticks were observed on animals at this kraal (Figure 2.8). *Rhipicephalus senegalensis* were regularly found on the animals with slight peaks in April 1990 and April 1991. Application of acaricide at local herd 2 was minimal.

Ticks counted on animals in this herd were 4,630. They included *A. variegatum*, 79 per cent, *R. senegalensis*, 10 per cent, *Boophilus*, 8 per cent and *H. rufipes*, 3 per cent (Table 2.6).

LOCAL HERD 3

Amblyomma variegatum ticks, *R. senegalensis* and *H. rufipes* were found throughout the year (Figure 2.9). The levels of *Amblyomma* ticks fluctuated probably because of the use of acaricides. *Amblyomma variegatum* numbers were highest in November 1989, August and November 1990 and May 1991. Low levels were observed in January, February and March (Figure 2.9). *Rhipicephalus senegalensis* levels were increased in April and May. *Amblyomma variegatum* and *R. senegalensis* were the most abundant ticks at local herd 3. *Boophilus* levels were low throughout the survey with complete absence of the ticks during February and July 1991 (Figure 2.9).

Amblyomma variegatum was the predominant tick species in this herd forming 52 per cent of a total of 4,935 ticks counted. *Rhipicephalus senegalensis* was next in abundance with 31 per cent, followed by *H. rufipes* with 10 per cent and *Boophilus*, 7 per cent of the total numbers (Table 2.6).

LOCAL HERD 4

Amblyomma variegatum ticks were found on animals in this herd throughout the survey period. The level of infestation with *A. variegatum* was highest in October and there was a second peak in May (Figure 2.10). *Amblyomma variegatum* levels were low between December-April after which the levels started to rise. The levels were also low in June-August. *Rhipicephalus senegalensis* ticks were found during most of the survey with slight peaks in May. However, there were very few *Rhipicephalus* ticks present between January and April. *Boophilus* and *Hyalomma* ticks were observed in very low numbers (Figure 2.10).

A total of 3,750 ticks were counted on animals in this herd. *Amblyomma variegatum* made up 47 per cent of the total, followed in abundance by *R. senegalensis*, 30 per cent, *Boophilus*, 12 per cent and *H. rufipes* 10 per cent (Table 2.6).

LOCAL HERD 5

The overall pattern of tick infestation at local herd 5 followed that of the other kraals, except that the numbers of *A. variegatum* ticks counted on the tagged animals tended to oscillate up and down more often (Figure 2.11), possibly in response to acaricide-treatment. *Amblyomma variegatum* and *R. senegalensis* ticks were found on animals throughout the survey period. *Boophilus* and *Hyalomma* ticks were found in much smaller numbers and these were very much reduced during certain periods of the year (Figure 2.11). *Amblyomma variegatum* numbers increased in June-July, May and October while the numbers decreased January-March. *Rhipicephalus* infestation levels increased in May and October but decreased the rest of the year (Figure 2.11).

The number of ticks counted at the herd were 3,435. As at other herds, *A. variegatum* was the predominant species of ticks found, making up 54 per cent of the total. The rest of the ticks counted included *R. senegalensis* 33 per cent, *H. rufipes* 8 per cent and *Boophilus* species, 4 per cent (Table 2.6).

ASSOCIATION OF *AMBLYOMMA VARIEGATUM* WITH *DERMATOPHILOSIS*

The most abundant species of ticks infesting animals in the five selected local herds were *A. variegatum* and *R. senegalensis* (Figure 2.7 and 2.11).

STATISTICAL ANALYSIS

The per cent infection rate of dermatophilosis was normalized using the arcsin percentage transformation technique. The time series data of dermatophilosis prevalence and tick species prevalence was cross-correlated using Pearson's parametric correlation. This was done for the same time scale and with a time lag of two months such that tick data were related to dermatophilosis data two months later. The value of two months was chosen from clinical experience and from experimental observations of A.R. Walker (pers. comm.) that in experiments with sheep infested with adult *A. variegatum* chronic lesions become fully established approximately two months after experimental infection.

The Pearson's correlation test was also used to compare the relationship, if any between the individual tick species and dermatophilosis. The results are summarized in Tables 2.7-2.10. Positive correlations of statistical significance were revealed between *A. variegatum* and dermatophilosis in local herds 1, 2 and 3; whereas a positive correlation was observed only once between *R. senegalensis* and dermatophilosis at local herd 2 and twice between *H. rufipes* and dermatophilosis at local herds 1 and 2.

LOCAL HERD 1

The prevalence of skin lesions, characteristic of dermatophilosis on the 130 animals in this kraal more than doubled, to 13 per cent in January 1990 with a further increase in April-August 1990 when over 30 per cent of the herd were affected (Figure

2.12). The highest infection rate of 38 per cent was recorded in June and that occurred approximately two months after the highest incidence of *A. variegatum* in that herd in April. Statistical analysis of Pearson's correlation coefficient (r) between *A. variegatum* and the arcsin transformed dermatophilosis lesion gave a highly significant relation ($r = +0.413$; $P<0.01$) (Figure 2.13). A higher correlation was observed ($r = +0.745$; $P<0.001$) with the two months lag (Figure 2.14).

The incidence of dermatophilosis in local herd 1 ranged from 2-38 per cent (Table 2.11).

LOCAL HERD 2

There was a drastic increase in the levels of ticks, especially *A. variegatum*, in February 1990 (Figure 2.15). The increase was followed by a rise in dermatophilosis lesions from 5 per cent or lower, to over 20 per cent of the herd (Table 2.12). This followed the presence of large numbers of *A. variegatum* on cattle in April (Figure 2.15).

A highly significant relationship was observed between *A. variegatum* and dermatophilosis ($r = +0.529$; $P<0.01$). A higher correlation was revealed with the two month lag data ($r = +0.711$; $P<0.001$).

LOCAL HERD 3

Animals in this herd were approximately 160 and the dermatophilosis incidence ranged from 2 - 11 per cent (Table 2.13) in September 1990 after a high *A. variegatum* activity a month earlier in August (Figure 2.16). Three dermatophilosis outbreaks in January 1990 and 1991, and July 1991, occurred two months after high levels of *A. variegatum* were recorded on the animals in November 1989 and 1990 and May 1991 (Figure 2.16). A significant correlation was shown between *A. variegatum* and dermatophilosis ($r = +0.501$; $P<0.01$). The two month lag data gave a slightly higher correlation ($r = +0.579$; $P<0.01$).

LOCAL HERD 4

The level of infestation with *A. variegatum* was highest in October 1989 and there was a second peak in May. Dermatophilosis was prevalent at this kraal in December-January and July (Figure 2.17). The incidence of dermatophilosis ranged from one to over 13 per cent in July 1990 (Table 2.14). The correlation between *A. variegatum* and dermatophilosis was not significant ($r = -0.01$; $P>0.05$). However, the two month lag data was very significantly correlated ($r = +0.49$; $P<0.01$).

LOCAL HERD 5

Dermatophilosis was more prevalent in this herd of approximately 75 cattle in May-June with a smaller peak in January (Figure 2.18). High levels of *A. variegatum* were recorded in May-June and September-October. The incidence of dermatophilosis ranged from 7 per cent to a peak of 29 per cent in June 1990. The infection rate was maintained between 22 - 29 per cent from April 1990 - August 1990 inclusive (Table 2.15).

No correlation was observed between *A. variegatum* and dermatophilosis in this herd ($r = +0.003$; $P>0.05$). There was also no correlation between the two month lag data ($r = +0.036$; $P>0.05$).

UNTREATED FRIESIAN CATTLE

Amblyomma variegatum ticks on this group of cattle increased from an average of five on three animals in August to an average of 139 in December, the highest number of ticks on an individual animal being 163 in December (Figure 2.19). This drastic increase in tick levels coincided with dermatophilosis, initial disease lesions being noticed in November (Figure 2.19). The lesions started on the lower limbs as small localized scabs which rapidly spread over most parts of the body, including the axillae, groin, dewlap and back within a month of its first appearance. By two months, generalized crusty lesions which progressively deteriorated were established (Plate 2.4). All the three untreated Friesians had developed clinical dermatophilosis by the end of December. The lesions always started on the limbs which were not tick-predilection sites (Plate 2.5). The disease was fatal, acute and generalized and unlike localized lesions in the groin of the Zebu-type cattle and N'damas which were infected with the disease.

ACARICIDE-TREATED ZEBU-TYPE CATTLE AND FRIESIANS

Although an average of four to ten *A. variegatum* ticks were found on the acaricide-treated Friesians in December 1990 and May 1991 respectively, none of them developed dermatophilosis (Figure 2.20). Similarly, the odd tick was found on the acaricide-treated Zebu-type cattle i.e. a tick on one animal in November 1990, February 1991, April 1991 and June 1991, nevertheless none of them came down with the disease (Figure 2.21).

ASSOCIATION BETWEEN RAINFALL, TEMPERATURE, RELATIVE HUMIDITY, THE PRESENCE OF TICKS AND DERMATOPHILOSIS

LOCAL HERD 1

Meteorological data at the Accra Airport, Ghana, for the period June 1989 - August 1991 were used to determine the relationship between the occurrence of ticks and dermatophilosis. Monthly rainfall, monthly mean temperature and mean monthly relative humidity values are presented in Figure 2.22 and Figure 2.23. Monthly mean temperatures for the period ranged from 25°C - 29.8°C, mean monthly relative humidity 74 - 87.5 per cent. There were two rainy seasons in a year: a major one in April-July with a minor one in October-November. Mean monthly rainfall ranged from 0.0 mm - 278 mm. Average rainfall for the 26 months the survey covered was 71 mm. Average rainfall for 1989 and 1990 was 55 mm and 47 mm respectively.

The association between rainfall and prevalence of ticks at local herd 1 is depicted in Figure 2.24. In 1990, a slight peak in *A. variegatum* in July and August followed increased rainfall in April-June. Pearson's parametric correlation showed no relationship between rainfall and the prevalence of ticks at local herd 1. Rainfall and incidence of dermatophilosis is shown in Figure 2.25. Periods of high rainfall in April-June 1990 and 1991 coincided with peaks of dermatophilosis. A slight peak of rainfall in November-December also coincided with a slight peak of dermatophilosis that year, but not the previous year. Moreover, statistical analysis revealed no correlation between rainfall and the disease incidence ($r = -0.056$; $P>0.05$).

The association between relative humidity and dermatophilosis in local herd 1 is given in Figure 2.26. Relative humidity was low in November-December during a slight peak of dermatophilosis and reached its peak in June-July 1990 after a peak of dermatophilosis in April-May. Statistical analysis showed no correlation between relative humidity and the disease ($r = -0.016$; $P>0.05$). Periods of high relative humidity did not coincide with periods of high levels of tick infestation (Figure 2.27). The relationship between relative humidity and prevalence of ticks at local herd 1 was not significant ($P>0.05$).

The association between temperature and prevalence of *A. variegatum* is illustrated in Figure 2.28. Periods of high temperatures coincided with high levels of tick infestation and as temperatures decreased from May-August 1991, tick activity and infestation also decreased (Figure 2.28). Statistical analysis by Pearson's correlation test revealed a significant correlation ($r = +0.448$; $P<0.01$).

LOCAL HERDS 3, 4 AND 5

These herds were all in close proximity to the meteorological recording station near Legon, University of Ghana and therefore the data from the three herds were pooled together. Monthly mean temperature (°C), mean monthly relative humidity (per cent) and monthly rainfall (mm) readings are presented in Figure 2.29 and 2.30. Monthly mean temperatures for the period ranged from 25.2°C - 29.9°C, mean relative humidity was 74 - 87.5 per cent. There were two rainy periods during a year, a major rainy season from April-July with a minor one before December. Mean monthly rainfall ranged from 0.0 mm - 314.4 mm. Average rainfall for the 26 months survey period was 77.9 mm. Average rainfall for 1989 was 51 mm and that for 1990 was 53.4 mm.

The association between rainfall and prevalence of ticks is given in Figure 2.31. Tick infestation levels picked up with increasing rainfall. The correlation between rainfall and prevalence of ticks was very significant ($r = +0.476$; $P<0.01$). From April-June 1990 during the rains, the incidence of dermatophilosis progressively increased until it reached a peak in July (Figure 2.32). Skin disease in December 1990 - January 1991 also coincided with a small peak of rainfall in December 1990. However, statistical analysis by Pearson's parametric correlation showed negative correlation which was not significant between rainfall and dermatophilosis ($r = -0.225$; $P>0.05$).

There was poor relationship between relative humidity and dermatophilosis at kraals 3, 4 and 5 as illustrated in Figure 2.33. In December 1989 - January 1990 when dermatophilosis was at a peak, relative humidity was at its lowest level. The height of relative humidity attained in July 1990, followed increased dermatophilosis levels in May and June 1990. No association was revealed, statistically, between relative humidity and dermatophilosis. Figure 2.34 depicts the association between relative humidity and *A. variegatum* ticks. A high level of relative humidity in October 1989 coincided with a peak in tick infestation. Another increase in relative humidity in May also coincided with high tick infestation levels. However, two major peaks of relative humidity in July 1990 and July 1991 did not occur at periods of high tick infestation. The height of tick infestation occurred in June 1990, a month after the peak of relative humidity. Statistical analysis gave a positive statistically non-significant correlation between relative humidity and *A. variegatum* infestation ($r = +0.252$; $P>0.05$).

The relationships between fluctuations in temperature and the activity and infestation levels of *A. variegatum* observed at local kraals 3, 4 and 5 are presented in Figure 2.35. High temperatures did not coincide with peaks of tick burdens. In March

1990 and February-March 1991 when temperatures were highest, tick infestations were low (Figure 2.35). No correlation was observed between fluctuations in temperature and tick burdens at local kraals 3, 4 and 5.

EXPERIMENT HERD

The ten untreated Zebu-type cattle were used to provide tick-infestation data. The meteorological information collected at Legon, Accra was used for the experiment group of cattle (Figure 2.29 and Figure 2.30). The association between rainfall and dermatophilosis at the experiment herd is illustrated in Figure 2.36. Periods of high rainfall did not coincide with high levels of disease. Statistical analysis by Pearson's correlation test showed negative but very significant correlation ($r = -0.507$; $P<0.01$). The relationship between rainfall pattern and tick burdens is presented in Figure 2.37. Seasonal patterns of rainfall coincided with peak tick burdens. The April-July rainy season coincided with the major tick activity and infestation periods of May-July 1990-1991. Tick activity occurred in November, two months after the rains in September 1990. Another period of tick activity occurred in January 1991, a month after a rainy spell in December. The association between rainfall and tick infestation was positive but low ($r = +0.174$).

The effect of relative humidity on tick activity and infestation in the experiment herd is shown in Figure 2.38. No relationship was observed between relative humidity and tick burdens. Peaks of tick infestation in April-June occurred before high levels of relative humidity were attained. Another peak tick activity in January 1991 did not coincide with high relative humidity levels. The effect of relative humidity on dermatophilosis at the experiment herd is given in Figure 2.39. In June 1990, a high relative humidity level coincided with a high incidence of dermatophilosis in June-July 1990. No other relationship between relative humidity and dermatophilosis was observed. Statistically, no correlation was revealed between relative humidity and dermatophilosis.

The association between temperature fluctuations and tick populations on the experiment herd during the survey is presented in Figure 2.40. High temperatures preceded high levels of tick burdens. High temperatures in November 1989 was followed by high tick burdens which reached a small peak in December 1989. Again high temperature levels in March and April 1990 was followed by high levels of tick infestation in April reaching a peak between May and June (Figure 2.40). The temperature height in November 1990 coincided with high levels of tick infestation the same month. This progressed through January and February 1991 when high tick levels were related to high temperatures. Statistical analysis by Pearson's parametric correlation showed a very significant correlation ($r = +0.522$, $P<0.01$).

DISCUSSION

Four genera comprising five species of ixodid ticks were found in varying numbers on cattle during a 26-month survey on the Accra Plains of Ghana. They were , *A. variegatum, B. decoloratus, B. annulatus, R. senegalensis* and *H. rufipes*. Mohammed (1978) and Bayer and Maina (1984) found similar genera of ticks in Nigeria.

FEEDING SITES

Only adult ticks were counted in the survey. Preferred sites of attachment for adult *A. variegatum* were along the ventral surface of the host including the groin, axillae, brisket and dewlap. In this survey 88 per cent (507 of 575) of *A. variegatum* ticks were found in the groin and axillae, a finding agreeing with observations of other workers (Hoogstraal, 1956; Yeoman and Walker, 1967; Walker, 1974; Mohammed, 1978). MacLeod (1975) listed 87 per cent of adults *A. variegatum* from the ventral surface i.e. dewlap, chest, axillae, abdomen and groin. Adult *H. rufipes* were almost entirely observed under the tail and the perineum. These findings agree well with those of Londt and co-workers (1979). The two *Boophilus* species were counted together and they had general distribution as recorded by MacLeod and colleagues (1977) and by Kaiser and colleagues (1982). *Rhipicephalus senegalensis* were almost entirely restricted to the ear. As Kaiser and his co-workers (1982) rightly pointed out, ticks shared the surface of their hosts remarkably well with each species having a preference for a different zone in an apparent evolutionary adaptation to reduce competition.

RELATIVE ABUNDANCE

Amblyomma variegatum was the most numerous tick species observed on all the five local herds and the experiment herd. This was followed in abundance by *Boophilus* on animals in the experiment group and local herd 2. A similar finding was made by Mohammed (1978) in the Northern Guinea Vegetation Zone in Nigeria. Hoogstraal (1956) and Walker (1974) stated that where they exist, *A. variegatum* is the predominant tick species found. *R. senegalensis* was next in abundance to *A. variegatum* on local herds 1, 3, 4 and 5, a finding different from that made by Mohammed (1978) and Bayer and Maina (1984) in Nigeria. Both workers observed more number of *A. variegatum* and *Boophilus* than *Rhipicephalus*. This difference in the relative abundance of the different species of ticks could be attributed to de-ticking practices. Animals in the local herds were de-ticked by manual application of acaricide solutions using pieces of cloth when tick levels built up. De-ticking was inconsistent and, moreover, the acaricide was applied to tick predilection sites such as the groin, axillae, brisket, dewlap and perineum, areas most affected by *A. variegatum* and *Boophilus* (Table 2.1). Whereas, *R. senegalensis* mainly affects the ears (Table 2.1) and therefore could easily have been overlooked during manual acaricide application. Herdsmen also de-ticked animals by manual removal of ticks during milking. Bigger ticks like *A. variegatum* and *Boophilus* species which were readily accessible would have been hand-picked rather than ticks like *Rhipicephalus* in obscured places i.e. the ears. This may explain why there were high numbers of *R. senegalensis* on four out of five of the local herds.

With no tick control at all in the experiment herds, *A. variegatum* was the main tick species encountered on animals in the different treatment groups, its infestation levels ranged from 67 - 75.5 per cent.

