Contagious Bovine Pleuropneumonia (CBPP) in the Maasai ecosystem of south-western Kenya: Evaluation of seroprevalence, risk factors and vaccine safety and efficacy.

Niwael Jesse Mtui-Malamsha

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Abstract

Contagious bovine pleuropneumonia (CBPP) is a bovine bacterial disease of major economic importance in sub-Saharan Africa. Vaccination has been recommended to control the disease in endemic areas such as the Maasai ecosystems of Kenya and Tanzania; however, the currently used live attenuated vaccine has been reported to have poor vaccine safety and efficacy. To compare standard (current) and an improved (buffered) version of the live CBPP-vaccine, several epidemiological studies were carried out in Maasai cattle in Kenya between 2006 and 2008. Specifically, the aims were to estimate CBPP seroprevalence at herd and animal level; to identify risk factors for seroprevalence at both levels; to investigate the spatial distribution of seroprevalence; to compare post vaccination adverse events in cattle vaccinated with a standard and a buffered vaccine, and finally to compare efficacy of the two vaccines to induce seroconversion and to prevent development of clinical signs suggestive of CBPP.

A cross-sectional study was carried out in 6872 cattle in 175 randomly selected herds from Loita and Mara divisions. A competitive ELISA revealed that 85% of the herds in the area had at least one seropositive animal and that seropositive herds were harbouring 11% seropositive cattle. A complement fixation test revealed that 46% of the herds had at least one seropositive animal and that seropositive herds were harbouring 4% seropositive cattle. A multivariable logistic regression analysis of the seroprevalence indicated that previous vaccination against CBPP, a history of CBPP outbreaks in the herd, animal age and the location of the herd in the division of Mara were positively correlated to seroprevalence. To investigate the observed difference in herd seroprevalence between the two divisions further, a spatial analysis was conducted. A SatScan test revealed clusters in Mara in areas identified by veterinary personnel as CBPP 'hot spots'. A logistic regression using spatial information identified that location in the midland agro-ecological zone or close to a river and vaccination were positively associated with seroprevalence.

To compare safety and efficacy of a standard and a buffered vaccine, two cohorts of approximately 40,000 cattle were used. The study showed that within 100 days post vaccination, 6.2 cattle per 1000 vaccinates developed adverse events, 4.1 of which were specifically attributable to vaccination and ranging from swelling of the tail to the tail sloughing off. This study revealed a slightly higher incidence of adverse events in cattle vaccinated with the buffered vaccine compared to the standard vaccine. A comparison of the efficacy of the two vaccines revealed that cattle vaccinated with the buffered vaccine had higher odds of seroconversion and lower odds of developing symptoms of CBPP, three and twelve months post vaccination respectively.

The epidemiological studies conducted clearly show wide spread seroprevalence in the Maasai cattle. Given the (spatial) heterogeneity observed, control measures should probably be targeted in areas of increased risk (clusters). However, positive association of vaccination and seropositivity call for better diagnostics tests that can differentiate vaccinated from infected animals. Vaccination with buffered vaccine resulted in increased seroconversion, decreased clinical signs indicative of CBPP post vaccination and low seroprevalence post 'outbreak'. Nevertheless, the increase in adverse events related to the buffered vaccine calls for further research into safer CBPP vaccines.

Declaration

I hereby declare that I composed this thesis and whenever work was performed by other people in the context of the larger project, I was fully involved in the design, fieldwork and analysis. Contributions made by other colleagues are fully acknowledged in the text. This work has not been submitted for any other degree or professional qualification.

Signature

Date:

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Abbreviations

ADRI:	Animal Disease Research Institute
AEZ:	agro-ecological zone
CBPP:	contagious bovine pleuropneumonia
cELISA:	competitive enzyme linked immunosorbent assay
CFT:	complement fixation test
CIRAD-EMTV:	French Agricultural Research Centre for International Development: Animal Production and Veterinary Medicine Department
CVL	Central Veterinary Laboratory
DIVA:	differentiation of infected from vaccinated
EMPRES	Emergency and Prevention System for transboundary animal and plants pests and diseases
FAO:	Food and Agriculture Organisation
Hsp:	herd specificity
IgG:	immunoglobulin subclass G
IgM:	immunoglobulin subclass M
ILRI:	International Livestock Research Institute
KARI:	Kenya Agricultural Research Institute
Kb:	kilobases
*Mmm*SC:	*Mycoplasma mycoides* subspecies m*ycoides* Small Colony
MRI:	Moredun Research Institute
OIE:	The World Organisation for Animal Health
OR:	odds ratio
RBC:	red blood cells
SE:	sensitivity
SP:	specificity
VVPC	Veterinary Vaccine Production Centre

Chapter 1 – General introduction

1.1 Contagious bovine pleuropneumonia

Contagious bovine pleuropneumonia (CBPP) is a transboundary cattle disease of major economic importance at herd and national levels. Annual losses amounting to €30.1 million were estimated in a recent study of the economic impact of CBPP in 12 endemically infected sub-Saharan African countries (Tambi *et al.*, 2006). These losses were attributed to mortality and reduction in beef and milk production and draft power. Further losses were attributed to reduced fertility and lost market opportunities due to trade bans (Karimuribo *et al.*, 1997; Rweyemamu and Benkirane, 1996).

1.2 Causative agent

1.2.1 Classification and general characteristics

Mycoplasma mycoides subspecies *mycoides* Small Colony (*Mmm*SC) is the causal agent of contagious bovine pleuropneumonia. It is an extra-cellular pathogen that lives in close association with the host cells. The organism is capable of self-replication, has a genome size of 1,211 kb and lacks a cell wall (Westberg *et al.*, 2004). *Mmm*SC shares many biochemical, immunological and genetic properties with six other mycoplasmas grouped under the so-called *Mycoplasma mycoides* cluster (Pettersson *et al.*., 1996). Other members of the cluster are *Mycoplasma mycoides* subsp. *mycoides* large colony, *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma capricolum* subsp. *capripneumoniae*, *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma* sp bovine group 7. *Mmm*SC belongs to the Class Mollicutes, Order Mycoplasmatales and Family Mycoplasmataceae. The Family Mycoplasmataceae contains two genera, *Mycoplasma* (urea negative) and *Ureaplasma* (urea positive) (Nicolet, 1996). Sequence analysis and phylogenetic studies of the 16S ribosomal RNA (16S rRNA) genes have shown that mycoplasmas are closely related to the Bacillus-Lactobacillus-Streptococcus group, suggesting that they have descended from Gram positive bacteria (Weisnburg *et al.*, 1989).

Mycoplasmas are highly host specific and adapted to a main host in which they are commonly pathogenic, but may colonize other hosts (Nicolet, 1996). *Mmm*SC, for example, is commonly found in cattle suffering from CBPP but has also been isolated from sheep suffering from mastitis and goats suffering from contagious caprine pleuropneumonia. It is not clear whether presence of *Mmm*SC in sheep and goats was causally associated with the observed diseases (Kusiluka *et al.*, 2000a; Srivastava *et al.*, 2000; Brandão, 1995).

1.3 Mode of transmission

CBPP is a lung disease primarily of cattle (*Bos indicus* and *B. taurus*), no wildlife reservoir has been reported (Provost *et al*., 1987). CBPP transmission is effected through exhalation of infectious aerosols from an infected animal and inhalation by a susceptible animal. The main reservoir of *Mmm*SC infection is cattle, from which bacteria have been isolated during the course of clinical disease (Kusiluka *et al.*, 2000b) and after clinical recovery (Windsor and Masiga, 1977b). Bacteria have been isolated from nasal swabs and sequestra. Trans-placental transmission has been suggested following isolation of *Mmm*SC from the foetus of an infected dam (Masiga *et al.*, 1972; Stone *et al.*, 1969). In addition, *Mmm*SC has been isolated from urine of acutely diseased cattle, with titres ranging between 10^2 and 10^8 organisms per millilitre of urine (Scudamore, 1976). *Mmm*SC has also been isolated from semen and sheath washings of two bulls (Gonçalves, 1994). The importance of infected urine or semen in the natural transmission of CBPP is unknown. Transmission through fomites and contaminated fodder has been suggested under experimental conditions (Windsor and Masiga, 1977a). Although water buffaloes are susceptible to CBPP, transmission of CBPP from buffaloes to cattle has never been reported.