Hyalomma rufipes ticks were found in low numbers. The most common ticks found in Nigeria by Bayer and Maina (1984) were in order of abundance *A. variegatum, Boophilus* species, *Rhipicephalus* spp. and *Hyalomma* spp. They found that *Hyalomma* spp. made up only a minor composition of total tick load. Their findings were similar to that observed in the present study. Merlin and colleagues (1988) working in Cameroon, found that *H. rufipes* had very low prevalence. MacLeod and co-workers (1977) found *B. decoloratus, R. appendiculatus, A. variegatum* and *Hyalomma* spp. to be present in that order of frequency in Zambia.

SEASONAL INCIDENCE OF TICKS

Amblyomma variegatum occurred throughout the year in the various kraals and the experiment herd with a decrease in the level of infestation in January-March and June-September followed by an increase in April-May and November-December. Two peaks of *A. variegatum* infestation were observed during the survey. Bayer and Maina (1984) reported that tick load was low during the dry season i.e. November-March but rose to a pronounced peak at the beginning of the wet season i.e. April-October and declined thereafter. Mohammed (1978) observed only one generation of adult *A. variegatum* during the year. The main activity was in the rainy season i.e. April-September. Between October and March, he observed, a very low incidence of *A. variegatum*. The two generations of *A. variegatum* ticks observed in the present study are in agreement with the observation of Wilson (1953) who stated that two or three generations of *A. variegatum* bred during a 12 month period in areas with two rainy periods each year. Petney and co-workers (1987) made a similar observation. There were two rainy seasons in April-July and October-December each year on the Accra Plains where the study was undertaken (Figures 2.22 and 2.29).

Merlin and colleagues (1988) found adult *A. variegatum* peak infestation in March-June while workers in Eastern and Southern Africa found peak adult *A. variegatum* infestation occurred from November-December-January. As Pegram and colleagues (1986) and Tatchell and Easton (1986) pointed out, substantial variations existed in the maximum abundance and the exact timing of peak occurrence of *A. variegatum* between years.

The prevalence of *Rhipicephalus, Hyalomma* and *Boophilus* ticks remained at a low level on the animals in the experiment herd. There were no definite patterns in the incidence of these ticks, however, *Boophilus* species were abundant in November but their numbers were reduced most part of the year. In the selected local herds *Rhipicephalus* attained peaks in April-May with low numbers the rest of the year. Under natural conditions cattle are challenged by more than one species of tick and therefore it is important to show whether there is cross-resistance between two species of ticks and the relationship between different ticks in establishment of disease. De Costa (1985) observed no cross-resistance between *R. appendiculatus* and *A. variegatum* on experimental cattle. Heller-Haupt, Varma and Langi (1981) working with laboratory animals observed that primary infestation with *R. appendiculatus* did not confer resistance to a secondary infestation with *A. variegatum*. They found that infestation with one species of tick conferred resistance to a secondary infestation with the same species, but either no resistance or only partial resistance to infestation with another species. A positive correlation between *A. variegatum* and dermatophilosis has been demonstrated in this study (Table 2.7). In contrast, the same relationship was established between *R. senegalensis* and dermatophilosis only once.

EFFECT OF ACARICIDE ON TICKS

A flumethrin-based pour-on acaricide[1] was used in the study. The initial three weekly application was changed to fortnightly application as a few ticks infested the cattle with the longer treatment intervals. In December 1990 and January 1991, an average of four *Amblyomma* ticks with five on an individual animal were observed on two acaricide-treated Friesians which were treated every two weeks with acaricides. In May 1991 an average of ten *A. variegatum* ticks with 12 on an individual animal were found on the acaricide-treated Friesians.

Hamel (1987) applied Bayticol at bi-weekly intervals on cattle under different management conditions in South Africa and found that it prevented the development of semi- and fully engorged *Amblyomma* ticks. He found very low incidence of adult ticks on treated cattle. Norval (1974) reported that Bayticol reduced tick populations on a farm to an acceptable and manageable level, particularly by interfering with the high reproductive potential of female *Amblyomma* ticks. Fortnightly application of Bayticol did not completely eliminate tick infestation in the experiment Friesian cattle, but it reduced it drastically, confirming the earlier observations of Norval (1974) and Hamel (1987). Nevertheless, Martinez (1991) implied that few ticks (less than five) were sufficient to provoke dermatophilosis and therefore total eradication of *A. variegatum* was necessary in preventing the disease in susceptible animals in theCaribbean. Our findings in the study on the Accra Plains of Ghana are contrary to Martinez's (1991) observation in the Caribbean. In the Ghanaian study susceptible animals infested with less than five ticks did not develop dermatophilosis.

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[1]flumethrin (Bayticol)

EFFECT OF RAINFALL AND HUMIDITY ON DERMATOPHILOSIS

The role of rainfall, water and high humidity has been stressed as important epidemiological factors in dermatophilosis by many workers. In the view of such workers, rainfall and high humidity, either directly or indirectly influenced the onset, the course, the severity and resolution of the disease. Most reported cases of dermatophilosis have been either during periods of heavy rainfall or wetting of the skin in conditions of high humidity (Chodnik, 1956; Plowright, 1956; Oppong, 1973; Oduye, 1976; Obeid, 1976; Stewart, 1972; Nobel *et al.*, 1976; Morrow *et al.*, 1989). Roberts (1963) and Oppong (1976) observed that moisture caused the release of zoospores from crusts and scabs which proceeded, under favourable conditions including wetness and humidity and initiated infection.

In an experimental *D. congolensis* infection in cattle, Oduye (1975c) found that simulated rainfall resulted in accelerated healing of experimental infections, a finding which confirmed Macadam's (1961) observation but refuted that of Chodnik's (1956). Moreover, *D. congolensis* was equally infective for both wet and dry scarified bovine skin. He speculated that the eruption of dermatophilosis lesions associated with early rain was not due to direct effect of rainfall on the skin. Strickland (1961) stated that the indirect effect of rain was due to the increased emergence of pupating insect larvae and an increase in the tick population which attacked cattle.

Martinez (1989, 1991), reported that in the Caribbean, rainfall increased tick populations but did not have a direct effect on dermatophilosis. Schultz's (1955) opinion that seasonal factors had no relationship with the disease is in contrast with those of many workers and the results of the present study.

Statistical analysis by Pearson's parametric correlation test revealed positive correlation between rainfall and tick populations and infestation, confirming earlier reports by Strickland (1961) and Macadam (1964, 1970). Petney and others (1987) stated that adult abundance of *A. variegatum* was highest during the periods of rains being independent of the timing of the rainy season. Other workers have pointed out that seasonal abundance of *A. variegatum* is dependent on the region, but in general, adult abundance is greatest during the rainy season (Yeoman and Walker, 1967; Tachell and Easton, 1986; MacLeod *et al.*, 1977; Pegram *et al.*, 1986; Bayer and Maina, 1984; Mohammed, 1978).

In contrast, although the disease occurred in the rainy season, there was no correlation between rainfall and dermatophilosis, a finding in agreement with earlier observations of Schulz (1955) and Braibant (1962). However, it refutes the findings of other workers who have associated rainfall with the disease (Chodnik, 1956; Plowright, 1956; Kelley and Bida, 1970; Coleman, 1967; Oppong, 1973). Moreover, Ainsworth and Austwick (1959) and Roberts and Graham (1966) emphasized the effect of constant wetting of the skin by rain in establishing *D. congolensis* infection.

Chodnik (1956) argued that high humidity alone was the sole factor responsible for the development and spread of lesions on individual animals. His statement has been refuted by the findings of this study because high humidity had no influence either on the disease or tick populations. Macadam (1964, 1970) reported that high humidity was not a direct factor on dermatophilosis, which agrees with the present findings. He also asserted that high humidity indirectly affected ectoparasite populations which directly aggravated the disease; we failed to show such a correlation.

ASSOCIATION BETWEEN *A. VARIEGATUM* AND *D. CONGOLENSIS*

Four months after exposure of Friesian cattle to ticks, the number of *A. variegatum* ticks on them was over three times the average number observed on the Zebu-type cattle (Table 2.3). These high *A. variegatum* levels coincided with dermatophilosis in the Friesians which occurred at the height of tick infestation in December (Figure 2.19). *In vitro* lymphocyte blastogenesis was reduced and the depressed uptake of [^{3}H]-thymidine by lymphocytes coincided with the high *A. variegatum* infestation (see Chapter 3).

The incidence of dermatophilosis occurred approximately two months after *A. variegatum* infestation. Statistical analysis of Pearson's correlation coefficient (r) between *A. variegatum* and dermatophilosis gave highly significant association on three out of five local selected herds. A higher correlation was observed between *A. variegatum* and dermatophilosis with the two month lag data on four out of the five farms, an observation confirming A.R. Walker's findings (Pers. comm.) that chronic lesions on experiment sheep infested with adult *A. variegatum* became fully established two months after infection.

Many workers have implicated *A. variegatum* with dermatophilosis. Plowright (1956), Oppong (1973, 1976), Oduye (1975b, 1976) and Lloyd (1976) reported on the association of *A. variegatum* and dermatophilosis in Africa. Outbreaks of dermatophilosis have been associated with introduction of *A. variegatum* on hitherto tick-free islands or new areas on islands in the Caribbean (Butler, 1975; Garris and Scotland, 1985; Barré *et al.*, 1988; Morrow and Compton, 1991).

Martinez (1991) discussed the possible role of *A. variegatum* in dermatophilosis in the Caribbean. He pointed out that goats imported from islands in the Caribbean without *A. variegatum* and dermatophilosis developed antibodies within two weeks of their arrival on another island, although they were housed in tick-free conditions. The antibody levels increased in the imported goats in subsequent weeks in spite of the fact that they were not exposed to ticks. None of the goats developed any visible lesions despite infectious contacts attested by the production of specific antibodies. However, Martinez (1991) stated that extensive dermatophilosis was established when some of the goats were exposed to adult *A. variegatum* infestation. Three control goats which were scarified with *D. congolensis* without ticks did not develop lesions.

He concluded that *A. variegatum* acted by favouring the development of lesions on carrier animals but not as a vector. He considered *A. variegatum* as a major influence on factors which interacted to induce dermatophilosis.

Oppong (1991) noted that circumstantial evidence of the involvement of ticks in the transmission and/or the spread of dermatophilosis is the general observation that in herds or areas where tick control measures are effective, the disease either did not occur or its incidence and severity were diminished. Hobday (1952), Plowright (1956), Matheron and others (1989), Samui and Hugh-Jones (1990b), Koney and Morrow (1990) reported that tick control significantly reduced the incidence of dermatophilosis in the herds of cattle with which they worked.

In our study, acaricide-treated animals in the experiment group did not show any clinical signs of dermatophilosis. The N'dama cattle were more resistant to tick infestation as fewer ticks were observed on them compared to the Zebu-types and the untreated Friesians. The N'damas also had less tick burdens in the second year of the study, than the previous year. In contrast, the Friesians were highly susceptible to tick infestation. Four months after they were exposed to ticks, they had three times as many ticks on them as the untreated Zebu-types. The heavy tick infestations in the Friesians coincided with the development of dermatophilosis. Unlike the N'damas which apparently had developed a high resistance to dermatophilosis through their ancestral association with tick infestations and the disease, the Friesians were very susceptible to both pathogens.

Specific association has been demonstrated between *A. variegatum* and dermatophilosis in this study, however, dermatophilosis lesions did not always coincide with *A. variegatum* attachment sites. It is possible therefore that the action of *A. variegatum* on its host was due to some systemic effect on the host whilst the ticks were salivating when feeding, such that the immune system of the host was modulated.

Experiments to assess the systemic effects ticks have on the immune system of their hosts are described in Chapter 3.

A clear seasonal incidence of *A. variegatum* ticks was also established in the study. High numbers of the ticks occurred in April-May and November-December. Planned acaricide use to control tick infestation and its influence on dermatophilosis could therefore be timed as discussed in Chapter 6 to limit the high seasonal incidence of ticks.

CHAPTER THREE

LYMPHOCYTE PROLIFERATIVE RESPONSES TO CONCANAVALIN A AND *D. CONGOLENSIS* OF LYMPHOCYTES DERIVED FROM EXPERIMENT GROUPS OF ANIMALS

Page No.

CHAPTER THREE

INTRODUCTION

Many contributory and overlapping factors have been incriminated in the establishment of dermatophilosis and also the severity of infection (Oppong, 1973; Oduye, 1976). Although the exact role of these factors in the disease process are poorly understood, a tropical climate, biting arthropods and intercurrent infections are thought to predispose animals to, and exacerbate, the disease (Oduye, 1976). Woodman and others (1990) placed the complex array of factors which have been implicated in chronic dermatophilosis into two broad and overlapping categories i.e. those that physically damage the integrity of the skin, thereby facilitating entry of *D. congolensis* and those that cause immunosuppression, thus hindering the normal host response.

Several studies have demonstrated that a humoral immune response occurs following both natural and experimental infections. However, antibodies are not protective against infection (Richard, Thurston and Pier, 1976; Lloyd and Jenkinson, 1980). Pulliam, Donald, Kelley and Coles (1967) reported that the chronic nature of the disease and ability of the organism to re-infect some animals indicated that antibody production alone could not effect recovery or prevent re-infection. Oduye (1974) observed no correlation between antibody titre and severity of infection. Complete immunity to dermatophilosis does not develop but accelerated healing occurs on re-infection (Ellis *et al.*, 1987; Sutherland *et al.*, 1988; How, 1989). Robert (1966) observed that this correlated with the onset of a delayed-type hypersensitivity response to *D. congolensis*. He therefore suggested that cell-mediated immunity (CMI) was involved in recovery.

Woodman and her colleagues (1990) examined the host cell-mediated immune response following experimentally-induced infection of rats with *D. congolensis* and concluded that *D. congolensis* was not itself immunosuppressive and that a rapid host cellular immune response was mounted.

Severe dermatophilosis in the tropics has been associated with the presence of arthropods feeding on the host (MacAdam, 1962, 1970) and specifically when the tick *A. variegatum* feeds (Plowright, 1956; Oppong, 1976; Norval, 1986; Martinez, 1989). However, all authors have emphasised that this relationship is not a simple one because the distribution of lesions do not always correlate with *D. congolensis* attachment sites.

Very little is known about either the mediators and effectors of bovine immunity to ticks or the tick-derived substances responsible for the induction of immunity (Brown, 1988). Tick resistance was passively transferred with viable lymph node cells, and it was reported that a T-lymphocyte enriched population transferred resistance more readily than a B-lymphocyte enriched population (Wikel and Allen, 1976). Wikel (1982a) demonstrated that lymphocytes from tick-infested guinea pigs showed significantly less *in vitro* responsiveness to the T-lymphocyte mitogens Concanavalin A and phytohaemagglutinin, while stimulation by the B-lymphocyte mitogen *Escherichia coli* lipopolysaccharide was unimpaired. These results provided evidence that ixodid tick infestation induces a variable, but significant, degree of reduced host immune responsiveness. Davis and Philpott (1980) postulated that the delayed hypersensitive lesions typically formed in host skin as a result of the repeated feeding of ticks will predispose hosts to dermatophilosis at the same localized sites. Barré (1989) postulated that, in addition, ticks have a systemic effect on the immune system of the host that will pre-dispose the host to dermatophilosis at sites other than tick feeding lesions. Muneer, Farah, Newman and Goyal (1988) defined immunosuppression as a state of decreased immune responsiveness to all foreign antigens leading to increased susceptibility to disease agents. They stated that many terms such as immune ablation (abrogation or permanent induration of immune response), immune tolerance (selective immunological unresponsiveness to a particular antigen), and immunodepression (failure to reach immunocompetence and reduction in already developed immune responsiveness) have been used to identify different types of impairement in function of the immune system.

An immunosuppressed state can be achieved by decreased function of one or more of the many interrelated arms of the immune system. The major components of the immune system generally affected by immunosuppressive agents are the humoral and the cellular responses. A number of tests can be used to measure humoral and cellular immune responses. For example, the cell-mediated immune response can be evaluated by a rosette technique, by measuring the response of cells to mitogens such as pokeweed mitogen (PWM), Concanavalin A (Con A) and phytohaemagglutinin (PHA), by evaluation of a delayed-type hypersensitivity reaction, response of T-lymphocytes to an allogenic lymphocyte (mixed lymphocyte reaction), stimulation of T-cell division by BCG vaccine, and by measurement of the cytotoxic activity of T-lymphocytes and tumoricidal action of natural killer cells.

A well documented phenomenon in the field of lymphocyte biology is lymphocyte stimulation, also known as lymphocyte activation or blast transformation. These refer to the morphological and metabolic changes which occur when lymphocytes are exposed to certain lectins, mitogens, allogeneic lymphocytes, or antigens to which previous exposure has occurred. One of the most obvious changes is that a high proportion of cells are stimulated to enter mitosis and one of the most frequently assessed metabolic changes is that of the rate of thymidine uptake (Ling and Kay, 1975).

Unlike mitogens, antigens only induce significant lymphocyte stimulation when the animal, from which the cells originated, has already been exposed to that antigen (Ling and Kay, 1975). Oppenheim (1968) showed that lymphocytes from patients with diseases associated with impaired delayed hypersensitivity do not transform well *in vitro*. Therefore, lymphocyte blastogenesis may be used as a measure of delayed hypersensitivity. The vast majority of antigens require T-lymphocyte interaction for the generation of an immune response (Miller and Mitchell, 1968). Therefore impaired T-lymphocyte function may compromise host defences against the establishment of disease.

It is possible that in addition to causing physical damage to the skin and breaking down the skin protective layer, ticks also cause some modulation of the host immune system. Ribeiro and colleagues (1990) stated that in addition to allowing the tick to feed, the salivary "drug store" may also help the pathogens that they transmit, by modulating host defences at the site of feeding.

The experiment herein discussed was designed using acaricide-treated i.e. tick-free and untreated i.e. tick-infested cattle to compare the response of lymphocytes derived from these experiment animals to T-lymphocyte mitogen Concanavalin A and *D. congolensis* antigen. The objective was to test the hypothesis that there is adverse modulation of the host immune responsiveness associated with tick infestation in cases of dermatophilosis.

MATERIALS AND METHODS

ANIMALS

An experiment herd of cattle was established as described in Chapter 2.

CELL CULTURE TECHNIQUES AND MATERIALS

Aseptic techniques were used in the preparation of cells for culture and throughout all the culture procedures. The water used for preparation of medium and of other solutions was of tissue culture grade (Appendix 3). Materials for culture use were sterilised by the appropriate method (Appendix 3). Prior to use, all foetal calf serum (FCS) was heated to 56°C for 30 minutes to inactivate complement and stored at -20°C. Glassware was washed to tissue culture standard (Appendix 3).

MONONUCLEAR CELL ISOLATION

Mononuclear cells were isolated from cattle blood, as follows:

The neck was swabbed with 70 per cent alcohol and 20 ml blood samples were collected from the jugular veins of the cattle into two 10 ml vacutainer tubes containing sodium heparin.