1.4 Disease manifestation

Following infection, the disease may take a variety of forms, depending on host susceptibility and virulence of the pathogen. Calves have been observed to get mild infections involving tendons and joints. Yearlings to three year-old animals show higher susceptibility than calves with lesions characterized by lung involvement (Masiga and

Windsor, 1978). Compared to *Mmm*SC isolated from CBPP outbreaks in Europe, *Mmm*SC isolated from CBPP outbreaks in Africa and Australia have been reported to be highly pathogenic (Pilo *et al.*, 2007; Nicholas *et al.*, 1996).

Peracute, acute, subacute and chronic forms of CBPP have been reported. Peracute cases may die without showing any clinical signs. Acute cases are characterized by rapid and severe pleurisy. Animals show dullness, anorexia and irregular rumination with high fever ranging from 40ºC to 42ºC (Provost *et al.*, 1987). Coughing is usually persistent and is slightly dry. When typical lesions develop, the signs become more pronounced with increasing frequency of coughing. Animals become prostrate or stand with back arched, head extended and elbows abducted due to laboured breathing. Peracute and acute cases are common during the early phase of an outbreak. Sub-acute and chronic forms occur in the majority of infected animals. In these forms, disease progression is slow and may take several weeks or even months before any clinical signs are observed. Articular localization of the pathogens is common in calves, causing lameness. Complications in calves may include endocarditis and myocarditis. In pregnant animals, abortion may occur.

When CBPP is introduced into a fully susceptible cattle population, the disease usually results in high morbidity (up to 100%) and nearly 50% mortality (Masiga *et al.*, 1996). The incubation period is varied. Experimentally infected animals have shown clinical signs of disease (fever) after three weeks (Hudson and Turner, 1963); or between 40 and 57 days *post* challenge (Wesonga and Thiaucourt, 2000). The incubation period after natural infection is not known.

1.5 Diagnosis

CBPP diagnosis relies on a combination of clinical examination, *post-mortem* and laboratory examination based on cultural methods and serological tests (FAO, 2002). Protein and nucleic acid-based molecular techniques are also available (Bashiruddin *et al.*, 1999; Gonçalves, 1994).

1.5.1 Clinical signs

Diagnosis of CBPP on the basis of clinical signs is possible during the acute phase when signs are typical. Adult cattle will present persistent coughing and laboured breathing due to pleuropneumonia while calves show lameness as a result of arthritis (FAO, 2002; Provost *et al.*, 1987). Symptoms are observed to decline in severity in proportion to the time that the disease has been present in the herd or region. No clinical signs are pathognomonic for CBPP. Therefore, it is important to eliminate other diseases presenting similar symptoms such as acute East Coast Fever or Ephemeral fever (FAO, 2002) from the differential diagnosis.

1.5.2 Post mortem lesions

Gross pathological lesions in the acute stage are characterized by fibrinous deposits on the parietal surfaces of lungs and distension of the interlobular spaces with straw coloured serofibrinous exudate (Trichard *et al.*, 1981). Lesions are usually unilateral, localised in the diaphragmatic lobe and present a characteristic marbling appearance (Masiga *et al.*, 1996; Provost *et al.*, 1987). Lesions are detectable on palpation, and upon incision, red and grey areas of hepatisation are revealed. In subacute cases, lesions are characterised by necrosis organized within lobules and interlobular septa and early sequestrum formation. Lesions in the chronic stage are characterised by well-defined sequestra surrounded by fibrous capsules. Adhesions, connecting thickened viscera and parietal pleura, are common (FAO, 2002; Amanfu *et al.*, 2000; Nicholas *et al.*, 1996; Trichard *et al.*, 1981).

1.5.3 Serological tests

The Complement Fixation Test (CFT) and competitive Enzyme Linked Immunosorbent Assay (cELISA) have been prescribed by the World Organization for Animal Health as herd-level serological diagnostic tests. In challenge trials, CFT positive test results have been reported from 7 days (Barber *et al.*, 1970) and up to 5 months post infection (Pearson and Lloyd, 1972).

Sensitivity (SE) of the CFT has been reported to vary with the stage of disease, being high during acute stages of disease when there is a high level of circulating complement-fixing immunoglobulin (Amanfu *et al.*, 2000; Barber *et al.*, 1970). A SE of 98% was estimated in a study from an outbreak area in Botswana, in which *post mortem* lung lesions were used as a gold standard (Amanfu *et al.*, 2000). However, a much lower SE of 64% was estimated in a study from Italy, where due to an eradication programme the disease had very low prevalence (Bellin *et al.*, 1998). A SE of 67% has been reported from an outbreak in the Owambo Mangetti area of Namibia in 1981, in which lung lesions at slaughter were used as gold standard (Trichard *et al.*, 1981).

A cELISA using a specific monoclonal antibody (117/5) has been developed and tested (Le Goff and Thiaucourt, 1998). No cross-reactions with other *Mycoplasma* species were reported during its development, and a relative SE of 96% and specificity (SP) of 97% were reported when CFT results were used as gold standard (Le Goff and Thiaucourt, 1998). Studies on comparison of CFT and cELISA based on macroscopic lung lesions indicated that more than 95% of animals that had lesions were positive in both tests (Amanfu *et al.*, 2000). Animals vaccinated with live attenuated CBPP vaccine mount immune responses, which can be detected by CFT or cELISA (Geiger, 2003; Thiaucourt *et al.*, 2000; Masiga and Mugera, 1973). Based on CFT and cELISA, immune responses following vaccination wane after three months (Thiaucourt *et al.*, 2000). Seroprevalence studies in areas where vaccination has been carried out have indicated a poor agreement between CFT and cELISA (Matua-Alumira *et al.*, 2006).

1.6 CBPP distribution

The chronology of CBPP outbreaks and eradication in different countries has been reviewed (Masiga *et al.*, 1996; Rweyemamu and Benkirane, 1996; Provost *et al.*, 1987). CBPP was widespread in Europe in the 18th century due to uncontrolled cattle movements that were caused by wars, transhumance and trade. The disease was introduced into the USA, Australia, Asia and southern Africa in the 19th century by

importation of infected animals from Europe. East, Central and West Africa are believed to have been infected in the 19th century by cattle from India (Provost *et al.*, 1987). CBPP was eradicated from most of western Europe, the USA and Japan by the end of the 19th and beginning of 20th century through vigorous control efforts involving stamping out and strict control of animal movements (Provost *et al.*, 1987). Some foci of disease remained in Spain, Portugal and Italy and flared up again in the 1980's and 1990's. However, effective control policies made eradication a success (Regalla *et al.*, 1996). Australia succeeded in eradicating the disease in 1973 after the disease being present in the country for 100 years. The control strategy was based on vaccination, animal movement control and implementation of a slaughter policy, backed-up with an efficient surveillance system (Newton, 1992).

According to the OIE, between 2007 and 2008 CBPP was wide spread in 12 countries in Africa (http://www.oie.int/wahis/public.php?page=disease_status_lists as accessed on 2nd April 2009). The countries were Uganda, Tanzania and Ethiopia in East Africa and Nigeria, Niger, Chad, Benin, Ghana, Burkina Faso, Togo, Democratic Republic of Congo and Angola in western and central Africa. CBPP had a zonal distribution as a result of CBPP control in countries such as Burundi, Namibia, Zambia, Kenya and Mali (Thomson, 2005). There are African countries that seem to have managed to eradicate CBPP; Zimbabwe, for instance, last reported the disease in 1904 and South Africa in 1924. Botswana was officially declared free of CBPP in 2008 and Sénégal declared itself provisionally free of disease according to the OIE pathway of declaration of CBPP freedom (http://www.oie.int/eng/info/en_ppcb.htm accessed on 6th April 2009).