Fourteen ml of blood from each animal was put in two 7-ml tubes and centrifuged at 400 g, 4°C for 20 minutes. The buffy coat was pipetted off and put in 8 ml phosphate buffered saline (PBS) containing heparin (10 units per ml). Four and half ml of the buffy coat and PBS mixture was gently layered on 2.5 ml of pre-chilled Ficoll Paque[1], in two 7-ml tubes and centrifuged with a force of 300 g at the interface at 15°C for 35 minutes. The cell-free supernatant from the gradient was discarded. The band below this, which was rich in lymphocytes, was collected, as was some of the Ficoll layer underneath, which contained monocytes and lymphocytes. The pellet at the bottom of the gradient which consisted of the majority of contaminating erythrocytes and granulocytes, together with some mononuclear cells and damaged cells, was discarded. The lymphocyte-rich suspension was washed in 5 ml PBS with centrifugation at 160 g (1500 rpm) for 10 minutes at 15°C to remove the Ficoll Paque. The supernatant was discarded and the lymphocytes were resuspended in 5 ml PBS and washed twice by centrifugating at 160 g, 15°C for 10 minutes to remove platelets. The supernatant fluid was decanted and the cell pellet finally resuspended in complete RPMI 1640 (Appendix 3) and poured into bijou bottles. Aliquots were taken for cell counts and to assess cell viability, while the remainder of cell suspensions were kept at 4°C.

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[1]Ficoll 400 plus sodium diatrizoate, total density 1.007 g ml^{-1}, Pharmacia Ltd., U.K.

CELL AND VIABILITY COUNTS

The determination of leucocyte concentrations was performed using an improved Neubauer haemocytometer, using the method described by Mishell, Shiigi, Henry and Chan (1980).

For viability counts, aliquots of cell suspensions were diluted by a factor of 10 with an 0.2 per cent solution of trypan blue[1] made up in PBS and stored at 4°C. The suspension was mixed gently and left to stand at room temperature for 5 minutes to allow uptake of the dye. The two chambers of a Neubauer haemocytometer were filled with samples from the suspension and at least 200 cells were counted in each. The numbers of dye-excluding (viable) and dye-absorbing (non-viable) cells were recorded and the percentage of viable cells calculated.

MONONUCLEAR CELL CULTURE

Foetal calf serum[2] was included in the medium at a level of 8 per cent. The value was chosen as a compromise between better viability, but greater background stimulation with higher levels of FCS (10 per cent and above) and poorer viability, but lesser background stimulation with lower levels of FCS (5 per cent or below) (Woodman, 1989). The background stimulation was inferred from the blast index of cell cultures in media alone. The complete culture medium used was RPMI 1640 containing 25 mM Hepes, 8 per cent heat-inactivated FCS, 5×10^5 M 2-mercaptoethanol, 2 mM L-glutamine, 100 units ml^{-1} sodium benzylpenicillin and 100 $\mu g\ ml^{-1}$ streptomycin sulphate (Appendix 3).

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[1]Sigma Chemical Co. Ltd., U.K.
[2]FCS-Myoclone Plus, Gibco Ltd., Scotland

CULTURES TO WHICH AUTOLOGOUS SERUM WAS ADDED

Later 8 per cent of serum from the animal under investigation was added to the complete medium. The cells were stimulated with antigen or mitogen where appropriate and cultured in flat bottom 96-well plates[1]. The wells in the 96-well plates were seeded with cells at an initial cell density of 2 x 10^5 per well (0.38 cm^2) in a total of 200 μl (1 x 10^6 ml^{-1}).

The outside rows of wells were filled with sterile PBS, rather than cell cultures due to their susceptibility to evaporation. When setting up cultures in multi-well plates, the cells were always added after the other constituents, to minimise the exposure time to unfavourable conditions. The plates were incubated at 37°C in a 5 per cent carbon dioxide - 95 per cent air humidifed atmosphere.

The cultures were replenished with fresh medium on day 3 and again on day 5 where cultures were maintained for examination on day 7. Half (100 μl) of the spent culture medium of each well was carefully drawn off without disturbing the cells which were settled at the bottom. This was then replaced with fresh medium, containing antigen, or mitogen where appropriate, such that the concentration of stimulant in the culture was maintained.

Lymphocyte cultures were also set up in which serum from the animals under investigation was added to their own cell cultures. Initially autologous serum was added to the cultures in place of foetal calf serum (FCS) but later it was changed so that own serum was added in addition to foetal calf serum.

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[1]Nunclon Suppliers, Gibco Ltd., Scotland

DERMATOPHILUS CONGOLENSIS ISOLATE

The isolate of *D. congolensis* used in these studies originated from a clinical case of dermatophilosis in a cow from the Accra Plains of Ghana in 1987 (A.N. Morrow, *pers. comm.*). A primary culture, which has been checked for purity was stored in Colombia broth[1] with 15 per cent glycol[2] at -40°C.

DERMATOPHILUS CONGOLENSIS CULTURE

A 100 μl aliquot of stock *D. congolensis* was thawed and streaked on to two blood agar (B/A) plates. These were incubated at 37°C for 48 hours in a candle jar and then for a further 24 hours at 37°C in air. Bottles of 15 ml brain-heart infusion - neutralized soya peptone (BHI/NSP) broth (Appendix 3), were seeded with a loopful of *D. congolensis*, taken from the last confluent streak on the B/A plate. After 72 hours at 37°C, 0.5 ml aliquots of broth culture were spread over B/A plates which were then incubated aerobically for 48 hours at 37°C. *D. congolensis* cocci were harvested from the plates by the addition of 7 ml of RPMI complete medium, followed by gentle rubbing over the surface of the culture with a bent pasteur pipette.

The bacterial suspension containing predominantly cocci, was left at 37°C for 60 minutes to allow debris to settle out, and the cocci rich supernatant decanted. An aliquot was removed for cell counts using a Weber Counting Chamber[3]. Aliquots were also checked for purity with a gram stain and microscopic observation and by culturing on B/A.

PREPARATION OF *DERMATOPHILUS CONGOLENSIS* ANTIGEN

The antigen used consisted of whole cocci and short filaments which were killed by addition to medium containing antibiotics. Woodman (1989) found that the

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[1]Gibco Ltd, Scotland
[2]BDH Ltd., U.K.
[3]Weber Scientific, U.K.

concentrations of antibiotics routinely used in the medium for cell culture were sufficient to rapidly inactivate *D. congolensis*. Growth, assessed by incubation on B/A plates, was completely inhibited following one hour of incubation at 37°C with complete RPMI medium containing 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Woodman (1989) also reported that penicillin and streptomycin at half the concentration used i.e. 50 units/μg ml^{-1} were equally as effective. She also demonstrated that, in contrast, *D. congolensis* grew well when incubated with the complete medium in the absence of antibiotics.

[^{3}H]-THYMIDINE INCORPORATION LYMPHOCYTE TRANSFORMATION TEST

Lymphocytes derived from Friesian cattle blood were cultured at an initial density of 10^5 cells ml^{-1} in 200 μl volumes of complete medium with or without a mitogen (Concanavalin A), *D. congolensis* antigen/cocci, in 96-well flat-bottomed plates. Five replicate cultures were set up for each treatment. On day three of culture, half of the spent medium was removed and replaced with fresh medium containing Concanavalin A or *D. congolensis* antigen/cocci, where appropriate, to maintain the concentration. On the first three occasions Concanavalin A stimulated cultures were pulsed with [^{3}H]-thymidine on day 3 while cultures containing *D. congolensis* antigen/cocci were pulsed on the fifth day. Subsequently all treatment cultures were pulsed for 4 hours with [^{3}H]-thymidine on day 5. The dose and labelling period used in the assays were previously determined, as described by Woodman (1989).

STOCK [^{3}H]-THYMIDINE (SPECIFIC ACTIVITY)

Five Ci $mmol^{-1}$ containing 1 mCi ml^{-1} was diluted by a factor of 100 with complete medium to 10 μ Ci ml^{-1}. Each well received 50 μl of the diluted label which

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[1]Amersham International Plc, U.K.

contained 0.5 μ Ci of [^{3}H]-thymidine, giving a final concentration of 2 μ Ci ml^{-1}. The labelled cultures were incubated, under normal culture conditions for 4 hours.

At the end of the incubation period the cells were harvested onto glass fibre filter paper (Whatman) and washed, using a Titertek cell harvester[2]. The filter papers were dried in a hot air oven; then each disc, which corresponded to one well of the plate, was transferred to a scintillation vial[3]. To each vial 1 ml of scintillation fluid[4] was added. The amount of titrium on the discs was determined with a liquid scintillation counter[5]. Each vial was counted for 5 minutes.

Results were expressed as counts per minute (CPM) which according to Woodman (1989), is more appropriate than disintegrations per minute (DPM) for this type of isotope counting. Woodman (1989) explained that DPM is a value derived from CPM, which takes into account the effects of chemical and light quenching. However, most of the quenching in this system is physical being caused by the filter, yet this would not be included in the machine's calculation of DPM. This is true wherever the activity remains on the filter as it does here, rather than eluting into the solution.

CYTOCENTRIFUGE PREPARATION

Cytosmears were prepared by dropping three replicate 100 μl sample cell suspensions of untreated control, antigen, or mitogen stimulated cultures into the wells of a cytocentrifuge[6]. The cells in each well were spun ontoclean glass slides by centrifugation at 110 g (1000 rpm) for 5 minutes. The slides were air-dried and fixed in methanol for 2 minutes and then stained with a 5 per cent solution of Giemsa's stain for 40 minutes (Appendix 3).

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[2]Skatron AS, U.K.
[3]Packard, Canberra, U.K.
[4]Optiscint Hi-Safe, LKB
[5]By the staff of the Ghana Atomic Energy Commission, Accra
[6]Cytospin 11 Shandon, Southern Products Ltd., U.K.

IN VITRO RESPONSE TO CONCANAVALIN A AND *D. CONGOLENSIS* BY LYMPHOCYTES FROM EXPERIMENT GROUPS OF ANIMALS

Lymphocytes were derived from the blood of two groups of experiment cattle, five acaricide-treated and five untreated animals in Group 1 and ten untreated cattle in Group 2. Twenty ml of blood was collected from all animals in each group on alternate months and cultures set up as described previously. In the first year, nine replicate samples of three different treatments of cell cultures were set up. The three treatments were cultures containing complete medium alone, containing *D. congolensis* cocci antigen, and containing Concanavalin A mitogen. In the second year only six replicate samples were used. On day 3 and 5 of culture, half of the spent medium was removed and replaced with fresh medium only, or fresh medium containing antigen or mitogen where appropriate to maintain the concentration. In the first year cytosmears were prepared for control cell cultures on day 5 of cell incubation. For Concanavalin A stimulated cells on day 3 and 5 and for *D. congolensis* antigen stimulated cells on day 5 and 7. In the second year of the experiment, spent media was changed on day 3 only, and cytosmears from all treatments were made on day 5 after which the cell cultures were discarded.

ADDITION OF AUTOLOGOUS SERUM TO CULTURE MEDIUM

In addition to using FCS alone in the complete medium, serum from the animal under investigation was added to the complete medium for some cultures to give a final concentration of 8 per cent. Cultures containing FCS alone and those containing FCS plus autologous serum were set up side by side. On day 3 of culture half of the spent medium (100 μl) was removed and replaced with medium containing the appropriate treatment i.e. fresh medium only, fresh medium plus autologous serum, fresh medium containing *D. congolensis* antigen, fresh medium containing *D. congolensis* antigen plus autologous serum and fresh medium with Concanavalin A and fresh medium with Concanavalin A plus autologous serum, to maintain the concentration. Cytospin smears were prepared for Concanavalin A stimulated cultured cells on days 3 and 5 of incubation and for control cell cultures and *D. congolensis*-stimulated cultures on day 5.

IN VITRO RESPONSE TO CONCANAVALIN A AND *D. CONGOLENSIS* BY LYMPHOCYTES FROM FRIESIAN CATTLE IN THE EXPERIMENT GROUP

Monthly blood samples were taken from the Friesians and lymphocytes derived from them as described for the other experiment groups. Cultures were set up with media containing FCS only and also with media containing FCS plus autologous serum.

In addition six replicate cultures of the three different treatment regimes viz., control, antigen- or mitogen-stimulated cells, were pulsed with [^{3}H]-thymidine. The first three samples taken in August-October, 1990 and which were stimulated with Concanavalin A were pulsed with [^{3}H]-thymidine on day 3 of incubation. Antigen stimulated cultures were pulsed with [^{3}H]-thymidine on day 5 and after that the cultures were discarded. After this all treated cultures were pulsed with [^{3}H]-thymidine on the 5th day of incubation only.

RAW DATA

Raw data pertaining to the results in this chapter are listed in Appendix Tables 1-39,41,42,45,46,48,51,53,54,55,57,60-78 and 93-95.

RESULTS

LYMPHOCYTE RESPONSE TO CONCANAVALIN A AND *DERMATOPHILUS CONGOLENSIS* OF EXPERIMENT GROUPS OF CATTLE

FOETAL CALF SERUM (FCS) SUPPLEMENTATION OF CULTURES

No differences were apparent in the responses to Concanavalin A of lymphocytes from the animals in the acaricide-treatment, the N'dama or tick-infested Zebu groups (Tables 3.1-3.4). The monthly median values from cultures supplemented with FCS ranged from 156-164 cells per 200 lymphocytes (78-82 per cent) (Table 3.5). Statistical analyses by Mann-Whitney tests revealed very significant differences (P<0.01) between day 5 cultures without antigen and day 5 cultures with Concanavalin A in all four cattle groups i.e. the acaricide treated, the N'dama and both untreated Zebu-type groups (Table 3.5). No significant differences (P>0.05) were noticed among the various treatment groups when day 5 cultures without antigen and day 5 cultures supplemented with *D. congolensis* were analysed (Table 3.6). Lymphocyte blastogenic values for day 5 cultures containing Concanavalin A, in all the treatment groups were similar when analysed by the Mann-Whitney test (P>0.05) (Table 3.7). The monthly median values of the percentages of transformed cells per 200 lymphocytes in day 5 cultures with Concanavalin A from animals in the experiment groups ranged from 58-90 pecent (Tables 3.1-3.4). Statistical analyses by Friedman's test revealed that there were no significant differences between the treatment groups when differences attributable to cows were compared (Table 3.8). Apart from untreated group 1, significant differences occurred between the other treatment groups when differences attributable to months were compared (Table 3.9).

AUTOLOGOUS i.e. OWN SERUM SUPPLEMENTATION OF CULTURES

The addition of autologous serum reduced the response to Concanavalin A, while the response to *D. congolensis* remained similar. Autologous serum suppressed the responses of lymphocytes from animals in the acaricide-treated group by 13 per cent, untreated group 1 by 36 per cent, untreated group 2 by 40 per cent and the N'dama by 34 per cent (Table 3.10). Statistical analysis by chi-square revealed no significant differences in median values of blastogenic lymphocytes from acaricide-treated and untreated cattle day 5 cultures in the presence of FCS and Concanavalin A (Table 3.11). In contrast, significant differences were noticed among treatments with cultures containing autologous serum (Table 3.12).

FOETAL CALF SERUM SUPPLEMENTATION OF CULTURES CONTAINING LYMPHOCYTES DERIVED FROM FRIESIAN CATTLE

ACARICIDE-TREATED GROUP

The monthly median values of lymphocyte blastogenesis in cultures supplemented with FCS ranged from 54-78 per cent per 200 lymphocytes for acaricide-treated Friesians (Table 3.13). Statistical analysis by Mann-Whitney test revealed very significant differences ($P < 0.01$) between day 5 cultures without antigen and cultures with Concanavalin A for all the treatment groups, but a significant difference was not observed between cultures without antigen and cultures supplemented with *D. congolensis* ($P > 0.05$) (Table 3.14). Three months after the tick-infested Friesians were put on acaricide treatment their lymphocyte responses to Concanavalin A improved drastically. The percentage reduction in lymphocyte responses to Concanavalin A between the acaricide-treated control group and the Friesians which were put on acaricide treatment for only three months was 7 per cent, their median percentage blastogenic values being 47 and 43.5 respectively.

AUTOLOGOUS SERUM SUPPLEMENTATION OF CULTURES CONTAINING CELLS DERIVED FROM FRIESIANS

The addition of autologous serum reduced the response to Concanavalin A, while the response to *D. congolensis* remained similar. The range of median values for day 5 cultures supplemented with Concanavalin A, using FCS was 53.5 - 78 per cent whereas when autologous serum was used, the range was 32.0 - 57.5 per cent (Table 3.15). The respective median values were 64 and 42 per cent; a very significant difference (Mann-Whitney $U = 2$, $n_1 = 9$, $n_2 = 11$, $P < 0.01$).

UNTREATED FRIESIAN CATTLE

LYMPHOCYTE RESPONSE TO CONCANAVALIN A OF TICK INFESTED FRIESIANS

Two months after exposure to ticks, the lymphocyte response of the tick-infested Friesians fell rapidly to between 33 and 56 per cent of that observed for the acaricide-treated group (Table 3.16). The negative slopes of the regression lines of best fit were -1.1 and -4.2 for the acaricide-treated and untreated groups respectively (Table 3.17; Fig 3.1). The difference between the slopes was significant ($t_{10} = 2.406$; $P < 0.05$).

The addition of autologous serum to the day 5 cultures reduced the response to Concanavalin A by 37-60 per cent (Tables 3.18 and 3.19). Statistical analysis by Wilcoxon's test for paired comparisons showed a significant difference between the blastogenic activity of lymphocytes from untreated Friesian cattle in day 5 cultures with Concanavalin A, supplemented with FCS and autologous serum ($P = 0.05$) (Table 3.20). The medians were 37.5 and 21.5 per cent for the FCS and autologous serum respectively and a comparison of the Mann-Whitney test confirmed the Wilcoxon's test result ($U = 4$, $n_1 = 4$, $n_2 = 4$; $P < 0.05$).

[^{3}H]-THYMIDINE UPTAKE BY LYMPHOCYTES IN CULTURES CONTAINING FCS

The [^{3}H]-thymidine uptake by lymphocytes derived from acaricide-treated and untreated Friesians were compared between cow donors irrespective of the month of sample collection and also between months of sample collection irrespective of the origins of the lymphocytes for culture media without antigen, with Concanavalin A and with *D. congolensis*.

Irrespective of the month of sample collection, no significant differences were revealed between untreated and acaricide-treated Friesians in the response to their lymphocytes uptake of [^{3}H]-thymidine in cultures without antigen and cultures with *D. congolensis* respectively (Mann-Whitney $U = 96.5$, $n_1 = 15$, $n_2 = 15$; $P>0.05$; $U = 95$, $n_1 = n_2 = 15$; $P>0.05$). In contrast significant differences were noticed between acaricide-treated and untreated Friesians when culture media containing Concanavalin A were compared (Mann-Whitney $U = 63$ $n_1 = n_2 = 15$; $P<0.05$). The median value for acaricide-treated Friesians was 37.6 and that for the untreated group was 20.6.