1.7 CBPP Control strategies

The USA, Japan and Western Europe managed to eradicate the disease through stamping out coupled with strict animal movement control (Masiga and Domenech, 1995; Provost *et al.*, 1987). Recently, Botswana managed to control and eradicate CBPP through stamping out (Amanfu *et al.*, 1998). However, stamping out may not be economically

feasible in endemic African countries and vaccination is the most frequently used control strategy in combination with animal movement control. To be effective, vaccination must be repeated initially at short intervals and thereafter annually over 3-5 years (FAO, 2002). CBPP vaccination was initially (1920's to early 1970's) based on broth T_1 vaccine Davies *et al*., 1968; Gilbert *et al*., 1970) which was later replaced by freeze-dried live attenuated *Mmm*SC vaccine T_1/44 vaccine (OIE, 2008). A streptomycin resistant variant (T_1sr) was developed and used in combination with Rinderpest vaccine (Rweyemamu *et al.,* 2000). Re-assessment of efficacy of two predominant vaccine strains (T_1/44 and T_1/sr) by means of a challenge trial was carried out following vaccination failures in Botswana (Thiaucourt *et al.,* 2000). The challenge trial was carried out in Cameroon, Kenya, and Namibia to cover the genetic diversity that exists among the pathogenic strains from different geographic areas. Susceptible cattle were vaccinated with either vaccine containing the minimum dose of 10^7 viable *Mmm*SC per dose, as recommended by the OIE (OIE, 2008). The efficacy varied from 33-67% regardless of the strain used. In a similar experiment, T_1/44 provided higher protection (95%) than T_1/sr (80.5%) in cattle vaccinated twice (Wesonga and Thiaucourt, 2000).

Based on a number of controlled experiments, CBPP vaccine efficacy ranged from 40% up to 95% (Thiaucourt *et al.*, 2000; Wesonga and Thiaucourt, 2000; Masiga and Windsor, 1978; Masiga and Windsor, 1974; Karst, 1972; Gilbert *et al.*, 1970; Davies *et al.*, 1968). Estimation of vaccine efficacy was based on the Hudson and Turner scoring system (Hudson and Turner, 1963). However, the method has not been used consistently and comparison of results across trials may not be meaningful (Mariner *et al.*, 2006).

Antibiotic treatment against CBPP is widely used. It is not part of any official control strategy due to suspicion that its use could facilitate developments of sequestra, increase the number of carrier animals, increase development of resistant strains, and mask the occurrence of clinical disease (Provost *et al.*, 1987). Masking of clinical disease will make diagnosis difficult, which may contribute to unrecognized infections and CBPP transmission. Nevertheless, antibiotics are widely used in pastoralist communities (Mariner *et al.*, 2006; Twinamasiko *et al.*, 2004; Msami *et al.*, 2001). At a meeting of

international experts organized by FAO in 2003 it was recommended that chemotherapy be reconsidered for CBPP control. Both in-*vivo* and in-*vitro* studies demonstrating usefulness of antibiotics for treating CBPP have been reported (Hübschle *et al.*, 2006; Twinamasiko *et al.*, 2004; Yaya *et al.*, 2004; Ayling *et al.*, 2000). In an in-*vitro* experiment, tilmicosin, danofloxacin, oxytetracycline, florfenicol and spectinomycin were found to be effective against a variety of strains of *Mmm*SC isolated from CBPP cases that had occurred in Africa and Europe (Ayling *et al.*, 2000). In a study carried out in Namibia, it was demonstrated that naïve animals kept in-contact to denofloxacin treated animals with CBPP had significantly fewer lesions, were less likely to die and to develop clinical disease than naïve animals kept in-contact to untreated animals with CBPP. In the same study, *Mmm*SC was isolated from a limited number of in-contact controls kept with the treated animals suggesting low spread of infection (Hübschle *et al.*, 2006). In a different trial, long-acting tetracycline was demonstrated to be effective in limiting clinical severity of the disease but ineffective in the prevention of persistence of viable *MmmSC* in treated animals (Niang *et al.*, 2007; Yaya *et al.*, 2004). Thus, the direct effect of tetracycline on the individual is positive (less clinical damage), but the indirect effect on the population may be negative (masking of signs leading to transmission).

Infection control methods act by reducing the effective reproductive number of the infectious agent in the population (Tambi *et al.*, 2006). The effective reproductive number is affected by contact between individuals, transmission probability per contact and duration of infectiousness. Animal movement control reduces the contact between infected and susceptible animals. Vaccination and treatment reduce susceptibility and infectiousness, respectively, at the individual level, thus reducing the probability of transmission per contact and reducing the prevalence of infection in the population. Because CBPP is a contagious disease, a reduction in prevalence will result in reduction in incidence thus further reducing prevalence in the population.

For the purpose of implementing different control strategies, Kenya was divided into different zones and zonation has undergone revisions in 1982 and 1998 due to changing

disease situations (Wanyoike, 1999). In 1982, for instance, there were three disease categories; category A (infected areas), category B (areas of risk but not infected) and category C (clean). The control strategy was based on vaccination and animal movement control in category A, animal movement control without vaccination in category B and no CBPP control measures in category C. In 1998, changes in the disease situation led to the formation of three alternative zones, i.e. zone I, II and III representing CBPP clean, recently infected and endemic areas respectively. In CBPP clean areas (Zone I), surveillance was carried out in all slaughter facilities accompanied by zoosanitary measures at livestock markets, borders check points and stock routes (Wanyoike, 1999). Disease control strategies for zone II were disease surveillance and vaccination in the event of a confirmed outbreak. In addition, zoosanitary measures were enforced. For zone III, the strategy was intensive vaccination and zoo-sanitary control measures. Wanyoike (1999) mapped the disease situation in Kenya over a period of ten years (1989-1998) based on suspected and confirmed CBPP outbreaks This information was synthesized and led to revision of zoning into infected, surveillance, buffer and clean zones (Kairu-Wanyoike *et al*., 2004). Guidelines for disease surveillance are outlined in the OIE Terrestrial Animal Health Code. Based on the current version, surveillance for disease involves reporting any signs of disease by veterinary services or livestock owners and an active programme of examination of a statistically determined sample from the host population in order to detect clinical signs or other indications of the occurrence of disease or transmission of infection (http://www.oie.int/eng/normes/mcode/en_chaptre_1.11.8.htm accessed on 9th July 2009). Suspicion of disease should be followed by actions such as submission of samples for laboratory confirmatory tests and enforcement of quarantine. Zoosanitary measures include issuance of cattle movement permits. It is recommended that cattle from infected areas should only be permitted to move to designated abattoirs. Intensive vaccination implied annual vaccination of all animals in the area with a vaccine containing at least 10^8 CFU of *Mmm*SC per dose over a period of 3-5 years.

1.8 CBPP in Maasai ecosystem of Kenya and Tanzania

There is no report of when the disease was first confirmed in Kenya, though it appears that there were suspected deaths as a result of pleuropneumonia-like disease in 1901 around Nairobi. Since then, the disease has spread widely in the country due to uncontrolled movement of infected animals (Kariuki, 1971). CBPP in the Maasai ecosystem was first reported in 1919 and it was widespread in Kenya by 1920 whereas in Tanzania the disease was confined in the Kenya-Tanzania Maasai ecosystem (Lwebandiza, 1969; Kariuki, 1971). By the 1970s, the disease was controlled in most parts of Kenya through quarantine, animal movement control and vaccination. However Maasailand (Kajiado and Narok) and other pastoralist communities remained as enzootic areas (Kariuki, 1971), maybe because of difficulty in controlling animal movement. The disease seems to have been cleared from the Maasai ecosystem, possibly through well-coordinated combined Rinderpest-CBPP vaccination programmes, between 1975 and 1982 when the area was categorized as clean area (Wanyoike, 1999). Re-emergence of the disease in the Maasai ecosystem of Kenya in 1990s did not only affect the Kenyan side but the disease was also re-introduced to the Maasai ecosystem of Tanzania due to illegal cattle movement (Msami *et al*., 2001). Control of animal movement is an important part of CBPP control strategies. Of the outbreaks traced in Kenya for example, 33.9% were found to be related to cattle movements (Wanyoike, 1999). Intensive vaccination, movement control and surveillance were still the major recommended control methods that were given priority when the disease re-emerged in the 1990s (FAO -EMPRES, 1995). The disease has not been cleared from Maasai land and the area is currently categorized as infected (Wanyoike-Kairu *et al*, 2004).

1.9 Aim and objectives of the study

CBPP control through vaccination has been unsatisfactory due to practical difficulties that range from inability to obtain uniform and consistent vaccine coverage to uncontrolled animal movement, poor vaccine efficacy and safety and poor vaccine stability *post* reconstitution (Thomson, 2005; Thiaucourt *et al.*, 2000; Wanyoike, 1999; Revell, 1973). Annual vaccination coverage between 1989 and 1998 in endemic districts

of Kenya ranged from 20 to 60% (Wanyoike, 1999). In Nigeria, overall vaccination coverage of 9.7% was observed between 1988 and 1999 (Aliyu *et al.*, 2000). Average annual CBPP vaccination coverage in endemically infected African countries between 1998 and 2003 ranged from 0.8 to 48% (Thomson, 2005) which is far below the recommended 100% vaccination repeated initially at short intervals (6 months) then annually for 3-5 years.

Field trials for estimation of vaccine efficacy have not been reported. However, based on field observations in the 1990s, it was reported that CBPP vaccines based on T_1/44 were not able to contain outbreaks (Amanfu *et al.*, 1998; Thiaucourt *et al.*, 2000). Low T_1/44 vaccine efficacy was suggested to be caused by sub-optimal titres attained during vaccine production that did not allow for losses during lyophilisation, storage and transport (Rweyemamu *et al.*, 1995). Vaccine culture viability was affected by a drop in pH following metabolism of glucose in Gourlay's growth media by *Mmm*SC (Windsor, 1978). Experiments have shown that inclusion of a buffer system based on 4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) in standard Gourlay's culture media was sufficient to prevent the drop in pH (Waite and March, 2001).

The current study was part of a Wellcome Trust-funded project (Grant No 075804) aimed at developing and validating new technologies for control of CBPP in East Africa. The project was devoted to comparing safety and efficacy of a standard and a buffered vaccine and to estimating socio-economic effects of CBPP control by vaccination. In addition, the project was devoted to developing and evaluating a genetically modified vaccine. This dissertation focuses on CBPP epidemiology and vaccine safety and efficacy aspects of the Wellcome Trust funded project. Two vaccines, a standard vaccine (Contavax®) currently used in Kenya for control of CBPP and a buffered vaccine (March, 2004) were compared. The buffer system in the new vaccine improves and prolongs viability of the *MmmSC* in the vaccine (Waite and March, 2001), and the higher titre of viable organisms in comparison with the standard vaccine is expected to contribute to improved vaccine efficacy.

Several epidemiological studies were carried out in the Maasai cattle of south-western Kenya between 2006 and 2008, including evaluation of seroprevalence before vaccination to establish a baseline and to identify risk factors for CBPP. Specifically, the aims of my work were

i. Estimating CBPP seroprevalence at herd and animal level (Chapter 2)
ii. Identifying risk factors for seroprevalence at both levels (Chapter 2)
iii. Investigating the spatial distribution of seroprevalence at herd level (Chapter 3)
iv. Identifying and quantifying adverse events *post* vaccination (Chapter 4)
v. Comparing *post* vaccination adverse events in cattle vaccinated with a standard and a buffered vaccine (Chapter 4), and finally,
vi. Comparing efficacy of the two vaccines to prevent development of clinical symptoms of CBPP and to induce seroconversion (Chapter 5).

The final chapter of my dissertation (Chapter 6) is devoted to an integrated discussion of results from all preceding chapters and leads to and outlook and recommendations for future work.

1.10 References

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Chapter 2 - CBPP seropositivity and associated risk factor study in the Maasai ecosystem of south-western Kenya

Abstract

A cross-sectional study was carried out aimed at estimating the seroprevalence of contagious bovine pleuropneumonia (CBPP) in the Maasai ecosystem of south-western Kenya, and at identifying risk factors associated with seroprevalence at herd- and animal level. The study involved 175 randomly selected herds in Mara and Loita divisions. Based on competitive Enzyme Linked Immunosorbent Assay (cELISA), 149 (85%) herds had at least one seropositive animal; based on Complement Fixation Test (CFT), 79 (45.7%) herds had at least one seropositive animal. Based on cELISA, animal-level seroprevalence was 11.2% and 9.7% for animals (n = 5963) from seropositive herds (n = 149) and animals (n = 6872) from all herds (n = 175), respectively. Based on CFT, animal level seroprevalence was 4.2 % and 1.9% for animals (n = 3170) from seropositive herds (n = 79) and animals from all herds, respectively.

Identification of risk factors associated with seroprevalence was carried out using multivariable logistic regression with correction for clustering at herd level in animal-level models. Univariable and multivariable analysis for herd-level seroprevalence identified CBPP vaccination as significant risk factor in models based on cELISA or CFT results, with higher risk for higher number of vaccinations. In the herd-level model based on cELISA results, the number of animals tested and division or identification, by herders, of trypanosomosis as one of the most important diseases affecting their animals, were also significant. The latter two variables were highly correlated. The animal-level model based on cELISA showed that division, listing of trypanosomosis among important diseases and age of the animal were significant risk factors for seropositivity, as well as an animal being old enough to have been present at the time of the most recent CBPP vaccination or outbreak. Age-related results suggest that age may be a proxy for accumulated exposure, even when known exposure is accounted for. In the model based on CFT, being old enough to have been present during the most recent CBPP outbreak was the only significant risk factor. The current study shows that agreement between cELISA and CFT test results at animal level was low, and that seroprevalence was significantly higher in herds reporting vaccination. These findings suggest that CBPP diagnosis and identification of risk factors is challenging in areas where vaccination is being practiced. There is a need therefore for research to develop better diagnostic tests, specifically tests that can differentiate vaccination from infection/disease when combined with the use of a DIVA (differentiation of infected and vaccinated animals) vaccine.

2.1 Introduction

A number of epidemiological studies have been conducted to determine CBPP prevalence in endemic countries in sub-Saharan Africa. Using the Complement Fixation Test (CFT), a seroprevalence of 10.5% was reported based on individual samples submitted to the Central Veterinary Laboratory in Kenya between 1988 and 1998 (Wanyoike, 1999). In a separate cross-sectional study of 457 samples collected from Kajiado District in Kenya (Matua-Alumira *et al.*, 2006), CBPP seroprevalences of 14.3% and 4.9% were estimated based on competitive Enzyme Linked Immunosorbent Assay (cELISA) and CFT, respectively. In an abattoir serological survey conducted in CBPP-endemic areas of Nigeria, cELISA and CFT seroprevalences of 32% and 27% , respectively, were observed (Aliyu *et al.*, 2003). In three separate cross-sectional studies, CBPP seroprevalence and risk factors in pastoral systems of Sudan (Zessin *et al.*, 1985; McDermott *et al.*, 1987) and Ethiopia (Bonnet *et al*., 2005) were described. However, such analyses have been lacking for the Maasai ecosystem, which is a transhumant pastoral system. Animals kept under pastoral systems are continuously at risk of contracting infectious diseases such as CBPP because of uncontrolled movement and extensive mixing in grazing, watering and saltlick areas. This is exacerbated by livestock markets, migration in search of pastures and water during dry seasons, and dowry or gift exchanges and other cultural practices (Zessin *et al.*, 1985; Mariner *et al.*, 2006).