Lymphocytes derived from tick-infested Friesians showed reduced proliferative responses as shown by [^{3}H]-thymidine uptake. The numbers of lymphocytes which incorporated [^{3}H]-thymidine fell linearly from 34,500 in August to 16,000 in March ($y = 37.11 - 2.64x$; $F = 5.70$; $P<0.05$) in marked contrast to the response curve of the lymphocytes from the acaricide-treated animals which rose from 30,700 in August to 43,500 in March with a transient high kick in October and November. All the points of the response curve of the lymphocytes from the acaricide-treated Friesians from October onwards fell outside the upper 95 per cent confidence limits of the linear regression of the response curve of the lymphocytes from the untreated cows as assessed by a [^{3}H]-thymidine incorporation assay when cultured with Concanavalin A (Figure 3.2).

Irrespective of the bovine donors of lymphocytes, there were no significant differences in [^{3}H]-thymidine uptake by lymphocytes in cultures containing *D. congolensis* (Mann-Whitney $U = 25$, $n_1 = n_2 = 8$; $P>0.05$). In contrast, very significant differences were revealed in cultures containing Concanavalin A (Mann-Whitney $U = 15$, $n_1 = n_2 = 10$; $P<0.01$). The medians for the acaricide-treated and untreated Friesians were 36.8 and 21.1 respectively.

Analysis of cultures containing FCS by the Kruskal-Wallis test revealed very significant differences between cultures without antigen, with *D. congolensis* and with Concanavalin A for lymphocytes derived from both acaricide-treated and untreated Friesians ($H = 19.27$; $k = 3$; $n_1 = 8$, $n_2 = 9$, $n_3 = 10$ $P<0.01$; $H = 19.57$; $k = 3$, $n_1 = 8$, $n_2 = 9$, $n_3 = 10$; $P<0.01$). Furthermore, no significant differences were shown by the Mann-Whitney test when cultures without antigen and those with *D. congolensis* were compared for both acaricide-treated and untreated Friesians ($P<0.05$) (Table 3.21 and Table 3.22) respectively.

[^{3}H]-THYMIDINE UPTAKE BY LYMPHOCYTES IN CULTURES CONTAINING AUTOLOGOUS SERUM

Irrespective of the month of sample collection no significant differences were revealed in [^{3}H]-thymidine uptake between acaricide-treated and untreated Friesians in autologous serum cultures containing *D. congolensis* (Mann-Whitney U = 104, $n_1 = n_2 = 15$; P<0.05). The medians for acaricide-treated and untreated Friesians were 241 and 224, respectively. Nevertheless, significant differences were shown in cultures without antigen and also in cultures with Concanavalin A between the acaricide-treated and untreated Friesians (U = 62.5; $n_1 = n_2 = 15$; P<0.05; U = 64; $n_1 = n_2 = 15$; P<0.05).

Irrespective of cow donors of lymphocytes, significant differences in [^{3}H]-thymidine uptake did not occur between acaricide-treated and untreated Friesians in autologous serum cultures containing Concanavalin A and *D. congolensis* (U = 19, $n_1 = n_2 = 9$; P>0.05) (U = 19, $n_1 = n_2 = 7$, P>0.05). The medians for acaricide-treated and untreated Friesians in cultures with Concanavalin A were 4.2×10^3 and 2.2×10^3 respectively. The medians for the two groups in cultures with *D. congolensis* were 78 and 91. In contrast very significant differences were noticed in cultures without antigen for the two Friesian groups (Mann-Whitney U = 6, $n_1 = n_2 = 8$, P<0.01), the median for the acaricide-treated group was 144 and that of the untreated ones was 100.

Analysis of cultures containing autologous serum by Kruskal-Wallis tests revealed very significant differences between cultures without antigen, with *D. congolensis* and those with Concanavalin A for lymphocytes derived from both acaricide-treated and untreated Friesians (H = 17.93; k = 3, $n_1 = 7$, $n_2 = 8$, $n_3 = 9$; P<0.01; H = 16.6; k = 3, $n_1 = 7$, $n_2 = 8$, $n_3 = 9$; P<0.01). Moreover, no significant differences were detected when cultures without antigen and those with *D. congolensis* were compared for both acaricide-treated and untreated Friesians (P<0.05) (Tables 3.23 and 3.24).

DISCUSSION

Cattle and laboratory animals can acquire an immunologically based resistance to tick infestation (Willadsen, 1980; Wikel, 1982c; Brown, 1988). Ribeiro and colleagues (1990) reported that during the long period of tick attachment, the host developed a complex inflammatory reaction which might be complicated by the development of immunity to tick antigens present at the feeding lesion. While unnatural hosts often develop effective anti-tick immunity, natural tick host associations are characterized by the absence or only partial expression of tick resistance despite a significant inflammatory reaction at the attachment site (Rebeiro *et al.*, 1990). Saliva of haematophagous arthropods contain substances that are antagonistic to many of their host's repair reactions which arrest blood flow at the feeding site or otherwise disrupt feeding (Ribeiro, 1987b). Ribeiro (1989) reported that saliva of the tick *Ixodes dammini* has mediators, such as prostaglandins and T-cell suppressors, that produce effects modulating the local environment.

Suppression of host immunity is an established evasive mechanism that enhances the survival of parasites in an immunologically hostile environment (Ogilvie and Wilson, 1976). Thomas and Neitz (1958) reported the possibility that ticks suppress the immune system of an infected host. They observed that cattle reared in an area of endemic babesiosis and anaplasmosis in South Africa lost their premunity to *Babesia bigemina* and *Anaplasma marginale* and succumbed when exposed to overwhelming burdens of the adult brown ear tick, *Rhipicephalus appendiculatus*. Wikel (1982a) conducted comprehensive *in vivo* and *in vitro* studies of the *Dermacentor andersoni*/guinea pig model and demonstrated that *D. andersonii* was capable of suppressing T-lymphocyte responses, but not B-lymphocyte responses. Rubaire-Akiki and Mutinga (1980) and Fivaz and Brown (1984) reported that *R. appendiculatus* induced a strong immunological response in rabbits and cattle, respectively. However, the host effects on the engorgement weight of the ticks and other parameters measuring resistance were only moderate suggesting that this tick may suppress host immune responses (Branagan, 1974; Kaiser *et al.*, 1982). Working in Zimbabwe, Fivaz (1989) showed that factor(s) in the salivary gland of *R. appendiculatus*, but not the closely related *R. zambeziensis*, were immunosuppressive. He concluded that immune suppression is not common in the genus *Rhipicephalus*. When Stewart (1983) added tick saliva from *Rhipicephalus evertsi* and *Boophilus microplus* at varying dilutions to bovine mononuclear cells in leucocyte transformation tests, he detected immunosuppression when compared with control tests. He obtained a dose-response curve which showed that tick saliva or extracts of salivary gland suppressed the leucocyte transformation up to a dilution of 1 in 9600 (34% suppression). Stewart (1983) obtained maximum leucocyte suppression of 80 per cent with *R. evertsi* extracts of salivary gland diluted to 1 in 40.

In the present study, the proliferative responses of lymphocytes derived from Zebu and N'dama cattle infested with ticks were depressed when autologous serum was added to culture medium containing their own cells and Concanavalin A, suggesting that factors in the animals' own serum inhibited the responses of T-lymphocytes. Wikel (1982a) showed a reduced *in vitro* responsiveness to Concanavalin A as a consequence of tick infestation which suggested that the *D. andersoni*/guinea pig interaction resulted in an alteration of reactivity apparently limited to T-lymphocytes. Dierks and Shepard (1968) made a similar observation in lepromatous leprosy patients i.e. those with large numbers of bacilli in lesions. They stated that the *in vitro* reactivity of lymphocytes from lepromatous patients to non-specific mitogens such as phytohaemagglutinin (PHA) was depressed. The depression was caused by a serum factor in these patients (Bullock and Fasal, 1968; Nelson *et al.*, 1971). Immune unresponsiveness is considered a key factor in the pathogenesis of lepromatous leprosy and the same may be true in the development of chronic dermatophilosis. *Demodex canis* infestations of dogs were used to describe the effects of follicle-mite infestation on the immune response. Lymphocytes from infested dogs were cultured *in vitro* with phytohaemagglutinin and poke-weed mitogen (Scott *et al.*, 1974). Lymphocytes from mite-infested dogs responded less intensely to these polyclonal activators than did similar cell populations from mite-free animals indicating the development of immunosuppression.

Four months after tick infestation, lymphocytes derived from tick-infested Friesians were hyporesponsive to T-lymphocyte blastogenesis indicating an endogeneous unresponsiveness. Similarly, the response of blastogenic activity was further depressed when autologous serum from the tick-infested Friesian cattle was added to their own cells. Moreover, the correlation betwen [^{3}H]-thymidine uptake and suppression of lymphocyte blastogenesis of the tick infested Friesians was earlier shown to be good. Reduced [^{3}H]-thymidine incorporation also coincided with the development of clinical lesions in the Friesians (see Chapter 2). Wikel (1982b) stated that an immunosuppressed host might display a reduced resistance response, and impaired immunological vigour and establishment of disease. The same may hold true for the association between *A. variegatum* and dermatophilosis.

Lymphocyte blastogenic activity of tick-infested Friesians showed an upward trend a month after the animals were put on acaricide treatment. The percentage reduction in lymphocyte blastogenesis of day 5 cultures containing Concanavalin A in FCS between the initial acaricide-treated Friesians and the tick-infested Friesians which were later treated with acaricide was 17 and 7 respectively, 1 and 3 months after the tick-infested Friesians were put on acaricide treatment. The improvement in lymphocyte blastogenic responses after tick control, occurred at a time when the Friesians had generalized dermatophilosis, indicating that *D. congolensis* had no effect on the reversal of lymphocyte blastogenesis, a finding in agreement with the observation of Woodman and others (1990) that *D. congolensis* by itself was not immunosuppressive. In contrast, depressed lymphocyte blastogenic responses reversed when tick infestation on infected Friesians was controlled. An indication that ticks were crucial in the lymphocyte immunosuppression observed.

Decreased immunological capabilites have been reported as a sequelae of infection by a number of pathogens. Suppression of the immune response occurred during infestation with *Leishmania tropica* (Farah *et al.*, 1976), *Schistosoma mansoni* (Colley, 1972), bovine respiratory syncytial virus infection (Sharma and Woldehiwet, 1991; Sharma *et al.*, 1990) and horn cancer affected cattle (Kuchroo *et al.*, 1981). Woodman and others (1990) showed that *D. congolensis* itself was not immunosuppressive, therefore the suppression seen in this study is probably a consequence of *A. variegatum.* Woodman (1989) stated that a chronic infection arises as a result of active immunosuppression, or because of a defect in some components of the immune response. Thus mononuclear cells would still be attracted to the site of *D. congolensis* infection as a result of the inflammatory response induced by tissue damage but would not be activated because of a defect in antigen-presentation or subsequent cell activation. Grainstein (1985) observed that T-suppressor cells would predominate following the induction of an immunosuppression pathway. However, some pathways result in specific suppression of the response to the induction antigen, while others cause a more general immunosuppressive effect.

A study of the nature of the immunosuppression effected by ticks was not attempted. With regard to the Friesians one wonders why the immunosuppression was specific for dermatophilosis; why did the Friesians not come down with another disease such as heartwater? On the other hand, although the untreated Zebu-type cows and N'damas were immunosuppressed with autologous serum, they did not develop generalized dermatophilosis. Inherited differences between the Friesians and the other two groups presumably predisposed the Friesians to develop acute generalized dermatophilosis. The overwhelming tick load on the Friesians compared to the other two groups (see Chapter 2), may have compromised also their resistance to infection.

Friesian cattle probably have a lower threshold for dermatophilosis than N'dama and indigenous Zebus.

In general concurrent infections are thought to depress cell-mediated responses (Turk, 1981) causing parasitised hosts to have a decreased ability to respond immunologically to unrelated agents (Ogilvie and Wilson, 1976). In the present study both acaricide-treated and untreated animals kept under the same management practices, were exposed to the same environmental conditions. Therefore it is likely that the ticks were inducing the immunosuppression. Bach (1975) reported that a degree of general immunosuppression may be caused by increased levels of cortisone induced by stress which may arise in response to various factors including the activity of biting arthropods. Marx (1975) observed that immunosuppressive effects may be directly attributable to the production of antibodies because antibodies can be immunosuppressive (Roitt *et al.*, 1985; Halliwell and Gorman, 1989).

Stobo and colleagues (1976) studied the *in vivo* and *in vitro* T-cell reactivity of patients infected with fungal organisms and they found a marked persistent T-cell dysfunction in a portion of the patients. They demonstrated that the dysfunction was not due to an absence of T-cells capable of reacting to antigens, but rather to the presence of a population of T-cells which suppressed other, potentially reactive cells. These could be T-suppressor cells which release immune modulating cytokines. Sharma and Woldehiwet (1991) reported that lymphocytes obtained from lambs experimentally infected with bovine respiratory syncytical virus had significantly reduced blastogenic responses to the mitogen phytohaemagglutinin. Sharma and colleagues (1990) attributed the depressed blastogenic response to the reduction in T-helper cells (CD_{4+}).

Wikel (1982c) and Barré (1989) suggested that reduced immunological function induced by ticks might occur through a number of different mechanisms such as immunological tolerance, stimulation of suppressor cell activity, altered macrophage function and/or selective destruction of lymphocytes. Barré (1989) was of the view that progression of dermatophilosis to a generalized state is probably due to a host imbalance which favours the growth of the pathogen. This imbalance may be induced by factors affecting local skin resistance such as water, immunological inhibitors found in tick saliva and/or factors affecting the general immune response. Martinez (1991) postulated that the activity of *A. variegatum* in dermatophilosis is due to biological activities similar to the immunosuppressive activity of the saliva of *D. andersoni* (Wikel and Osburn, 1982) and *Ixodes dammini* (Ribeiro *et al.*, 1985). He stated that lymphoproliferative tests performed on peripheral mononuclear cells of goats showed 15 to 60 per cent inhibition of the proliferation induced by Concanavalin A by addition of 1 to 20 μl of *Amblyomma* saliva per ml of culture medium. He speculated that the immunosuppression he observed was due to the presence of prostaglandin PGE_2 and prostacyclines he demonstrated in saliva of *A. variegatum.*

Langerhans cells have been implicated in the trapping and presentation of tick salivary gland antigens (Allen *et al.*, 1979) to sensitized T-cells which then secrete chemotactic factors that recruit circulating basophils, bound with anti-tick antibody, to infiltrate the local site and effect the immune resistance response (Brown, 1988). Latif and others (1990) reported that animals sensitized to *R. appendiculatus* showed increased numbers of Langerhans cells in the epidermis as well as dermis. It is possible that overwhelming amounts of tick salivary gland antigen were presented to sensitized T-cells and that T-helper cells were exhausted. On the contrary, substances in tick saliva e.g. prostaglandins either directly inhibited T-helper cells function or stimulated T-suppressor cells thus interfering with or altering normal immunoregulatory activities resulting in immunosuppression. Prostaglandins have been reported to have both stimulatory and suppressive properties (Phipps *et al.*, 1991).

Woodman (1989) reported that localisation and rapid resolution of dermatophilosis may be attributed, in part, to activated antigen-specific T-helper cells. A defect in this activation might enhance the persistence and spread of *D. congolensis* giving rise to chronic generalized lesions.

D. congolensis antigen did not stimulate lymphocytes derived from any of the experiment groups of cattle including those infested with ticks and infected with dermatophilosis. Woodman and others (1990) working with rats demonstrated a strong and specific proliferative, dose-dependent response of spleen mononuclear cells they isolated from rats experimentally infected with *D. congolensis*. They reported that the presence of *D. congolensis* caused some stimulation of mononuclear cells regardless of the origin of cells but that the [^{3}H]-thymidine uptake by mononuclear cells from infected rats was significantly greater than those by cells from naive rats. The lack of response in the present study might be attributed to the difference in animals species used in the two experiments and also the source of lymphocytes. Woodman and co-workers (1990) derived the mononuclear cells from the spleen of rats while lymphocytes were derived from peripheral blood of cattle in the present study. The methods of preparation of *D. congolense* antigen in the two experiments were basically similar because the procedure followed by Woodman and her colleagues (1990) was adopted in the present study.

Sutherland and collegues (1991) observed a difference in response to vaccination between pen trials and field studies with dermatophilosis in sheep such that field trials did not give satisfactory protection against vaccinated animals. They attributed the differences in protection between pen trials and field studies with the *D. congolensis* vaccines they used for factors such as differences between *D. congolensis* strains in the field and the strain used for the pen trials. In the housed animals, the vaccine they used was based on the same strain of *D. congolensis* with which the animals were challenged. In contrast, in the field studies, the sheep were vaccinated with a strain of *D. congolensis* first isolated from an area 100 Km from where the vaccinated sheep were exposed to a local strain. Comparisons on cultural characteristics between the two strains showed differences in the haemolysis pattern on blood agar. The isolate of *D. congolensis* used in the present study was obtained from an infected cow about 78 Km from the experiment group of animals. In the light of Sutherland and colleagues (1991) findings, it is possible to speculate that a difference in antigenicity between strains accounted for the lack of response of lymphocytes to the *D. congolensis* antigen.

CHAPTER FOUR

RESPONSE OF MONONUCLEAR CELLS DERIVED FROM CLINICALLY AFFECTED ANIMALS TO CONCANAVALIN A AND *D. CONGOLENSIS*

Page No.

CHAPTER FOUR

INTRODUCTION

Results reported in Chapter 3 indicated that factor(s) or substances(s) in the serum of cattle infected with ticks were capable of suppressing their *in vitro* lymphocyte blastogenic reponses. Similar suppression have been reported by others. For example, Corbett and colleagues (1975) reported that serum from dogs with chronic generalized demodectic mange significantly inhibited the *in vitro* proliferative response of normal canine lymphocytes to phytohaemagglutinin, indicating that soluble suppressive factors were induced during the course of mite infestation.

Salivary-gland antigen from ticks has been implicated in the induction and elicitation of host immune response to ticks. Wikel (1981) and Brown and others (1984) showed that salivary-gland antigen derived from ticks induced a significant degree of resistance in guinea pigs never previously exposed to tick infestation. Willadsen and Williams (1976) evoked strong immediate skin responses in cattle by the injection of a protein from *B. microplus* described as an esterase with a molecular weight of 60 kDa. Brown and others (1984) used serum from guinea pigs expressing resistance to *A. americanum* to identify a single protein from the salivary gland secretions of this tick estimated to be 20 kDa. Furthermore, purified salivary-gland antigen containing the 20 kDa species immunized guinea pigs against feeding by *A. americanum* (Brown and Askenase, 1986).

Brown (1988) is of the opinion that few other reports have been published describing the characterization of tick products capable of inducing host immunity or eliciting skin responses in tick sensitized animals. Inhibitory, substances such as prostaglandin have been isolated from tick saliva (Higgs *et al.*, 1976; Dickinson, 1976; Ribeiro *et al.*, 1985) and these substances have been shown to be immunosuppressive (Martinez, 1991; Ribeiro *et al.*, 1985, 1990). Ribeiro and colleagues (1985) stated that saliva ejected by adult *Ixodes dammini* contained antihemostatic, anti-inflammatory and immunosuppressive components, properties that appeared to facilitate blood-feeding success of this tick during its prolonged period of host attachment.

Prostaglandins, in particular, produced a spectrum of effects that hindered as well as helped feeding. It prevented macrophage activation and neutrophil activity. Thus ticks antagonize their hosts hemostatic and inflammatory responses by the actions of several distinct salivary components (Ribeiro *et al.*, 1985).

The present study examined the response patterns of lymphocytes from clinically infected animals to Concanavalin A and also investigated the effect serum from clinically affected animals had on immune responsiveness of lymphocytes derived from clinically uninfected animals in the presence of Concanavalin A.

MATERIALS AND METHODS

Six Zebu-type cattle with clinical dermatophilosis of varying severity were selected from the herds at two of the kraals visited each month and were bled into heparinized tubes. Purified lymphocytes obtained by density gradient centrifugation of the collected blood were cultured at the initial density of 1×10^6 cells ml^{-1} in 200 μl volumes of complete medium for five days in tissue culture media to which either Concanavalin A at the concentration of 2 μg ml^{-1} or *D. congolensis* whole cell antigen at the concentration of 1×10^7 ml^{-1} was added. Tissue culture media to which autologous serum was added were also set up. Aliquots of cell suspensions were removed on day 5 and cytosmears prepared for microscopic examination.

A further investigation was carried out to find the effect serum from infected animals had on blastogenic activity of cell cultures derived from clean animals i.e. animals neither infested with ticks nor infected with disease. Serum from two of the affected animals was added to lymphocyte cultures derived from two of the acaricide-treated animals (Animals 159 and 164). Autologous serum from cow 159 and cow 164 was also added to medium containing their own cells.

RAW DATA

Raw data pertaining to the results in this chapter are listed in Appendix Tables 18-20.

RESULTS

Three of the clinically affected animals which came from the same kraal showed normal responses in Concanavalin A - stimulated day 5 cultures; median values ranged from 70-72 percent. However, readings of the lymphocyte response to Concanavalin A for the other two animals which came from another kraal, were low, the range of median values being 48-56 per cent (Table 4.1). No significant differences were observed between day 5 cultures containing no antigen and cultures containing *D. congolensis* in FCS and autologous serum, respectively ($P>0.05$) (Table 4.2). However, there were significant differences ($P<0.05$) between day 5 cultures containing no antigen and cultures containing Concanavalin A in FCS and autologous serum, respectively (Table 4.2).

THE RESPONSES TO CONCANAVALIN A IN CULTURES TO WHICH AUTOLOGOUS (OWN) SERUM WAS ADDED

Cultures to which autologous serum were added were depressed in all six treatments (Table 4.3). The percentage depression of the FCS response ranged from 61 to 37 (Table 4.4), results similar to that seen in the tick-infested experiment groups of animals. Statistical analysis of the number of blastogenic cells by the Mann-Whitney test showed that the difference was real ($U = 0$; $n_1 = n_2 = 6$; $P<0.05$), fewer lymphocyte cells being transformed in the presence of autologous serum as compared to the cells proliferating in the presence of FCS, the medians being 70 and 35.5 per cent.

LYMPHOCYTE BLASTOGENSIS TO CONCANAVALIN A AND *D. CONGOLENSIS* OF TWO ACARICIDE-TREATED ANIMALS USING SERA FROM TWO CLINICALLY INFECTED ANIMALS

Both sera from the clinically infected animals depressed the response of the lymphocytes to a much lower level than was observed when autologous serum i.e. serum from cows 159 and 164 was added to the lymphocyte cultures (Table 4.5). Reduction in response of lymphocytes to autologous serum and sera from the two infected animals relative to the activation in FCS was as follows. Lymphocytes from acaricide-treated cow 159 were depressed 18, 56 and 48 per cent by its autologous serum, infected cow 1 serum and infected cow 2 serum respectively. Lymphocytes from the second acaricide-treated cow 164, were depressed 33, 42 and 42 per cent by its autologous serum, infected cow 1 serum and infected cow 2 serum, respectively (Table 4.6).

There were very significant differences between values of blastogenic cells in day 5 cultures containing no antigen and Concanavalin A (Mann-Whitney $U = 0$; $n_1 = n_2 = 8$; $P<0.01$). However, there were no significant differences between values of blastogenic cells from cows 159 and 164 in day 5 cultures containing FCS and autologous serum (Mann-Whitney $U = 24.5$; $n_1 = n_2 = 8$; $P>0.05$). There were also no significant differences between values of blastogenic cells in day 5 cultures containing FCS and autologous serum from infected cows 1 and 2 ($U = 6$; $n_1 = n_2 = 4$; $P>0.05$) (Table 4.7).

Despite the low numbers of animals used, a significant difference was demonstrated in the values of lymphocyte blastogenic activity in Concanavalin A day 5 cultures supplemented with FCS or autologous serum and sera from infected cows 1 or 2, the medians being 73.0 and 45.5 respectively ($U = 0$; $n_1 = n_2 = 4$; $P<0.05$).

DISCUSSION

Nelson and colleagues (1971) used lymphocyte blastogensis to evaluate the immune responses of patients with leprosy. They found that cells from Malays and

Indians with lepromatous leprosy and from Malays with tuberculoid leprosy transformed as well as cells from normal controls when cultured in normal reference serum. Cells from lepromatous Malays and Indians transformed significantly less than cells from normal controls when cultured in autologous serum. Normal lymphocytes transformed significantly less well when cultured in serum from lepromatous or tuberculoid Malays or from lepromatous Indians than when cultured in serum from normal controls. In the present study, three of the six clinically affected animals showed normal lymphocyte blastogenic responses when cultured in medium containing FCS whereas two others were significantly hyporesponsive to lymphocyte stimulation when their cells were cultured in medium containing FCS. However, cultures to which autologous serum was added were depressed significantly in all six treatments the median depressions ranging from 37.2 - 60.8 per cent of the FCS responses indicating that there were substances or factors in the serum which caused the T-lymphocyte hyporesponsiveness to the mitogen. Furthermore, when sera from two animals infected with ticks and dermatophilosis were added to culture medium containing lymphocytes derived from two "clean" i.e. acaricide-treated animals, it was observed that both sera depressed the response of the lymphocytes to a lower level than was observed when FCS and autologous serum was added to the culture medium. This is additional evidence that factors in the serum of animals clinically infected with ticks and dermatophilosis are capable of suppressing *in vitro* lymphocyte proliferation of cells derived from "clean" animals.

Several workers have reported the involvement of a serum factor in impairment of lymphocyte transformation. Heilman and McFarland (1966) reported that in the case of tuberculosis, incubation of the cells from a tuberculous individual in normal serum will restore the response of that patient's lymphocyte to the PPD stimulus. Conversely, the incubation of lymphocytes from a normal PPD-positive subject in the serum from a tuberculous patient will suppress the response of the cells to PPD.

Dhingra and colleagues (1982) observed a significantly lower percentage of rosette forming cells of 2-amino ethyl isothorium bromide (AET) treated sheep red blood cells when peripheral blood lymphocytes were incubated with 50 per cent serum from animals affected with horn cancer than in serum from controls. The suppression effect of horn cancer sera on E_{AET} rosette forming cells indicated the presence of a certain factor or factors, in the sera of horn cancer affected animals. Similar E-rosette inhibitory activity have been reported in the sera from patients with human breast carcinoma (Whitehead *et al.*, 1977). Whitehead and colleagues (1977) demonstrated that sera from tumor-bearing individuals contain a certain factor, or factors which inhibited the normal capacity of T-lymphocytes to form rosette with sheep erythrocytes either by masking or altering the E-receptor sites.

Calderon and Hay (1984) found that the lymphoid cells of mice suffering from a chronic *Trichophyton quinckeanum* infection conferred protection when transferred to recipient mice. However, the adoptive immunity was abrogated when the cells were transferred along with autologous donor serum. They showed that the inhibitory serum factor was dermatophyte antigen rather than antibody. Fischer, Ballett and Griscell (1978) demonstrated that the serum of patients with chronic mucocutaneous candidiasis inhibited the patients lymphocyte transformation response to *Candida albicans* and that the inhibition was caused by a polysaccharide antigen of *Candida albicans* in the serum which disappeared under anti-fungal therapy. Thus antigens from skin pathogens may enter the circulation and inhibit cell-mediated immunity. In the present study the nature of the serum factor which depressed lymphocyte blastogenesis was not determined. However, Calderon and Hay (1984) and Fischer and colleagues (1978) postulated that inhibitory serum factors in their experiments were dermatophyte antigen and Candida antigen respectively. Jones and Artis (1981) suggested that immunological tolerance may develop in dermatophyte infections due to an overload of dermatophyte antigen. Turk (1981) also suggested that a high antigenic-load contributed to the lack of host resistance in the chronic skin diseases lepromatous leprosy, mucocutaneous candidiasis, diffuse leishmaniasis and secondary syphilis.

Tick salivary gland antigens contain inhibitory substances that affect the immune responsiveness of tick-infested animals (Ribeiro *et al.*, 1985; Ribeiro *et al.*, 1990; Barré, 1989). Ribeiro and colleagues (1985) demonstrated that *Ixodes dammini* saliva prevented *in vitro* T-cell activation. Ribeiro (1987a) attributed the immunosuppressive activity of *I. dammini* saliva to prostaglandin PGE_2 in the saliva, although he recognized that other substances may be involved. He stated that such immunosuppressive activity may decrease host immune responses to salivary antigen. Wikel (1982) and Wikel and Osburn (1982) showed that tick-infested animals demonstrated reduced lymphocyte responsiveness to mitogens.

Bach (1982) showed that prostaglandin PGE_2 and prostacyclin inhibited cell-mediated processes such as mast cell degranulation and that prevented release of other mediators of inflammation. In the present study, sera from tick-free, acaricide-treated animals did not depress blastogenesis, significantly whereas sera from tick-infested, dermatophilosis cattle did. It was also shown in Chapter 3 that depressed blastogenic responses of lymphocytes from tick-infested Friesians was reversed once the cattle were put on acaricide treatment and hence became tick free. Two months after acaricide treatment, the previously tick-infested Friesians were basically tick free but had dermatophilosis; their lymphocyte responses were only decreased by 7 per cent compared to those of the control group - the removal of ticks coincided with the reversal of lymphocyte responses. In the light of Woodman's statement (1990) that *D. congolensis* does not immunosuppress, and the fact that removal of ticks from the Friesian cattle coincided with reversal of depressed lymphocyte responses, it is tempting to suggest that substance(s) in tick saliva were responsible for the inhibiting factor(s) in the serum.

INTRODUCTION

Animals develop resistance to ticks after natural infestations or after immunisation with tick-derived antigens (George *et al.*, 1985; de Castro *et al.*, 1985; Opdebeek *et al.*, 1988). Resistance to tick infestation is manifested by significantly fewer ticks engorging on resistant animals and by a reduced engorgement weight for those few ticks which did engorge (Smith *et al.*, 1989).

Both antibody and cell-mediated immunity are involved although the degree of involvement of either may depend on the species of tick and host animal (Smith *et al.*, 1989). The production of antibody in response to tick infestation has been reported in a number of tick host associations but it has not always been possible to correlate antibody titres with the degree of resistance or indeed to show that antibody is involved in protective immunity (Wikel *et al.*, 1978; Brown, 1988).

A delayed-type hypersensitivity reaction characteristic of cell-mediated immunity was demonstrated after intradermal inoculation of tick salivary gland extracts into resistant animals (Wikel, *et al.*, 1978; Girardin and Brossard, 1985). Smith and colleagues (1989) asserted that such a reaction, if it provided a significant negative correlation with the number of ticks on individual animals, could be substituted for the laborious task of counting ticks in assessing the resistance status of animals. Wikel (1980) had earlier postulated that host resistance to tick infestation represented a cutaneous hypersensitivity state which had a protective function.

The presence of a cell-mediated immune component in some diseases is evaluated *in vivo* by an animal's skin reaction to intradermal inoculation of an antigen similar to or which shares similar antigenic properties with the organism causing the disease. In leprosy the tuberculoid type represents the high resistant, self-limiting form, while the low resistant, progressing type is found in lepromatous leprosy (Dohms, 1991). Circumstantial evidence indicated that patients with lepromatous leprosy had a depressed capacity to express or to develop cell-mediated immunity as demonstrated by depressed delayed skin reactivity to mycobacterial antigens, lepromin and tuberculin (Godal *et al.*, 1971).

Purified protein derivative (PPD) of the tubercle bacillus, is currently used as the diagnostic reagent in the tuberculin skin test (Corrin *et al.*, 1987). Fifis, Plackett, Corner and Wood (1989) reported that PPD contained a highly complex mixture of antigens and the antigenic components responsible for the observed reaction were largely uncharacterized. They purified a *M. bovis* antigen from culture filtrate by chromatofocussing and pointed out that the specificity of the purified antigen was far superior to that of the crude culture filtrate. The purified antigen also elicited strong *in vivo* and *in vitro* cell-mediated responses. Woodman and colleagues (1990), observed cross-reaction between PPD antigen and *D. congolensis* cocci. They used PPD as positive control when studying the cell-mediated immune responses in rats to *D. congolensis* and they just happened to find that PPD caused significant stimulation of mononuclear cells (MC) from *D. congolensis*-infected rats which responded far more strongly to it than did MC from naive rats. However, this cross-reaction was uni-directional since MC from PPD-sensitized rats did not respond more strongly to the cocci than did cells from naive rats. The fact that experimental infection with *D. congolensis*, sensitized animals to PPD of *M. tuberculosis* poses some questions particularly because tuberculosis is a zoonotic disease. What effect, if any, does natural dermatophilosis or vaccination have on the tuberculin skin test?

Gregson (1970) provided evidence that saliva and attachment cement derived from *D. andersoni* were antigenic in man. Both substances added to cultures, induced *in vitro* antigen-specific blastogenesis of peripheral blood lymphocytes derived from himself. Such reactivity is an *in vitro* correlate of immune responsiveness. A cell-mediated component of guinea pig resistance to ticks was demonstrated by Wikel and colleagues (1978). Salivary gland antigen initiated *in vitro* lymphocyte blastogenesis when added to cultures of lymph node cells from tick resistant hosts.

Many attempts to establish the chronic, generalized form of dermatophilosis experimentally have all been unsuccessful (Mémery and Thiery, 1960; Abu-Samra and others, 1976). This suggests that under most conditions, the host is able to mount an effective response whereas the chronic form of the disease may occur when the normal host response is compromised by a range of interacting factors.

This chapter concerns attempts to characterize skin reactivity of two groups of cattle, one group infested and the other uninfested with ticks, to PPD after the animals have been sensitized to the antigen, and to demonstrate antigen-specific *in vitro* blastogenesis of lymphocytes derived from the experiment groups of cattle to salivary gland antigen and PPD. The responses of the cattle to experimental infection with *D. congolensis* under field conditions were also investigated.

MATERIALS AND METHODS

SKIN REACTIVITY AND RESPONSES OF LYMPHOCYTES OF CATTLE TO PURIFIED PROTEIN DERIVATIVE (PPD) AND *D. CONGOLENSIS* FOLLOWING SENSITIZATION

Fifteen cattle in an experiment group comprising five N'dama and five Zebu-type that were tick infested, and five Zebu-type animals that were tick free were sensitized to purified protein derivative of *M. tuberculosis* (PPD). The animals were simultaneously injected intradermally and subcutaneously with 0.1 ml of an emulsion consisting of equal volumes of Freund's incomplete adjuvant and PPD of *M. bovis* (1 mg/ml). The site was the loose skin over their ribs.

Subsequently, to determine whether the cattle were sensitized to PPD, skin tests were carried out. All 15 animals were injected intradermally with 0.1 ml of tuberculin (1.0 mg/ml PPD of *M. bovis*) at a marked site in the skin fold at the base of the tail 6 weeks after the initial sensitizing injection. The skin thickness at the sites was measured prior to injection and 72 hours after with calipers calibrated in millimetres.

Lymphocyte responses to PPD were checked before the cattle were sensitized to PPD and were checked again 3 weeks after the initial-sensitizing injection. All the 15 cattle were re-sensitized by injecting 1 ml of an emulsion containing equal volumes of tuberculin (*M. bovis* 1 mg/ml) and Freund's complete adjuvant intradermally 7 weeks after the initial sensitizing injection. One week later, their lymphocyte responses to PPD was checked once more.

Lymphocyte cells were derived from the 15 sensitized animals as described in Chapter 3 and were cultured in 96-well plates in complete medium either alone, or with Concanavalin A at a concentration of 2 $\mu g\ ml^{-1}$ or with PPD at a concentration of 5 μg^{-1} or 10 $\mu g\ ml^{-1}$. On day 3 of culture, half the spent medium (100 μl) was replaced with the appropriate medium to sustain the concentration in the culture medium. On day 5 of culture (100 μl) three replicate samples from replicate wells were taken for cytospin preparations.

Culture media were also set up in which lymphocytes derived from the 15 animals sensitized to PPD were cultured in 96-well plates in complete medium either alone or with Concanavalin A at the concentration of 2 $\mu g\ ml^{-1}$ or with two different preparations of *D. congolensis* antigens, Dc_1 and Dc_2.

Antigen Dc_1 was the spore or whole cell antigen used in tissue cultures in Chapter 3. It was used at the concentration of 10^7 per ml and was killed by addition of RPMI with antibiotics so that the spores were not viable and could not play an active role in the cell cultures but were merely inert antigenic structures.

The medium antigen, Dc_2 was produced by growing dermatophilus in RPMI complete medium without antibiotics for 6 days. The medium was then spun to remove cells and, thereafter, filter sterilized.

IN VITRO REPONSES TO TICK SALIVARY GLAND ANTIGEN AND CONCANAVALIN A BY LYMPHOCYTES FROM CATTLE

Lymphocytes were derived from 16 cattle made up of four N'dama, four Zebu-type and two Friesians which were tick infested, and four Zebu-type and two Friesians that were tick free. The animals were from the experiment groups decribed in Chapter 2.

The lymphocyte blastogenesis technique used was the same as described in Chapter 3. The cells were cultured in complete medium alone, or with Concanavalin A at 2 μg ml^{-1}, or with two different concentrations of salivary gland antigen i.e. 5 μg ml^{-1} and 10 μg ml^{-1}, or with salivary gland antigen plus Concanavalin A.

The salivary gland antigen was kindly donated by Dr. Alan Walker of the C.T.V.M, University of Edinburgh, Scotland. Forty pairs of salivary glands were dissected from fed male and female *A. variegatum* ticks. The harvests were sonicated in Tris/HCl buffer 0.05 M, pH 8.5 and freeze-dried, but they were neither centrifuged nor filtered. The protein concentration was about 500 μg/vial. The contents of a vial were dissolved in complete medium to the required concentration of 5 μg ml^{-1} and then filtered to sterilize it.