The CFT and cELISA are the OIE prescribed herd level tests for CBPP and they are said to have specificity of 98% and 99.9%, respectively; Sensitivity for both tests is said to be about 70%. Vaccinated animals are seronegative by CFT within 3 months post vaccination (http:www.oie.int/eng/norms/mmanual/2008/pdf/2.04.09_CBPP.pdf as accessed on 9th July 2009). According to Thiaucourt and colleagues (2000), immune responses measured by cELISA following vaccination also wane after 3 months (Thiaucourt *et al.*, 2000). No guidelines have been given by the OIE with regard to interpretation of CFT and cELISA results when both tests are performed on the same

samples. Depending on the intention of testing, parallel or serial interpretation can be carried out. Parallel interpretation, i.e. a sample is considered positive if at least one of the tests is positive, could be used when the intention is to increase sensitivity at the cost of specificity. Conversely, serial interpretation, i.e. a sample is only considered positive if both tests are positive, would increase specificity at the cost of sensitivity. (Dohoo *et al*, 2003).

The availability of epidemiological data on CBPP in Maasai cattle would assist in the development of surveillance and control strategies specific for this herding system. The objective of this study was therefore to conduct an epidemiological survey of CBPP in the Maasai ecosystem of south western Kenya. Specifically, the study aimed to:

i. Estimate CBPP seroprevalence at herd and individual animal levels
ii. Identify risk factors associated with CBPP seroprevalence at herd and individual animal levels

2.2 Materials and methods

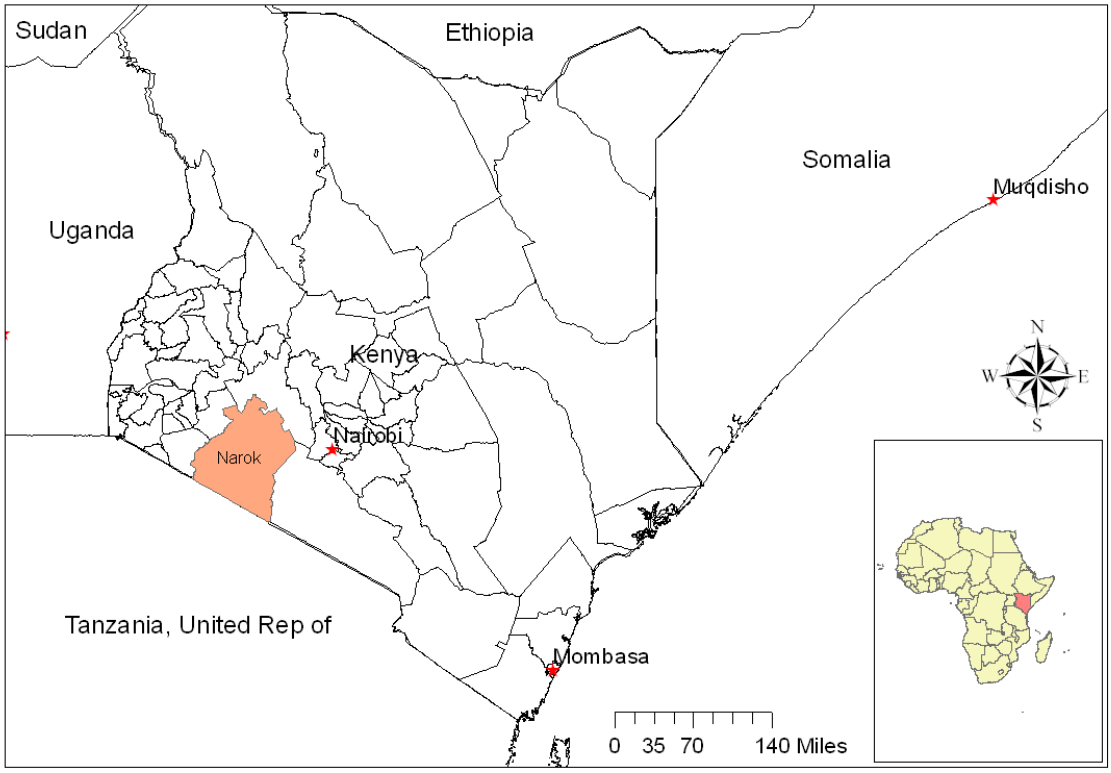
2.2.1 The study area

The study was carried out in the Loita and Mara divisions of Narok District, which is situated in the Rift valley province of south western Kenya (Figure 2.1). The district lies between 34˚45'E and 36˚00'E and 0˚45'S and 2˚00'S (Serneels *et al.*, 2001) and borders the Loliondo district of the United Republic of Tanzania to the south, Nakuru to the north, Kajiado to the east, and Transmara and Bomet to the west. The district occupies 15,098 km^2 and its population is estimated at 365,750 based on a 1999 census (www.statoids.com accessed on 6th April 2009). The area experiences bimodal rainfall with long rains between March and May and short rains between October and December. On average, the area receives between 500-800 mm rain annually, with high local variations. The rains are unreliable and evaporation levels are high (Jaetzold and Schmidt, 1983).

Mara and Loita divisions are inhabited predominantly by Maasai people of two ethnicities, the Purkos and Loitas, whose livelihoods depend largely on pastoral and agro-pastoral activities. Maasai cattle and small ruminants are routinely confined to paddocks known as *bomas* overnight, with kids and calves being accommodated in the same huts (*manyatas*) as their owners. During the dry season, migrations involving cattle more than 2 years old are usually carried out by the *morani* (young warriors) under supervision of elders, whilst the milking herd, sheep and goats remain at the main homestead. The study area was selected because it falls within a nationally designated infected zone (Wanyoike-Kairu *et al*., 2004) and has a history of CBPP outbreaks (H. Kiara, personal communication, 2008).

The current field study was carried out between July and September 2006. Although this is normally the dry period, for this particular year there was prolonged rainfall. As a result, both pasture and water were sufficient and both herders and their animals were quite accessible.

Figure 2.1: Map indicating location of the study district in Kenya



2.2.2 Selection of participants

A cross-sectional survey was carried out on the basis of two sampling frames. One sampling frame consisted of herders registered with the Ilkerin-Loita integral development programme for Loita Division. A similar sampling frame, with herders' names, locations and sub-locations (these are official administrative units) and types and numbers of animals, was developed for Mara Division, where a comprehensive register for herders was not available. OIE recommends the use of CFT or cELISA as herd-level test and suggests that sample size should be adjusted to compensate for low sensitivity of the tests (http://www.oie.int/eng/normes/mcode/en_chapitre_1.11.8.htm; last accessed 14th July 2009). The sample size was calculated using Win Episcope 2.0 (http://www.clive.ed.ac.uk) based on 95% confidence level, 5% absolute precision and

an expected prevalence of 5% at herd level. The number of herds to be sampled in each division was determined by proportional allocation based on number of animals in the area. Within each division, sub-locations were purposively selected to attain a wide geographical representation. Within sub-locations, participants were selected randomly from lists of herders. Sampling units comprised single herds and the sample size for detecting at least one positive animal within the herd was calculated on the basis of an expected within-herd prevalence of 10%, with 95% confidence level. The seroprevalence used was within the range found in the literature (Mariner *et al.,* 2006; Matua-Alumira *et al.,* 2006; Zessin *et al.,* 1985). For the purposes of the study, a herd was defined as the animals kept in one enclosure. Animals in a herd could therefore belong to more than one herder. For each herd, an effort was made to include animals of different ages and both genders. Herders that were included for the current study had not reported CBPP vaccination at least six months prior to the study in order to avoid seropositivity resulting from vaccination.

Fifty-four of the randomly selected herders were excluded from the study because they had migrated, their cattle had been vaccinated within the past six months or because their animals shared a *boma* with another selected herder. Replacements were selected using residual random numbers. If animals of a replacement herder were part of the same *boma*, another herd was chosen from the same vicinity.