MULTIPLE EXPERIMENTAL INFECTION OF CATTLE WITH *D. CONGOLENSIS* AND SUBSEQUENT INTRADERMAL TESTS USING *D. CONGOLENSIS* ANTIGENS

Fifteen cattle used in this skin reactivity test to PPD were used to investigate experimental infection to *D. congolensis*. An area about 8-10 cm square was shaved over the shoulder or rib cage of each animal using a razor blade. The shaved area was washed with water and scarified with a wire-mesh until it was hyperaemic. Two to 3 mls of a broth culture of *D. congolensis* was applied to the shaved area. The infected animals were observed regularly and the state of scab formation and detachment was recorded. The animals were re-infected one month after the initial infection, then 2 weeks after the second infection and finally 5 weeks after the third infection. Each time, the site of infection was changed and a different site on the opposite side of the animals were infected on various occasions. In all, the animals were infected four times. They were examined clinically every day and the evolution and resolution of the lesions were noted.

After the experimental infection, the cattle were inoculated intradermally at the base of the tailfold with two different antigens of *D. congolensis*, antigen M and antigen S. Antigen M was injected at the right side and antigen S at the left side at 0.1 ml each per site. Antigen M was medium-derived antigen, (DC_2) the same as used in cultures when medium antigen was used. *D. congolensis* was grown for 6 days in RPMI with all the additives except the antibiotics. It was then centrifuged and the supernatant removed and filtered through a 0.2 μm filter. Antigen S was sonicated whole cell antigen reconstituted at a concentration of 100 mg/100 ml.

The skin thicknesses at the sites was measured prior to injection and 24 and 72 hours after, respectively with calipers calibrated in millimeters. The same operator measured the skin thicknesses before and after inoculation of antigens.

RAW DATA

Raw data pertaining to the results in Chapter 5 are listed in Appendix Tables 40,43,44,47,50,52,56,58, 59 and 96-98.

RESULTS

REACTION TO SKIN TESTING AFTER PPD INOCULATION

All cattle developed increases in skin thickness that ranged from 0.5 to 4.2 mm (Table 5.1). The median increases were 2.6 mm, 1.6 mm and 1.2 mm for the acaricide-treated Zebu-types, untreated Zebu-types and untreated N'damas, respectively. The median percentage increments in skin thickness were 42, 31 and 21 for the acaricide-treated Zebu-types, untreated Zebu-types and untreated N'damas respectively (Table 5.1). Statistical analysis by the Kruskal-Wallis test revealed no significant differences in the pre-inoculation skin thicknesses between the three groups, the medians of which were 5.2, 5.1 and 5.4 mm, respectively (P>0.05) (Table 5.2). Likewise, no significant differences were revealed in skin reactivity in the three groups of cattle 72 hours after inoculation (P>0.05) (Table 5.2). Although there were differences in skin reaction diameters between the acaricide-treated Zebu-types, and the untreated Zebu-types and N'dama groups, they were not statistically significant (P>0.05) (Table 5.2).

RESPONSE OF SENSITIZED LYMPHOCYTES TO PPD AND CONCANAVALIN A

There were no obvious differences between the three groups of cattle in their responses to PPD and Concanavalin A, when day 5 cultures were compared (H = 0.335, k = 3, n = 12; P>0.05) (Table 5.3). In contrast, the Kruskal-Wallis statistical test revealed very significant differences between the four treatments i.e. day 5 cultures without antigen, with Concanavalin A, with PPD at the concentrations of 5 μg^{-1} and 10 μg^{-1}, respectively (H = 20.142, k = 4, n = 9; P<0.01). The overall medians were 10, 62, 10, 12 for day 5 cultures without antigen, with Concanavalin A, with PPD at the concentration of 5 μg^{-1} and 10 μg^{-1} respectively (Table 5.3). No significant differences between treatment groups were observed (H = 0.773, k = 3, n = 9; P>0.05) when day 5 cultures containing Concanavalin A were omitted.

The slope of the line of best fit of the blastogenic data from day 5 cultures without antigen was negative (-0.33) (Table 5.4). Similarly, the line of best fit of the data from day 5 cultures with Concanavalin A was negative (-1.98) (Table 5.4). In contrast, the lines of best fit of the data from day 5 cultures with 5 μg^{-1} and 10 μg^{-1} of PPD were both positive (+1.55 and +2.22, respectively). The difference between the values induced by 5 μg^{-1} and 10 μg^{-1} PPD was not significant (Mann-Whitney U = 35, n_1 = n_2 = 9; P>0.05). The data were therefore pooled and the calculated regression of the percentage responses of the lymphocytes and the number of injections was linear, positive and significant ($F = 8.64$; $v_1 = 1$ $v_2 = 16$; $P<0.01$) (Figure 5.1).

The median values of transformed lymphocytes from Concanavalin A treated cultures from the three groups of cattle were 62, 47 and 64 per cent for the acaricide-treated Zebu-types, the untreated Zebu-types and the N'dama respectively. The differences in the proportions were very, very significant (Chi-square = 14.147, $v = 2$, $P<0.001$) and was attributable to the low value obtained from the untreated Zebu-type cattle because the difference between the value for acaricide-treated Zebu-types and N'damas was not statistically significant (Chi-square = 0.177, $v = 1$, $P>0.05$).

RESPONSE OF LYMPHOCYTES DERIVED FROM ANIMALS SENSITIZED WITH PPD TO CONCANAVALIN A AND *D. CONGOLENSIS*

There were no obvious differences between the three groups of cattle in their responses to Concanavalin A and *D. congolensis*, when day 5 cultures were compared (Table 5.5). No statistical differences were observed between the three groups of cattle when day 5 cultures without antigen, with Dc_1 and Dc_2 were compared (Kruskal-Wallis, $H = 2.047$, $k = 3$, $n = 9$; $P>0.05$). In contrast, the Kruskal-Wallis statistical test revealed very significant differences between treatment groups, irrespective of cattle i.e. day 5 cultures without antigen, with Dc_1 and Dc_2 respectively ($H = 9.751$, $k = 3$, $n = 9$; $P<0.01$). The overall medians were 10, 15 and 12 for day 5 cultures without antigen, with Dc_1 and Dc_2 respectively (Table 5.5). The only significant linear relationship between percentage of transformed lymphocytes and the number of intradermal tests was found with cultures seeded with Dc_2 and was linear and positive ($\hat{y} = 15.46 + 2.67x$, $F = 10.96$, $v_1 = 1$, $v_2 = 7$; $P<0.05$) (Figure 5.2).

Irrespective of cattle groups, comparisons were made between the different treatments i.e. day 5 cultures with antigen, with Dc_1 and Dc_2. Statistical analysis by the Mann-Whitney test revealed very significant differences between day 5 cultures without antigen and those seeded with Dc_1 (Mann-Whitney $U = 6$; $n_1 = n_2 = 9$;

P<0.01). Similarly, very significant differences were revealed between day 5 cultures without antigen and those containing Dc_2 (Mann-Whitney U = 10.5; $n_1 = n_2 = 9$; P <0.01). In contrast, a comparison between day 5 cultures containing Dc_1 and cultures with Dc_2 was not significant (Mann-Whitney U = 20.5; $n_1 = 9$, $n_2 = 9$; P>0.05). The median values of transformed lymphocytes from Concanavalin A treated cultures from the three groups of cattle were 64, 54 and 63 per cent for the acaricide-treated Zebu-types, the untreated Zebu-types and the untreated N'damas respectively (Table 5.5). Comparisons were made between the three groups of cattle by Chi-square. Differences between acaricide-treated Zebu-types and untreated Zebu-types were significant (Chi-square = 4.134, v = 1, P<0.05). In contrast, no significant differences were observed between the acaricide-treated Zebu-types and the untreated N'dama nor between the untreated Zebu-types and the untreated N'dama (Chi-square = 0.43 and 3.336 respectively, P<0.05).

RESPONSE OF LYMPHOCYTES TO TICK SALIVARY GLAND ANTIGEN

A statistical analysis by the Kruskal-Wallis test revealed very significant differences between day 5 cultures without antigen, with Concanavalin A alone, with Concanavalin A plus tick saliva, and with tick saliva alone (H = 15.019, k = 4, n = 5; P<0.01) (Table 5.6). There were no obvious differences between day 5 cultures without antigen and those containing tick saliva antigen alone. Likewise, no apparent differences were seen between day 5 cultures with Concanavalin A and those with Concanavalin A plus tick saliva antigen (Table 5.6). Statistical analyses using the Mann-Whitney U test confirmed these observations (U = 5.5; $n_1 = n_2 = 5$; P>0.05) for the culture without antigen and tick saliva alone and (U = 4; $n_1 = n_2 = 5$; P>0.05) for the culture with Concanavalin A and Concanavalin A plus tick saliva antigen. Breed differences were also not significant (H = 0.593; k = 5, n = 4; P>0.05).

Comparisons were then made within the groups of animals, using the Chi-square test to evaluate the blastogenic activity of their lymphocytes to tick saliva antigen. No significant differences were observed between day 5 cultures without antigen when compared with day 5 cultures containing tick saliva antigen alone in the acaricide-treated Zebu-type (Chi-square = 0, v = 1, P>0.05), the untreated N'dama group (Chi-square = 0.256, v = 1, P>0.05), acaricide-treated Friesians (Chi-square = 0.448, v = 1, P>0.05) nor the untreated Friesians (Chi-square = 0.027, v = 1, P>0.05). In contrast, very, very significant differences were revealed between day 5 cultures without antigen and cultures containing tick saliva antigen in untreated Zebu-type cattle such that the percentage of transformed cells was higher in cultures with added tick saliva (Chi-square = 13.636, v = 1, P<0.001) (Table 5.7).

Day 5 cultures containing Concanavalin A were compared between the cattle groups using the Chi-square test. Very, very significant differences were noticed when all the groups were compared (Chi-square = 71.647, v = 4, P<0.001). The median of day 5 culture containing Concanavalin A for the untreated Friesians was low, 33.5 per cent (Table 5.6) and therefore it was omitted and the other four groups compared. A very significant difference was still observed (Chi-square = 12.440, v = 3, P<0.01). No significant differences were revealed when the medians for the acaricide-treated Zebu-type cattle and the untreated Friesians were omitted and those of the other three groups were compared (Chi-square = 3.497, v = 2, P>0.05). A very significant difference was noticed between day-5 cultures containing Concanavalin A of acaricide-treated Zebu-type cattle and untreated Zebu-type cattle (Chi-square = 8.674, v = 1, P<0.01). In contrast differences noticed between day-5 cultures containing Concanavalin A of the two untreated cattle groups i.e. Zebu-type and N'dama were not significant (Chi-square = 2.394, v = 1; P>0.05).

MULTIPLE EXPERIMENTAL INFECTIONS WITH *D. CONGOLENSIS*

All cattle experimentally infected developed crusts which were hard to remove at the infected sites. The crusts resolved spontaneously in 12-18 days after the first infection, 5-16 days after the second infection, 7-15 days after the third infection and

11-21 days after the fourth infection (Table 5.8). Medians of days in which the experimental infection with *D. congolensis* resolved in the three groups of cattle are given in Table 5.9. Irrespective of cattle groups, differences between infections were very, very significant (Kruskal-Wallis, H = 26.60. k = 4, n = 15; P<0.001). The correlation of the days to resolution on the number of infections was negative and very significant (r_5 = -0.35, P<0.01), the relationship, however was not linear but curvilinear, the deviations from the linear correlation being very, very significant (F = 20.70; $v_1 = 2$, $v_2 = 56$; P<0.001) (Figure 5.3)..

Irrespective of the number of infections, differences between cattle groups were not statistically significant (Kruskal-Wallis, H = 3.888, k = 3, n = 20; P>0.05). Irrespective of cattle groups, comparisons of resolution between infections 1 and 2, 1 and 3 were very significant (P<0.01, Table 5.10). However, the difference observed between the rate of resolution between infection 1 and 4 was not significant (P>0.05) (Table 5.10). In short, accelerated immune responses occurred after the second and third infections but resolution of the fourth infection was similar to that of the first. Days to complete resolution of infections irrespective of number of infections were compared using Kruskal-Wallis test. Significant differences were observed for the acaricide-treated and untreated Zebu-type cattle (P<0.05) (Table 5.11), and very, very significant differences were detected overall (P<0.001) (Table 5.11). Differences attributable to the different cattle groups in duration of resolution of infections were not significant as revealed by the Kruskal-Wallis test (P>0.05) (Table 5.12).

REACTION TO SKIN TESTING TO *D. CONGOLENSIS* ANTIGENS AFTER EXPERIMENTAL INFECTION OF 15 CATTLE WITH *D. CONGOLENSIS*

Very minimal positive skin reactivities were observed in the 15 cattle 24 hours post-inoculation with either *D. congolensis* antigen M or antigen S. The median increases in skin thicknesses induced by antigen M were 0.8, 1.0, 1.1 mm for acaricide-treated Zebu-types, untreated Zebu-types and untreated N'damas, respectively (Table 5.13). The corresponding values, 24 hours post-inoculation of antigen S were 1.2, 1.0, 0.1 mm for acaricide-treated Zebu-types, untreated Zebu-types and untreated N'damas respectively (Table 5.14). Irrespective of cattle groups, no significant differences were observed in skin thicknesses 24 hours after inoculation with antigen M (Kruskal-Wallis $H = 0.26$, $k = 3$, $n = 5$; $P>0.05$). In contrast, very significant differences were revealed in skin thicknesses 24 hours after inoculation with antigen S (Kruskal-Wallis $H = 8.723$, $k = 3$, $n = 5$; $P<0.01$). The medians in skin thicknesses being 1.2, 1.0 and 0.1 mm for the acaricide-treated Zebu-types, untreated Zebu-types and untreated N'damas respectively (Table 5.14). A Mann-Whitney statistical test showed no significant differences 24 hours post-inoculation with antigen S between skin reactivity of acaricide-treated Zebu-types and untreated Zebu-types (Mann-Whitney $U = 11.5$, $n_1 = n_2 = 5$; $P>0.05$).

Differences between groups of cattle were not significant at 72 hours post-inoculation with either antigen M or antigen S, respectively (Kruskal-Wallis $H = 0.74$ and $H = 1.005$). The medians were 0.0, 0.6 and 0.1 mm for acaricide-treated Zebu-types, untreated Zebu-types and untreated N'dama respectively 72 hours after inoculation with antigen M (Table 5.15). The medians of skin reactivities were 0.0, 0.3, 0.0 mm for acaricide-treated Zebu-types, untreated Zebu-types and untreated N'dama respectively 72 hours after inoculation with antigen S (Table 5.16).

DISCUSSION

INTRADERMAL SKIN TESTS

Intradermal skin tests using purified protein derivative of *M. bovis* are used as the standard test for detecting tuberculosis in cattle and other livestock. Lepper and colleagues (1977) evaluated the efficacy and sensitivity of two different doses 0.1 mg and 0.2 mg of bovine PPD to detect tuberculosis in beef cattle in Australia. They found that 0.2 mg dose of PPD detected 95 per cent of tuberculous cattle and caused false positive reactions in 19 per cent of cattle without lesions, reactions being shown positive when the increase in skin thickness was ≥ 2 mm. The 0.1 mg dose detected 80 pecent of tuberculous cattle and caused 11 per cent false-positive reactions. No visible lesions of tuberculosis were found in either of their control groups although 11 per cent reacted to 0.2 mg and 5 per cent to 0.1 mg bovine PPD at 72 hours.

Corrin and colleagues (1987) checked tuberculosis in red deer and reported that a review of skin test and post-mortem data indicated that the sensitivity of the intradermal test using PPD was approximately 85 per cent. They concluded that the 85 per cent sensitivity of the PPD test in deer was comparable to that reported for the tuberculin test in cattle. In the current study, skin reactivity after initial sensitisation and intradermal inoculation with PPD of *M. bovis* 6 weeks after in three groups of experimental animals resulted in increases at the test sites. The skin reaction diameters were larger in acaricide-treated Zebu-type cattle than two other untreated cattle groups i.e. Zebu-type and N'dama. In the two untreated groups, the N'dama reacted less to the skin test than the Zebu-types. These findings suggested tick-free animals were more sensitive to PPD skin test whereas tick-infested N'damas were more resistant i.e. less reactive to PPD skin test. However, statistical analysis revealed no significant differences between the cattle groups in their response to the skin test.

Ritchie (1953) put the significant level of sensitivity to bovine PPD at 4 mm or greater. Going by that assessment only two of the acaricide-treated Zebu-types reacted to the intradermal test to PPD in this study (Table 5.1). However, Corner (1981) stated that for practical purposes, a 2 mm increase in skin reactivity to PPD should indicate a positive response to the test. By this assessment, three of the acaricide-treated Zebu-types, two untreated Zebu-types and one untreated N'dama reacted positively to the PPD test (Table 5.1).

Response of cattle to intradermal inoculation with *D. congolensis* antigens S and M were minimal. None of the animal's skin reaction diameters was 2 mm to either of the antigens 24 and 72 hours after inoculation. There were slight increases in skin thicknesses 24 hours to both antigen S and M in all the treatment groups, but these increases had decreased by 72 hours post-inoculation indicating that they were not delayed-type hypersensitivity reactions. Skin reactions to needle injections could have accounted for the initial skin reactions.

As pointed out in Chapter 3, there could be strain variations in *D. congolensis* with different antigenicity. *D. congolensis* used in the preparation of vaccines was the same isolate used in the preparation of antigens for the lymphocyte blastogenesis study. Poor antigenicity of the *D. congolensis* isolate also could have accounted for lack of reactivity after intradermal inoculation.

The tuberculin skin test using intradermal injection of PPD is used in many countries to diagnose tuberculosis in cattle and as a tool for a campaign for the eradication of tuberculosis (Ketterer *et al.*, 1981; Corner, 1981; Minden *et al.*, 1986). Radford and co-workers (1988) stated that the causative agent of bovine tuberculosis, *M. bovis*, is closely related to *M. tuberculosis* the aetiology of the disease in humans. In their view eradication of the disease from cattle has been hampered by the lack of sensitivity and specificity of the bovine skin test currently used for detection of infected animals. They attributed the non-specificity of the tuberculin test to cross-reaction with antigens from other environmental mycobacterial or nocardial species. Minden and colleagues (1986) stressed the view that PPD, currently used for the study of immunological reactions to mycobacterial antigens, is known to be a complex mixture of proteins that were isolated from culture media in which tuberculin bacilli were grown. Although useful under many conditions, PPD lacks specificity.

Minden and others (1986) showed that guinea pigs immunized with mycobacteria, including non-tuberculous mycobacteria, had significant reactions to 250 tuberculin units of PPD. Duffield and colleagues (1989), Corner (1981), Auer (1987) have reported on cross-reactivity of PPD with other bacterial antigens particularly other mycobacteria to which test animals had been exposed. Auer (1987) stressed that the tuberculin test suffers from the disadvantage of a 72 hour period separating two procedures with each animal tested and also it lacks accuracy. He reported sensitivity and specificity of 81.8 and 96.3 per cent respectively.