2.2.3 Questionnaire development, pre-testing and interviews

A questionnaire was designed to determine herders' knowledge on livestock diseases (particularly CBPP control measures), livestock contacts and movements and herd dynamics. One herder was interviewed for each cattle enclosure. The questionnaire contained mostly closed-ended (categorical) questions to facilitate data analysis, minimize variation and improve precision of responses. Six interviewers fluent in English and Maasai were recruited and trained on use of the questionnaire as a method of data collection. Based on the recommendation that pre-testing should be performed in the same population in which the actual study will be carried out (Thrusfield, 2005),

pre-testing of the questionnaire was carried out in Sekenani sublocation of Mara division between June 26 and July 1, 2006. The performance of the questionnaire was evaluated in terms of the time taken to complete the interviews and the ambiguity, mutual exclusivity and relevance of questions, and modifications were made as necessary. The duration of interview was more than three hours during pre-testing. After modification of the questionnaire, the actual interviews took approximately 70 minutes. The questionnaire was developed, evaluated and carried out in partnership with S. Wanyoike, University of Reading, UK, as part of a multidisciplinary Wellcome-Trust funded project. The questionnaire (Appendix i.) had three sections. The first section was a general one with herders' information, while the second detailed important livestock diseases, CBPP occurrence and control measures. The third section focused on herd demography and cattle contact structure and movements.

Face to face interviews were carried out between July and September 2006 in all selected herds. On arrival at the relevant households, interviewers introduced themselves and outlined the project objectives to the household head before requesting permission to interview. The interviews were carried out in the Maasai language and the responses back-translated into English by the interviewers.

2.2.4 Sera collection and analysis

Four bleeders were recruited and trained on the bleeding protocol to ensure high standards in handling and labelling of samples. For each selected animal, body condition was recorded based on a nine-unit scoring system and scores were collapsed into three categories (good, fair or poor condition). About eight ml of blood was drawn from the jugular vein using sterile 21 gauge needles (Becton Dickinson Vacutainer Systems, Plymouth, UK) and 9 ml plain vacutainer tubes (Greiner Bio-One North America inc, USA), which were labelled with unique herd and animal numbers. Clotted blood was centrifuged at 800g for 5 minutes using a portable centrifuge (MSE, UK) and sera were collected into serum tubes (MS Kay and Company, New Delhi, India). These were stored in a refrigerator for a maximum of 7 days before being transferred in cooler boxes

to Kenya Agriculture Research Institute (KARI), Muguga, Kenya, where they were kept at frozen at -20°C until analyzed.

The serum samples were analysed using the cELISA kit described by Le Goff and Thiaucourt (1998) developed and supplied by CIRAD-EMTV. Briefly, two plates were used for each sample run, the U-bottom (dilution) and *Mmm*SC precoated plates (*Mmm*SC = *Mycoplasma mycoides subsp. mycoides* Small Colony, the causative agent of CBPP). For the plate layout, columns A1-H1 and A2-H2 were used for control samples while test samples were in wells A3 (sample no 1) to H12 (sample no 80). Each of the plates had a strong positive control sample dispensed in wells B1, B2, C1 and C2; weak positive control sample in wells D1, D2, E1and E2; negative control sample in wells H1 and H2. Wells A1 and A2 were for conjugate control while F1, F2, G1, and G2 were for monoclonal control samples.

100 µl of dilution buffer was dispensed into all wells of the dilution plate (Greiner, UK). Then 11 µl of each test sera or control samples were added into wells to give roughly a 1:10 dilution. 110 µl of monoclonal antibody (mab) 117/5 anti-*Mmm*SC (diluted 1:120 in kit dilution buffer) was added to all the wells except A1 and A2 where 110 µl of kit dilution buffer was added. 100 µl of the contents of each well was transferred into the corresponding wells of the *Mmm*SC precoated plate. The plate was then incubated at 37ºC for one hour with gentle agitation. The plate was then washed twice with kit wash buffer, after which 100µl of horseradish peroxidase-conjugated rabbit anti-mouse IgG (diluted 1:100 in kit dilution buffer) was added to each well and the plate incubated at 37ºC for 30 minutes. After a further three washes with the kit wash buffer, 100 µl of TMB substrate (supplied with the kit as ready to use solution) was added and the plates were incubated at 37ºC for 20 minutes. The reaction was stopped by addition of 100 µl 0.5M sulphuric acid; the plates were gently shaken to homogenize the coloured solution. The underside of plate was wiped and optical density (OD) of individual reactions was measured at 450 nm using a plate reader (PR2100, Sanofi, Pasteur diagnostic, USA). The percentage inhibition (PI) value for each sample was calculated by the following formula

$$PI = \{ (OD_{mab} - OD_{testserum}) / (OD_{mab} - OD_{conjugate}) \} \times 100\% \quad [1]$$

OD_{mab} is optical density for the monoclonal antibody; $OD_{testserum}$ is optical density for the test serum; $OD_{conjugate}$ is optical density for the conjugate. All samples that showed a PI of 50% or higher were considered positive for CBPP (Le Goff and Thiaucourt, 1998).

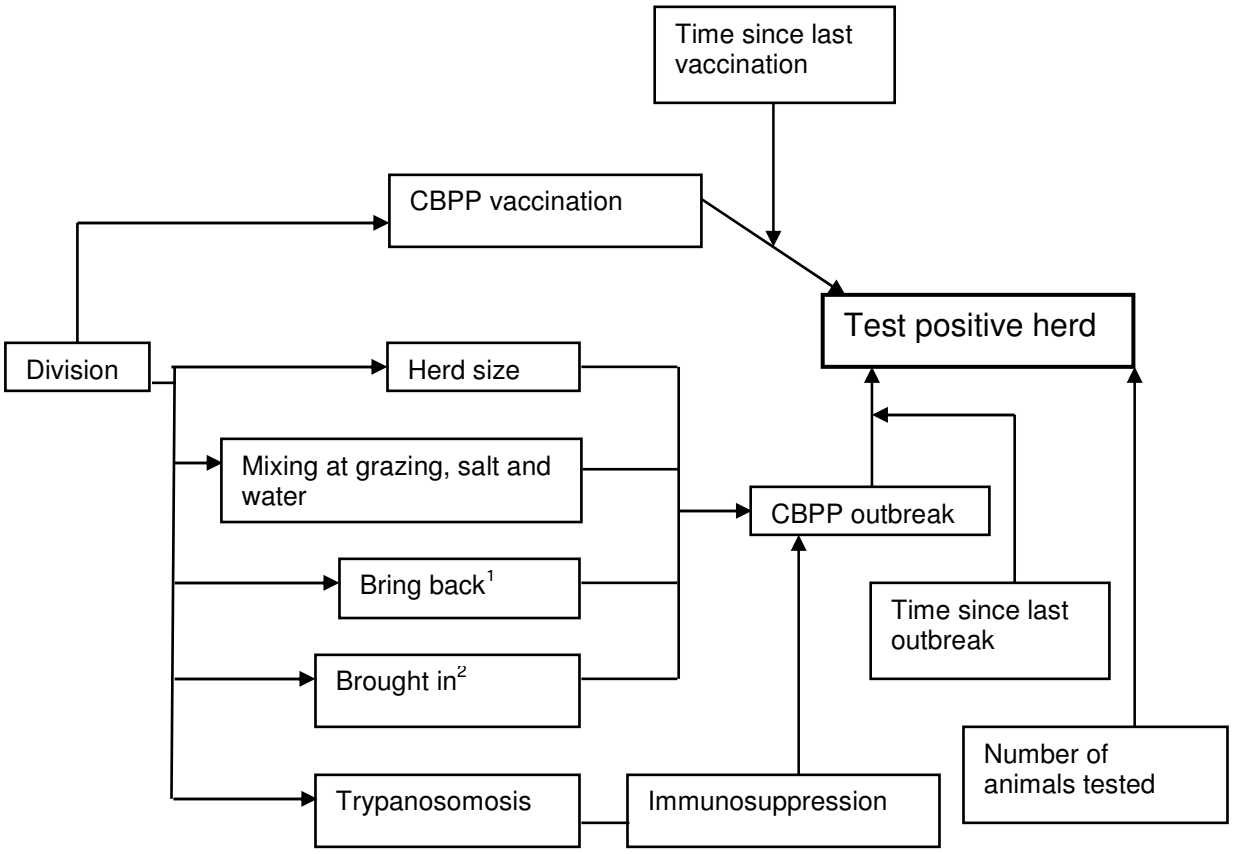
Serum samples were also analysed by CFT. Preparation for CFT commenced at least two days before the test was performed. Fifty ml of sheep blood was collected into graduated bottles containing an equal volume of Alsever's solution and stored at 4ºC for at least 2 days before use. On the day of the test, the blood cells were pelleted at 700g for 10 minutes and washed three times with Veronal buffer containing calcium and magnesium (VCM), with 1 volume of sheep red blood cells (RBC) washed in 9 volumes of VCM. The RBC fraction was then reconstituted in VCM to make a 6% suspension. To this, an equal volume of haemolytic serum (BioMérieux, France) diluted at 14 µl in 10 ml VCM was added to make 3% RBC suspension. The suspension was then incubated for a minimum of 30 min at room temperature, with gentle agitation to sensitize the RBC. Meanwhile, 10 µl of test sera were diluted in 90 µl of VCM buffer to make a 1:10 dilution in U-bottomed 96-well plates. 25 µl of the diluted sera was transferred to the corresponding well of the test plate. To each well of the test plate 25 µl of *Mmm*SC antigen (KARI-Muguga) diluted at 1:40 in CVM buffer (titre of antigen is dependent on the batch), followed by 25 µl of complement (BioMérieux) diluted 1:20 in VCM buffer was added to each well. Plates were then incubated at 37ºC for 30 minutes with gentle shaking, after which 25µl of the sensitized sheep RBC suspension was added to each well. After a further incubation at 37ºC for 30 minutes with shaking, plates were held at 4ºC overnight and results were recorded the following day. Interpretation of the results was in line with recommendations of OIE. Scoring (1-4) was based on subjective assessment of the degree of haemolysis, as evidenced by settling of RBC. With complete inhibition of haemolysis (4), all the cells settle at the bottom of the well and clear