The lines of best fit of the data from day 5 cultures with 5 μg^{-1} and 10 μg^{-1} of PPD were both positive (+1.67 and +0.71). The calculated regression of the percentage responses of the lymphocytes with 5 μg^{-1} and 10 μg^{-1} PPD, and the number of injections was linear, positive and significant ($F = 7.61$, $v_1 = 1$, $v_2 = 16$; $P<0.05$). This finding indicated that following subsequent injection with PPD, lymphocytes derived from the injected cattle responded better than previously. The response to PPD stimulation of lymphocytes increased with the number of injections.

Irrespective of cattle, very significant differences were revealed when day-5 cultures of lymphocytes derived from cattle sensitized with PPD were compared when grown without antigen or with Dc_1 and Dc_2. The *D. congolensis* antigens significantly stimulated the lymphocytes in contrast to the fortuitous findings of Woodman and colleagues (1990) who used lymphocytes from rats sensitized to PPD and *D. congolensis* antigens. The apparent contradiction is perhaps explained by species differences, for example, cattle are often infected with *D. congolensis* whereas, so far as I can ascertain, rats are not naturally infected.

The implication of these findings in tuberculin skin testing of dermatophilosis-infected animals is not clear at the moment. Will *D. congolensis* give false-positive reactions by cross reacting with PPD? One can speculate that some common antigens may be shared by *D. congolensis* and *M. tuberculosis*, to bring about this cross-reaction. As a matter of fact, some relationship probably exists between the two organisms because they both belong to the same order, Actinomycetales, but in different families (Brock, 1979). It is not perhaps surprising therefore that *D.*

congolensis antigen did cause significant stimulation of lymphocytes derived from cattle sensitized to PPD.

Ketterer and colleagues (1981) reported that numerous isolates of mycobacteria other than *M. bovis* have been investigated in cattle for pathogenicity and ability to sensitize to tuberculins. They reported that non-specific reactions to the single caudal-fold tuberculin test in cattle have a variety of causes. For example, infection with *Corynebacterium pyogenes* have been suspected. In their view reactions caused by contact with saprophytic mycobacteria of the *M. avian* complex are a potential problem in the eradication of bovine tuberculosis from northern areas of Australia.

The occurrence of non-specific reactors to the tuberculin test can cause serious problems in a campaign for the eradication of bovine tuberculosis. Further investigations should be carried out to confirm or refute cross-reaction between *D. congolensis* and PPD since tuberculosis is a potential problem in both humans and animals in many areas where cattle are infected with dermatophilosis.

RESPONSE TO TICK SALIVA ANTIGENS

Very, very significant differences were revealed by the Chi-square test between day-5 cultures without antigen and cultures containing tick saliva antigen alone in untreated Zebu-type cattle. This finding indicated that lymphocytes derived from tick-infested Zebu-type cattle responded to an *in vitro* stimulation by tick saliva antigen derived from *A. variegatum*.

In contrast, lymphocytes derived from acaricide-treated Zebu-type cattle, tick-infested N'damas, tick-infested Friesians and acaricide-treated Friesians, were not stimulated by tick saliva antigen *in vitro*. Wikel and others (1978) and Wikel (1982b) found that antigens they isolated from the salivary gland of adult female *D. andersoni* initiated *in vitro* lymphocyte blastogenesis when added to cultures of lymph node cells from guinea pigs which had developed resistance to *D. andersoni*. Our findings that lymphocytes from tick-infested Zebu-type cattle responded to *in vitro* lymphocyte blastogenesis when tick saliva antigen was added to the culture medium confirmed and showed that the earlier observations of Wikel and others (1978) and Wikel (1982b) were applicable to *A. variegatum* - tick-infested zebu-cattle interactions.

Nevertheless, lymphocytes derived from tick-infested N'damas and tick-infested Friesians did not respond to *in vitro* stimulation, contrary to expectation. This implied that both the tick-infested N'damas and the Friesians were not tick resistant. However, it was shown in Chapter 2 that the untreated N'damas were tick-resistant as demonstrated by the low numbers of ticks they picked up during the survey period. The reason why this group of cattle did not respond to the *in vitro* test is difficult to explain. Were they desensitized? Bendyev and Khudainazarova (1976) reported that cows exposed to long, repeated infestations became desensitized to infestation with *Hyalomma asiaticum* (Schulze and Schlottke) but why were the tick-infested Zebu-types not desensitized?

The tick-infested Friesians on the other hand, were unresponsive to the *in vitro* lymphocyte stimulation by tick saliva antigen because they did not develop resistance to tick infestation. During the short period they were kept in the study, tick numbers steadily built upon them (see Chapter 2).

Lymphocytes derived from acaricide-treated Zebu-type cattle and Friesians i.e. control animals not exposed to ticks, cultured in the same medium and with the same concentration of salivary gland antigen were not stimulated. Wikel and colleagues (1978) and Wikel (1982b) made similar findings in their experiment with guinea pigs and *D. andersoni.* They observed a low stimulation index of lymphocytes from control animals and concluded that salivary gland antigen did not possess non-specific mitogenic activity.

In vitro lymphocyte blastogenesis, specifically induced by salivary gland antigen derived from *A. variegatum* was shown in this study to develop in tick-infested Zebu-type cattle. Antigen-specific *in vitro* lymphocyte blastogensis has been proposed as an analogue of cell-mediated immune responsiveness (Oppenheim, 1968); however, B cells are also capable of undergoing *in vitro* antigen specific blastogenesis (Grey *et al.*, 1972). Unfortunately, in the present study, facilities to characterize lymphocytes of T and B cells were not available.

The *in vitro* reaction of peripheral blood lymphocytes derived from tick-infested Zebu-type cattle in an antigen-specific manner to salivary gland antigen derived from *A. variegatum*, indicated the presence of an antigen-reactive cell population.

EXPERIMENTAL INFECTION OF ANIMALS

Although Makinde and Wilkie (1979) demonstrated antibodies in response to crude antigens of *D. congolensis* as early as 5 days during experimental infection of cattle antibodies to *D. congolensis* have not been known to influence development of the lesions of dermatophilosis or to prevent their formation in previously infected animals. Infected animals normally responded to infection by producing antibodies but the levels never corresponded with the severity of the infection (Merkel *et al.*, 1972; Pulliam *et al.*, 1967).

Experimental infections of animals with *D. congolensis* have resolved spontaneously without progressing into the chronic state. A role for antibodies in the termination of experimental infection has been attributed by some workers.

MacAdam (1970) observed that experimentally induced lesions of dermatophilosis lasted 15 days in cattle, 18 days in ponies, 42 days in goats and 30 days in sheep. In the present study lesions in experimentally infected animals resolved spontaneously in 12-18 days after the first infection, 5-16 days after the second infection, 7-15 days after the third infection and 11-21 days after the fourth infection. The second and third infections resolved faster than the first, but the last infection healed at approximately the same time as the first infection. Fast healing of the second and perhaps the third infections indicated an anamnestic response. In contrast, the delay in lesion resolution after the fourth infection suggests that the animals were desensitized after the repeated infections. The animals were infected four times, the second infection being one month after the initial one, then 2 weeks after the second infection and finally 5 weeks after the third infection.

Makinde and Ezeh (1981) had similar results with respect to the healing of lesions. They experimentally infected four cattle with *D. congolensis* and noted that it took 20-25 days for first infection and 10-15 days for second infection to heal. The time of the second healing coincided with the periods the serum antibody titres were high. They concluded that the rapid healing which they observed during the second infection indicated an anamnestic response, which was much higher than the primary one. Resolution of lesions after the second and third infestations in this study showed similar anamnestic responses as observed by Makinde and Ezeh (1981).

Abu-Samra, Imbabi and Mahgoub (1976) experimentally established infection by inoculation of *D. congolensis* on to scarified skin sites in rabbits, calves, goats, sheep, donkeys and camels. In all species the experimental infection remained localised to test sites and healed completely within 20-52 days. Abu-Samra and others (1976) reported that experiment animals were resistant to re-infection with *D. congolensis* by scarification 21 days after the inital infection. They found no lesions on freshly scarified areas and there was no evidence of spread of the original lesions. They concluded that infection may confer some degree of transitory resistance. In the present study, we were able to re-infect the 15 experimental cattle, one month, 6 weeks and 12 weeks after the inital infection. Ellis and colleagues (1987, 1989) observed that the resolution of lesions in Merino sheep commenced 21 to 38 days after the primary challenge with *D. congolensis* zoospores, but resolution began 13 days after a subsequent infection.

Barré (1989) stated that the least understood aspect of the pathogenesis of dermatophilosis is how it becomes generalized to affect the whole body thus inducing loss of condition with eventual death. He pointed out that generalization of infection had not occurred following experimental infection even when factors considered to be involved in the aetiology of the disease, such as rain or tick infestation were reproduced. Tick-infested Zebu-type and N'damas in this study were exposed to ticks over 1½ years and their immune systems were modulated (see Chapter 3). One would therefore have expected that *D. congolensis* infection in immunosuppressed animals should have progressed differently than that in acaricide-treated animals whose immune systems were not immunosuppressed. However, both the acaricide-treated and tick-infested animals recovered spontaneously from the experimental infection. Thus progression of the disease to the generalized state does not depend on immunosuppression alone. Furthermore, generalization of dermatophilosis does not occur in certain breeds (Coleman, 1967; Chodnik, 1956, Barré, 1989). Were the breeds used in the survey resistant to dermatophilosis, especially the acute, generalized form, unlike the Friesians?

The resolution of dermatophilosis lesions in most animals is thought to be mediated by the animals immune system (Roberts, 1967; Lloyd and Jenkinson, 1981). It is puzzling why chronically affected animals are unable to resolve infection. Ellis and others (1989) speculated that chronically infected sheep may have quantitative or qualitative differences in their inflammatory or immunological defence mechanisms when compared with sheep that recovered spontaneously from the disease. Chronic dermatophilosis may result when a range of intervening factors hinder the normal host response such that the spread of infection goes unchecked. Defects in the normal host immune defence mechanisms and interactions of other factors may allow establishment and progression of dermatophilosis.

In vitro responsiveness of lymphocytes derived from tick-infested Zebu-type cattle to the mitogen Concanavalin A were significantly reduced compared to those from acaricide-treated Zebu-types. These findings confirmed the earlier observation in

Chapter 3 that tick infestation caused a degree of immunosuppression in the host. In Chapter 3, it was demonstrated that autologous serum from tick-infested Zebu-type cattle was essential for the immunosuppression. However, with longer periods of tick infestation, immunosuppression occurred in the absence of autologous serum. It is not therefore surprising that active tick control has been shown to be correlated with low prevalence of dermatophilosis.

CHAPTER SIX

GENERAL DISCUSSION

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Four genera of ticks namely *Amblyomma*, *Boophilus*, *Hyalomma* and *Rhipicephalus* were identified on cattle in a survey on the Accra Plains of Ghana. Mohammed (1978) and Bayer and Maina (1984) found the same four genera of ticks in Nigeria. In contrast, only three genera of ticks namely *Amblyomma*, *Boophilus* and *Hyalomma* were identified on cattle on the Accra Plains of Ghana in an earlier survey by the Animal Research Institute (ARI), Achimota, Ghana (1970-71). Five species of ticks including *A. variegatum, B. decoloratus, B. annulata, H. rufipes* and *R. senegalensis* were identified on cattle at the same time in the present survey. In contrast, only three species of ticks namely, *A. variegatum, H. rufipes* and *B. decoloratus* were identified on cattle on the Accra Plains of Ghana by the ARI (1970-71). The genus *Rhipicephalus* and two additional species, *B. annulatus* and *R. senegalensis* were identified in the present study compared to the results of the ARI (1970-71).

The present study also provided information on the relative abundance and distribution on the host of the different species of ticks. *Amblyomma variegatum* was found to be the predominant tick species infesting cattle in the area, with high incidences of infestation in the rainy seasons, a finding in agreement with those of ARI (1970-71) and Bayer and Maina (1984). The prevalence of *A. variegatum* had a highly significant positive correlation with dermatophilosis in four of five local herds studied.

Both *R. senegalensis* and *H. rufipes* significantly correlated with dermatophilosis in a few of the local herds studied. This finding deserves comment because to the best of my knowledge, this is the first report correlating *R. senegalensis* and *H. rufipes* with dermatophilosis. Reports in the literature on tick association with dermatophilosis have invariably incriminated *A. variegatum* as the only tick species involved in the disease process (Plowright, 1956; Chodnik, 1956; Coleman, 1967; Oppong, 1973; Morrow *et al.*, 1989). In the light of this finding, investigations into the role of ticks, other than *A. variegatum*, especially *R. senegalensis* and *H. rufipes*, in the pathogenesis of dermatophilosis are warranted. It is possible that the association noticed in this study between *R. senegalensis* and *H. rufipes* with dermatophilosis was by chance and hence incidental. Nevertheless, Fivaz (1989) demonstrated that *R. appendiculatus* was immunosuppressive to cattle and rabbits and the same activity may be associated with *R. senegalensis*. Moreover, Kusel 'Tan (1967) has isolated *D. congolensis* from *H. asiaticum*.

Amblyomma variegatum was found throughout the year with seasonal peak levels of infestation in April-May and October-November/December. Peak levels of *R. senegalensis* occurred in April whereas *H. rufipes* was found at low levels throughout the year but on occasions, had a small peak in November. These findings contrast with those of Smith (1969) and Kaiser and others (1982) who noted a lack of seasonality in the prevalence of ticks in their studies in Eastern Uganda. Kaiser and colleagues (1982) stated that all species of ticks were present throughout the year with no seasonal variation in abundance of the 2- and 3-host species. They pointed out that yearly rainfall was well distributed averaging 1,400 mm in the area of their study, a factor, which probably accounted for their conclusions.

The seasonal variation observed confirmed the earlier findings of Wilson (1946), MacLeod and others (1977), Mohammed (1978) and Bayer and Maina (1984) of seasonal variation where monomodal rainfall occurred.

The finding that dermatophilosis developed two months after severe tick infestation was not unexpected as it confirmed A.R. Walker's earlier clinical observations (*pers. comm.*, 1991). For the first time, a definite timescale has been established between severe tick infestation and development of dermatophilosis. The significance of this finding is that it will help to formulate the strategy to minimize outbreaks of dermatophilosis discussed later. Lloyd and colleagues (1990) showed that long-acting oxytetracycline can be used to conveniently halt outbreaks of dermatophilosis and allow time for changes in management designed to reduce recurrence of the disease. With the knowledge from the present study that dermatophilosis occurred two months after severe tick infestation, strategic use of long-acting oxytetracycline 4-6 weeks after severe tick infestation, in tick resistant cattle, can halt or minimize outbreaks of dermatophilosis until changes in management such as application of acaricides are effected.

At the present time vaccines against *D. congolensis* are not the answer in the control of dermatophilosis. In the future advances may be made in vaccines against *D. congolensis* and other skin pathogens using new and appropriate technology such as recombinant vector vaccines.

Potentiation of vaccines by non-specific immunodutation has been proposed. For example, Andrew (1991) remarked that cytokines have potential health benefits and that, one, interleukin-2 which stimulates proliferation of T-lymphocytes during an immune response promoting the clearance of virus from animals with depressed immunity, is associated with adjuvant affects in vaccines. The cytokine bovine Y-interferon, has been used to prevent experimentally induced shipping fever in cattle and in the treatment of neonatal diarrhoea. A sensitive sandwich enzyme immunoassay for bovine Y-interferon has been developed and used in the diagnosis of bovine tuberculosis in cattle. Andrew (1991) stressed that present knowledge of immune function is such that it may be possible to design vaccines intelligently and to use cytokines, produced *in vitro* by recombinant DNA technology and drugs to manage the immune responsiveness to animals.

Much of the current activity in the development of improved vaccines centres on the identification and application of epitopes of high immunogenic potential and the incorporation of the relevant genes into suitable non-pathogenic vectors. For example, Makinde and Gyles (1991) analysed the protein composition of *D. congolensis* by the use of sodium dodecyl sulphate-polyacrymide gel electrophoresis (SDS-PAGE) and found that protein profiles of *D. congolensis* can be used to distinguish individual isolates from one another. They stated that defining the immunogenicity of surface exposed proteins of *D. congolensis* may enhance the development of an effective vaccine and help in developing recombinant DNA technique for further studies of *D. congolensis*.

The costs associated with the production or purchase of vaccines and their administration by trained personnel greatly outweigh the cost of application of pour-on acaricides by individual farmers and, therefore, it is not a forgone conclusion that the best method of controlling dermatophilosis in developing countries like Ghana will be vaccination. Vaccines designed to be administered orally e.g. Newcastle disease vaccine can really compete in cost-benefit with pour-on acaricides.

RESISTANT BREEDS OF CATTLE

In the absence of suitable vaccines against *D. congolensis*, the logical measures to be taken in controlling dermatophilosis will be the use of indigenous breeds of cattle which are relatively resistant to ticks and dermatophilosis together with well planned tick control programmes backed up with long-acting oxytetracycline therapy.

The existence of certain cattle breeds more resistant to tick infestation than others has been reported by several workers (Riek, 1962; Wharton, 1976; Utech *et al.*, 1978). Strother and colleagues (1974) demonstrated that fewer larvae, nymphae and adults of *A. americanum* engorged on purebred Brahman than on Hereford cattle. Significant differences in tick resistance within the same breeds have been reported (Riek, 1962; Utech *et al.*, 1978). Riek (1962) observed varying degrees of resistance in pure-bred *Bos taurus* and *Bos indicus* cattle and their crossbreeds, resistance being so highly developed in some animals of each of these groups that very few, if any, ticks matured on them. N'dama cattle are known to be resistant to both ticks and dermatophilosis (MacAdam, 1970; Koney and Morrow, 1990).

In the present study, the N'damas were as expected, less infested with ticks compared to the untreated Zebu-types and the Friesians. Clear-cut differences in innate responses in resistance to ticks and dermatophilosis were observed during the study, the spectrum ranging from highly susceptible Friesians, unsusceptible Zebu-types to resistant N'damas. One of the untreated group of Zebu-type cattle was also more resistant than the other as exemplified by the levels of tick infestation on the two groups, a finding indicating that within the indigenous Zebu breeds some animals are more resistant than others and confirming similar observations (Riek, 1962; Utech *et al.*, 1978; Kaiser *et al.*, 1982). Despite their high resistance to ticks and dermatophilosis unfortunately, N'dama cattle on the Accra Plains of Ghana are shunned by farmers because of their smaller size and lower productivity. Nevertheless, Brumby and Gryseels (1984) reported that ILCA's studies with N'dama cattle in ten African countries have indicated that the growth and reproduction of these animals were much higher than had hitherto been acknowledged.