supernatant is seen, whereas in-complete haemolysis (1) results in no sedimented RBC and a red supernatant fluid. Samples that scored 1 or 2 at the 1:10 dilution were considered negative, while those with scores of 3 or 4 at this dilution were re-titrated to obtain end point titres. In titrations, a reading of 3 or 4 was considered positive.

2.2.5 Data analysis

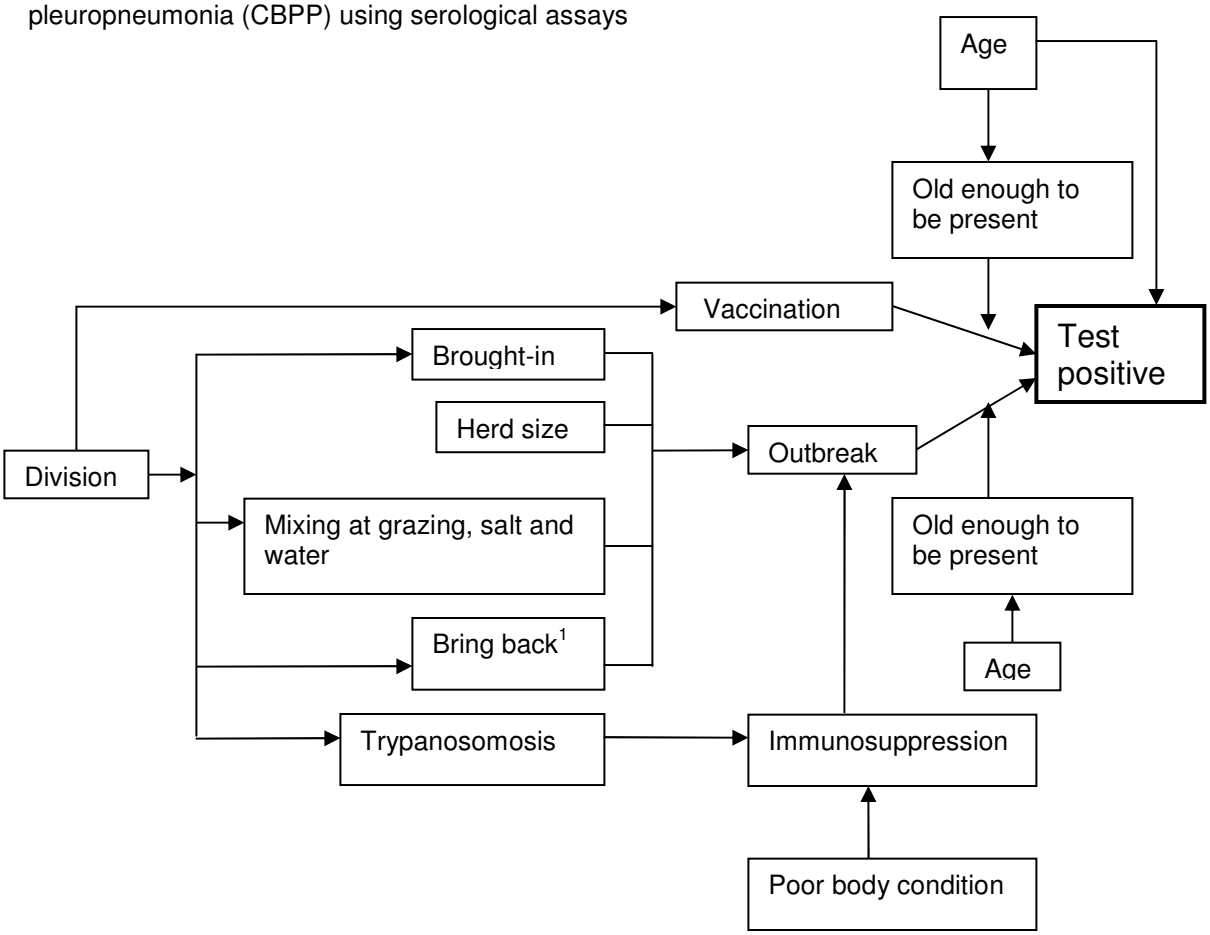
Models were developed to identify factors associated with CBPP seropositivity at herd and animal levels. Separate models were developed for cELISA and CFT tests. Model building was guided by a hypothetical causal diagram (Figure 2.2a and 2.2b) based on a literature review. A history of CBPP outbreaks or vaccination were considered as the primary factor that could result in positive test results (Barber *et al.*, 1970; Niang *et al.*, 2007; Marobela-Raborokgwe *et al.*, 2003; Trichard *et al.*, 1981). Transmission between herds is driven by contact structures such as mixing during grazing, at salt licks and watering points, and by mixing with animals brought back from market (Mariner *et al.*, 2006; Zessin *et al.*, 1985). Trypanosomosis causes immunosuppression, which may make animals more susceptible to disease or unable to mount a good immune response upon vaccination (Ilemobade *et al.*, 1982). Age was used as a proxy measure of accumulated exposure (Boelaert *et al.*, 2005). At individual animal level, it was hypothesised that poor body condition could be associated with CBPP, either as a cause of increased susceptibility to disease or as a result of disease. A herd was defined as test-positive if at least one animal in the herd was test-positive and the herd-level model was corrected for number of animals tested per herd. The dependent variable at animal level was whether the animal was positive or negative for a given test. Two animal level models were developed; the first model included herds with at least one positive animal, based on the assumption that without presence of at least one seropositive animal, there would be no exposure to CBPP and hence no opportunity to identify risk factors for infection. In this model, seropositive test results were thought to be due to infection and not to vaccination, because herds that vaccinated in the past 6 months had been excluded from the study and vaccine titres are claimed to wane 3 months after vaccination. The second model included all herds, based on the idea that serostatus may not be an accurate indicator of exposure, and to allow for consideration of a possible impact of vaccination on serostatus. CBPP outbreak and trypanosomosis reports were based on herders' knowledge of clinical disease and no confirmatory tests were carried out.