Hewetson (1972) showed that resistance to tick infestations was heritable. However low productivity of indigenous breeds of cattle on the Accra Plains means it will take a long time to establish nucleus herds of productive cattle through crossbreeding. Nevertheless, from the field experience of the author, some indigenous cattle are high producers as exemplified by cows calving yearly even under the present low management systems. Sandford, Wissocq, Durkin and Trail (1982) reported that the mean calving interval for 357 records from 1974-1979 was 495 $\pm$ 16 days for indigenous cattle they studied in Senegal. Similarly, A.G. Lamorde (*pers. comm.*, 1991), for example, cited a case of a Zebu cow in Nigeria which produced 17 litres of milk per day. Proper management practices including better and adequate nutrition, availability and access to water, control of ecto- and endoparasites and provision of good health care will improve the productivity of indigenous animals.

Unavailability of suitable dry season feed is in my estimation the main constraint in livestock production on the Accra Plains of Ghana. Proper and adequate feeding of livestock should therefore entail fodder conservation and efficient use of under-utilized agricultural by-products such as cocoa pods, waste cocoa beans and cotton seed cake, all of which are in abundance and are currently discarded. Carefully organized breeding programmes can then be fruitfully executed enabling indigenous breeds with high production potential to be selected and used in crossbreeding programmes.

Productivity of livestock depends on their survivalbility because the only useful thing which can be got safely from a dead animal is its hide. In Ghana exotic breeds of animals usually die when they were exposed to severe tick challenge, tick-borne parasites and dermatophilosis (Koney 1984; Oppong 1991). More attention must be focussed on improving productivity of local breeds which are more resistant to diseases which kill imported breeds.

Spickett (1987) pointed out that the use of tick-resistant cattle to control dermatophilosis through population control of ticks will probably not be sufficiently effective on its own and has to be integrated with strategic acaricidal control, taking into consideration factors such as the preservation of immunity through the maintenance of threshold tick numbers.

EXOTIC BREEDS OF CATTLE

Exotic breeds can only be kept successfully in the tropics under management which incorporates regular tick control (Koney, 1984; Callow, 1983). A successful case of a pure-bred Holstein herd in a relatively favourable climatic area at Vom in Nigeria is under the management of the West African Milk Company Ltd., Nigeria (Wamco). The Management Agent is the Dutch Dairy Development BV of the Netherlands. To minimize tick infestation, the management embarked on an intensive tick control programme as a precursor of an intensive disease prevention programme that included dermatophilosis. The cow herd was treated with acaricides every five days utilizing a shuttle programme with two products: an organophosphate[1] in the dry season and a pyrethroid[2] during the rainy season. An intensive fly control programme whereby weekly application of a deltamethrin based pour-on product[3] in combination with a strict cleaning and disinfection programme was also implemented. The cows were on adequate and balanced diets in which grazing rhodes grass pasture was supplemented with maize silage and farm-mixed concentrates.

Although the management were in their third year of operation they were not making profits. To supplement income from milk production, the farm cultivated maize which was sold for human consumption.

Such high capital intensive farming systems have inherent drawbacks with regard to start-up and operational costs, including costs of acaricides and managerial skills.

The question arises whether huge sums of foreign exchange should be spent in importation of acaricides to maintain exotic breeds of cattle or whether it would be cheaper and more profitable to import milk and milk products because developing countries can increase their cereal, e.g. maize and rice production for export after meeting domestic consumption and use some of the income to import milk and milk products which are cheaper than acaricides. Von Massow (1989) explained that developing countries imported dairy products because they were relatively cheap on the world markets and also it was easier to import milk products than to provide them locally, given the state of existing marketing channels and general infrastructures.

Other social and political issues are also raised! Do people need milk and milk products? Can they not get animal protein from other sources such as fish and eggs? Only a handful of well-placed people in society in Ghana regularly consume milk and

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[1]Asuntol
[2]flumethrin, Bayticol
[3]deltamethrin, Spot on

milk products. Von Massow (1989) pointed out that dairy products are not usually considered as basic a staple as, for example, grain and in some African countries including Ghana total milk consumption per person is less than 20 kg per year.

Many herdsmen and cattle owners on the Accra Plains of Ghana are aware of the destructive effect ticks have on livestock such as severe udder damage and tick worry which reduce the milk yields that are the source of livelihood of the herdsmen. The herdsmen are therefore very keen on tick control but they have to depend on acaricides being supplied by city-dwelling cattle owners.

Traditionally tick control is limited to calves and/or milking cows and it is left to the initiative of the herdsmen. Hand dressing is used i.e. acaricide-solutions are applied manually with pieces of cloth to tick attachment sites. The frequent general unavailability of acaricides in the country is also a limiting factor in tick control. A combination of the application of Bayticol at peak levels of tick infestation and manual deticking of selected animals at periods when ticks are not plentiful can be successfully employed to keep tick numbers down to acceptable levels.

STRATEGIC USE OF ACARICIDES IN TICK CONTROL

Spickett (1987) pointed out that the use of tick-resistant cattle to control dermatophilosis through population control of ticks will probably not be sufficiently effective on its own and has to be integrated with strategic acaricidal control, taking into consideration factors such as the preservation of immunity through threshold tick numbers.

Seasonal prevalence of ticks on the Accra Plains of Ghana was established in this study to be in April-May and October-November/December (see Chapter 2). With this knowledge of the periods of the year when ticks are plentiful, it is possible to plan the strategic application of acaricides to coincide with peak tick-infestation levels. For example, an acaricide with long residual activity such as Bayticol should be applied at the beginning of April, mid-May, in mid-October and then mid-November i.e. four times in a year to coincide with periods of severe tick burdens. Hamel and Duncan (1986) used Bayticol in field trials on tick-infested cattle in Zimbabwe and found that application of the acaricide every 2-3 weeks, showed only minor re-infestation rates with the odd flat tick to be counted. Findings in the present study on the use of Bayticol confirmed the earlier field observations of its efficacy by Hamel and Duncan (1986), Hamel (1987) and Hamel and Amelsfoort (1985). It is proposed that for strategic reduction of tick numbers a 4-6 weekly application scheme be used at the height of tick infestation in the rainy season, care being taken to maintain low tick numbers because the above proposed schedule is not an eradication programme. Alternate policies of strategic tick control to maintain enzootic stability were reported by Bigalke (1980) and Norval (1981). Bigalke (1980) asserted that in areas where both *B. bovis* and *B. bigemina* were present, dipping created an unstable situation, particularly for *B. bigemina.* He speculated that although no serological tool was then available to study the epidemiology of heartwater, field observations suggested the existence of situations of enzootic stability and instability similar to those of bovine babesiosis. An identical situation may prevail on the Accra Plains of Ghana. Bigalke (1980) recommended that in order to maintain enzootically stable conditions, it is necessary to farm with tick resistant cattle, an additional advantage being less frequent exposure of ticks to acaricides and consequently a reduced selection pressure for resistance.

DRUG RESIDUES IN ANIMAL PRODUCTS

Drug resistance to acaricides by ticks and drug residues in milk and meat are potential problems associated with intensive use of drugs including acaricides (Brander and Pugh, 1971; Drummond, 1983). Normally when drugs, for example, antibiotics are used on animals, drug withdrawal periods are observed during which time products from treated animals are not sold for human consumption. Wharton

(1976) cited as an example of drug residues, unacceptable acaricide residue levels in animal products which caused the withdrawal of organochlorides.

For fear of drug residue levels in milk or meat, many countries will not import such products from areas where drugs are intensively used and facilities for analysing residue levels are lacking.

The management of "Wamco" claimed that milk samples from their herd were analysed every three months in the Netherlands and no drug residue problems had been encountered.

RESISTANCE TO ACARICIDES BY TICKS

Primary reliance on acaricides for tick control has caused difficulties because broad range acaricide resistance has created serious control problems in many regions in several countries (Brander and Pugh, 1971; McCosker, 1979; Drummond, 1983). Bigalke (1980) reported that resistance to *B. microplus* was a particularly serious problem in Australia and that in South Africa, resistance to a wide variety of pesticides had been encountered on a limited scale on *Boophilus* species. Wharton (1976) lamented that the resistance of ticks to acaricides poses an increasing threat to livestock production in many countries because of their almost complete dependence on acaricides for tick control. He speculated that in Africa tick resistance to acaricides would ultimately lead to severe dislocation of the cattle industry because of the serious problems caused by the three-host ticks, *Amblyomma, Rhipicephalus* and *Hyalomma* and the diseases they transmit. All three genera of ticks Wharton (1976) cited were encountered on the Accra Plains of Ghana in the present study. Single-host ticks such as *Boophilus* present a lesser problem.

Fischer (1983) estimated the cost of discovering and developing a new pesticide to be $ 6.5 M (US) excluding capital investment. Wharton (1976) stressed that the adaptability of ticks limits the commercial return from acaricides and might lead to a situation in which chemical companies would find the development of acaricides not economically viable. Fortunately, since then pour-on acaricides have been developed.

The emergence of resistance to acaricides by ticks (Brander and Pugh, 1971; Wharton, 1976; Bigalke, 1980; Drummond, 1983) and cost of producing new acaricides have focussed attention on the need for alternate methods of tick control that are less dependent on acaricides.

NATURAL OR BIOLOGICAL CONTROL OF TICKS

It was the view of Spickett (1987) that the main drive to use natural or biological means to control ticks has been socio-economic and environmental pressures in the developed world resulting from the use of toxic and increasingly expensive chemicals and the emergence of resistance to acaricides by ticks. In addition, in the developing world there is the impetus of the scarcity of foreign exchange. Spickett (1987) explained that natural control methods have evolved around three main biological entities influencing the fluctuations of tick populations and these include: vegetation and microclimate, host factors and direct effects, including parasites.

The introduction of tick resistant grasses has had some successes. Spickett (1987) stated that research into this area had been aimed more at investigating the tick repellent properties than on large scale assessments of the effects on tick populations. Plant species exhibiting potential as tick deterrents are *Melinis minutiflora* (molasses grass), *Pennisetum cynodon* (gambo grass) (Thompson, Ron and Romero, 1978) and certain *Stylosanthes* species (Sutherst, Jones and Schnitzerling, 1982); all these species are present in Ghana. Spickett (1987) asserted that the *Stylosanthes* species are probably the most effective, possessing glandular trichomes which form viscous secretions incorporating volatile chemicals toxic to tick larvae. Wilkinson (1977) remarked that various ticks are associated with particular types of vegetation presumably because these protect free-living tick stages from the sun and drying winds and thus reduce dessication. Thompson and others (1978) confirmed this statement when they investigated the possible role of anti-tick grasses in maintaining or eliminating field tick populations and found that *M. minutiflora* severely reduced tick populations while *Andropogon* maintained a low tick populations over time. Thompson and co-workers (1978) postulated that it could be possible to combine grass species which have ability to repel larval ticks in tick control system utilizing very limited acaricide applications and good management practices to give low cost, efficient tick control for traditional livestock producers who lack the resources for conventional tick control methods.

To date work done on tick resistant grasses has been with the one host tick *B. microplus*, therefore Spickett (1987) doubted the value of *Stylosanthes* as a direct control of *Amblyomma*, as large nymphal and adult stages are not affected. However, the incorporation of *Stylosanthes* in an integrated biological system would not only decrease the number of *Amblyomma* ticks but would increase the nutritive value of the pasture.

It is important to emulate innovations like planting tick-resistant grasses in developing countries to reduce tick infestation of pastures especially since legumes like *Stylosanthes* have high nutritive value. Unfortunately the present husbandry and traditional management practices on the Accra Plains of Ghana whereby cattle graze on communal lands are not conducive for pasture development. As Innes (1977) correctly pointed out land is under communal ownership and arable land is apportioned to those who need it by a custodian of the land i.e. family heads or traditional rulers. Individual grazing rights to specific areas do not exist and cattle may be grazed anywhere, provided they are kept away from growing crops. The situation in most heavily populated areas is quite different.

The Fulani on the Accra Plains of Ghana recognize that there are natural herbs which are lethal to ticks and therefore in the absence of chemical acaricides, they use these local herbs to control tick infestations. Preliminary field trials conducted by the author to investigate the acaricidal properties of these herbs indicated anti-tick activity. The reports are yet to be published. Sadly, however, further financial support for the project has not been forthcoming.

A major hindrance in tick control is the cost of acaricides and the necessary capital infrastructure i.e dip tanks and spray races. Areas with endemic tick infestation such as Ghana, should exploit the possibility of growing pyrethroids locally by buying the seeds and necessary patent rights. There may be different strains of pyrethroid plants but once successfully cultivated, acaricides and insecticide might be produced locally not only to control ticks but also to control mosquitoes which are tremendously important to human health.

VACCINATION AGAINST TICKS

Several attempts to artificially immunize hosts with tick extracts were reviewed by Willadsen (1980). Ackerman and colleagues (1980) used extracts derived from whole tick and digestive tract antigens from *D. variabilis* to induce resistance in rats. They found no fatality among ticks, feeding on vaccinated animals in their investigation with two or three antigen inoculations but they observed that reproduction of ticks which infested immunized rats were impaired because the fecundity as measure by oviposition and hatchability, was adversely affected. McGowan and others (1980) used an extract from a whole tick homogenate of *A. maculatum* in investigating the resistance of immunized rabbits. They found that ticks fed on immunized animals failed to engorge and suffered a reduction in engorgement weight and egg mass weight. Willadsen (1980) in his review was skeptical about vaccination against ticks and stated that attempts to immunize artificially against ticks have been, at best, partially successful and moreover, none of the methods of immunization used so far were likely to be acceptable and economically sound in a practical situation. Nevertheless, Spickett (1987) was very optimistic about vaccination against ticks based on observations by other workers. He remarked that research into the isolation of specific antigens and consequent vaccination against ticks should be given priority, especially when the possibility of large quantities of antigens by molecular cloning techniques are considered. He stressed that vector control principles are not aimed at eradication but at containing tick populations to low numbers. When vaccination against ticks becomes a reality, a combination of strategic use of acaricides based on the seasonal prevalence of ticks on the Accra Plains established in the present study could be used aimed at containing tick populations to low numbers.

IMMUNOSUPPRESSION

Muneer and others (1988) defined immunosuppression as a state of decreased immune responsiveness to all foreign antigens leading to increased susceptibility to disease agents. They stated that immunosuppressive agents damage the elements of the immune system and may alter the host's protective and immunoregulatory functional balance. In animals immunosuppression may be caused by infectious and non-infectious agents. Some immunosuppressive agents may decrease the susceptibility of the host to a particular pathogen. For example, cyclophosphamide has been found to decrease the resistant of chickens to lymphoid leukosis. Unfortunately, however, these agents may increase host susceptibility to many other infectious agents.

European breeds of cattle infected with *D. congolensis* in Europe usually do not come down with dermatophilosis. Lloyd (1981) demonstrated that Ayrshire cattle which had apparently never shown any clinical symptoms of dermatophilosis, nevertheless possessed antibodies to *D. congolensis*. Friesian cattle or other breeds of cattle in Europe are not so often exposed to the immunomodulating and stressful conditions such as the severe tick infestation, drought and malnutrition seen in West Africa.

Friesian cattle in the present study developed severe generalized lesions four months after they were exposed to tick challenge. The lesions developed fast and progressed rapidly into a generalized fulminating form within one month contrary to the slow progressing chronic lesions characteristic of Zebu cattle on the Accra Plains of Ghana (see Chapter 2).

Massive tick infestation levels coincided with clinical dermatophilosis which also corresponded with poor responsiveness of the tick infested Friesians as measured by *in vitro* Lymphocyte Transformation Test (LTT) in the presence of the mitogen Concanavalin A and confirmed by [^{3}H]-thymidine incorporation of their lymphocytes (see Chapter 3). Wikel and others (1978) and Wikel and Osburn (1982) showed that lymphocytes derived from *D. andersoni* infested guinea pigs were immunosuppressed. Our findings in this study confirm the earlier findings of Wikel and colleagues (1978) and Wikel and Osburn (1982). It is possible that the stress from severe tick infestation alone overwhelmed the immune responses of the Friesians. On the other hand, the innate susceptibility of the Friesians to tick infestation may have been responsible for the low threshold of the Friesians to dermatophilosis.

Acaricide-treated Friesians and Zebu-type cattle were virtually free from ticks and did not develop dermatophilosis confirming an earlier observation in similarly treated cattle on the Accra Plains of Ghana by the author in 1984.

The immune responses of tick-infested Zebu-type cattle and N'damas as measured by lymphocyte transformation tests were initially suppressed by the addition of autologous serum (see Chapter 3) not by FCS. Later the tick-infested Zebus were immunosuppressed using media containing FCS (see Chapter 5), a finding that confirmed the earlier results of Wikel and others (1978) and Wikel and Osburn (1982) in their guinea pig/*D. andersoni* model. Although both the Zebus and N'damas were immunosuppressed, they did not come down with generalized dermatophilosis after repeated experimental infections with *D. congolensis*, indicating that there were other factors in addition to immunosuppression which influenced the establishment and progression of acute generalized dermatophilosis observed in the Friesians in the present study. Serum collected from animals infested with ticks and infested naturally with dermatophilosis in the field suppressed lymphocyte responses of cells from "clean" animals (see Chapter 4), confirming earlier observations that factor(s) in the serum of animals infested with ticks were immunosuppressive (see Chapter 3). Nevertheless, suppression of the immune system of tick-infested Zebu-type cattle and N'damas did not result in severe generalized dermatophilosis after repeated experimental infections. The findings of this study add and support the observations of earlier workers that pathogenesis of dermatophilosis is complex (Ellis *et al.*, 1989; Woodman *et al.*, 1990; Martinez, 1991).

In the past dermatophilosis was more prevalent in West Africa at the onset of the rains in water-deprived, starved and fatigued trade animals being trekked from the Sahel to coastal towns whereas today cattle are trucked to the abattoirs and when slaughtered are in a better condition than those slaughtered 25 years ago (G.R. Scott, *pers comm.*, 1991).

In conclusion, the results of this study point to a complex interplay of multiple factors that include starvation, concurrent diseases, other environmental stresses and stress of ectoparasites play a significant role in the disease dermatophilosis. In particular, *A. variegatum* infestation appears to be the dominant stressor as judged by its modulating effect on the hosts immune systems. The close correlation between *R. senegalensis* and *H. rufipes* with overt dermatophilosis has hitherto not been reported.

Based on established findings of seasonal abundance and distribution of ticks on the Accra Plains of Ghana in the present study, and host susceptibility to ticks, it is recommended that the use of selected indigenous breeds of cattle which are more resistant to ticks than exotic breeds be integrated with strategic use of acaricides to reduce tick infestation to acceptable levels. Thus reducing the prevalence of dermatophilosis. Any tick control measures involving the use of acaricides should take into consideration the effect the control programme will have on enzootic stability. It is proposed that an acaricide with long residual effects such as Bayticol be applied four times a year at the beginning of April, mid-May, mid-October and mid-November to coincide with periods of high tick infestation. Manual deticking i.e. hand dressing should be used in the dry season to supplement use of the Bayticol.

Proper nutrition of cattle, particularly, dry season feeding has been emphasized in addition to exploration of biological means of tick control such as tick-resistant grasses some of which have high nutritional values.

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