Figure 2.2a: Hypothetical herd-level causal diagram for detection of contagious bovine pleuropneumonia (CBPP) using serological assays



1. Bring back (from market) expresses if animals not sold at the market are brought back home (Yes/No)
2. Brought in expresses total number of animals brought into the herd in the past 12 months through purchase, gifts and dowry (0; 1-9; ≥10)

Figure 2.2b: Hypothetical animal-level causal diagram for detection of contagious bovine pleuropneumonia (CBPP) using serological assays



1. Bring back (from market) expresses if animals not sold at the market are brought back home (Yes/No)

The animal level model was corrected for clustering of animals within a herd (Twisk, 2003) as in other studies (Boelaert *et al.*, 2005; Schnier *et al.*, 2004; Zadoks *et al.*, 2001).

Descriptive statistics and model analyses were performed using the STATA statistical package (Intercooled 8th version; StataCorp: Texas, USA). Univariable logistic regression analysis was performed using independent variables selected on the basis of the hypothetical causal diagram. The multivariable model was built by backward elimination with P value set at ≤ 0.10 for retention in the model. Cross tabulations were carried out between independent variables to analyze interdependence among variables

at herd level. Animal-level models were built in the same manner as herd models but the final multivariable model was based on logistic mixed-effect regression with herd included as random effect. The general equation is given by:

$$\text{logit}\,(p)=\beta_0+\sum\beta_i X_i+ b_j + \varepsilon; \qquad [1]$$

where p = outcome (positive or negative test results for cELISA or CFT; separate analyses were performed for the results from each diagnostic test); β_0 = intercept; β_i = regression coefficient for risk factor i; X_i = value of risk factor i; b_j = random effect for herd j included only in the animal model; ε = error term. Regression coefficients were the natural logarithm of the odds ratio (Dohoo *et al.*, 2003). All tests were two tailed and a P value <0.10 was considered significant.

The approximate attributable fraction (AF) was estimated for primary factors of interest i.e. vaccination and outbreak, using a general equation:

$$AF= (OR-1)/OR \qquad [2]$$

where OR = odds ratio, based on the assumption that there is causal relationship (Dohoo *et al.*, 2003).

Herd specificity (HSp) of 65% was estimated based on cELISA specificity of 99%. An HSp of 42% was estimated based on CFT specificity of 98%. The HSp was estimated based on the general equation:

$$HSp = Sp^n \qquad [3]$$

where n = median number of animals tested (43), Sp= specificity of individual test; 0.99 for cELISA and 0.98 for CFT. The latter was estimated based on 33,000 sera from healthy herds that had been tested in Italy (Bellin *et al.,* 1998).

Model diagnostics were based on the detection of herds with undue influence on the model. Each herd was dropped from the animal model and the model was re-run to observe the changes on the estimates (Schnier, 2004). Model diagnostics were performed using the SAS statistical package (SAS 9.1; SAS Institute Inc., Cary, NC, USA).

Agreement of the cELISA and CFT animal-level results was assessed using the Kappa statistic (Dohoo *et al.*, 2003).

Risk factors and categorization of variables that were considered for inclusion in the models are described in Table 2.1.

Table 2.1: Description of variables included in the models for identification of risk factors for contagious bovine pleuropneumonia

Dependent variable	Description
Age	1: ≤1 yr; 2: >1 and ≤2 years; 3: >2 and ≤ 3 years; 4: >3 yrs
Bring back from market	Animals not sold at the market brought back home (Yes/No)
Brought in	Total number of animals brought into the herd in the past 12 months through purchase, gifts and dowry
Body condition	Body condition scores (1: good; 2: fair; 3: poor)
CBPP outbreak	CBPP outbreak reported in the past 10 years (Yes/No)
CBPP outbreak risk	Reflection of an animal being old enough to have been present during last CBPP outbreak (0: absent; 1: present)
CBPP outbreak number	Number of years in which CBPP outbreak in the herd has been observed by herder in the past 10 years (1: none; 2: one; 3: more than one)
CBPP vaccination	CBPP vaccination carried out in the past 10 yrs (Yes/No)
CBPP vaccination risk	Was the animal old enough to have been present during the last CBPP vaccination (0: no; 1: yes)
CBPP vaccination number	Number of years in which CBPP vaccination was carried out in the past 10 years (1: none; 2: one; 3: more than one)
Division	Administrative units (Loita and Mara)
Gender	Animal gender (1: male; 2: female)
Herd size	Herd size (1: ≤42 cattle; 2: 43 to 99 cattle; 3: ≥100 cattle)
Mixing at grazing	Number of herds mixing at grazing point (0: <10; 1: ≥10)
Mixing at saltlick	Number of herds mixing at salt lick point (0: ≤5; 1: >5)
Mixing at water	Number of herds mixing at water point (0: ≤5; 1: >5)
Number of animals tested	Number of animals tested per herd (centred at 39 animals)
Time since last CBPP outbreak	2006 minus year of last CBPP outbreak expressed in years (1: ≤1; 2: 2 ; 3: 3 to 10; 4: not experienced in last 10 years)
Time since last CBPP vaccination	2006 minus year of last vaccination expressed in years (1: ≤1; 2: 2 ; 3: 3 to 10; 4: not experienced in last 10 years)
Trypanosomosis	Trypanosomosis listed among important diseases by herder (Yes /No)

2.3 Results

2.3.1 Study population

In total, 179 herders were interviewed but complete data was available for only 175 of these, i.e. 132 from Mara and 43 from Loita. Herd size ranged from 6 to 450 cattle with a median of 75. The majority of herders (81%) reported vaccinating against CBPP in the past ten years, during which time CBPP outbreaks were reported by 42% of herders. The majority of herders had brought in animals through purchases, gifts or dowry in the year preceding the study (Table 2.2).

Table 2.2: General characteristics of the study herds participating in a survey of contagious bovine pleuropneumonia seroprevalence in the Maasai ecosystem of south-western Kenya

Variable	Levels	Herds (%)	
Bring back from market	Yes	149	(90)
	No	26	(10)
Brought in (animal number)	0	44	(25)
	1-9	79	(45)
	≥10	52	(30)
CBPP outbreak	Yes	74	(42)
	No	101	(58)
CBPP outbreak number	0	101	(58)
	1	63	(36)
	≥2	11	(6)
CBPP vaccination	Yes	141	(81)
	No	34	(19)
CBPP vaccination number	0	34	(19)
	1	108	(62)
	≥2	33	(19)
Division	Mara	132	(75)
	Loita	43	(25)
Herd size	≤42	62	(35)
	43 to 99	49	(28)
	≥100	64	(37)
Mixing at grazing (number of herds)	<10	132	(77)
	≥10	40	(23)
Mixing at salt lick (number of herds)	0 to 5	111	(65)
	>5	61	(35)
Mixing at watering (number of herds)	0 to 5	63	(37)
	>5	108	(63)
Time since last CBPP outbreak (years)	1	23	(13)
	2	13	(7)
	> 2	38	(22)
	no outbreaks	101	(158)
Time since last CBPP vaccination (years)	1	32	(18)
	2	23	(13)
	> 2	86	(49)
	no vaccination	34	(19)
Trypanosomosis	Yes	98	(56)
	No	77	(44)

In total, 6998 animals were bled from the 175 herds, with a range of 2 to 47 animals per herd and a median of 43. A subset of 6872 were analysed by both tests and included in the statistical analysis. The study sample was dominated by zebu animals in fair to good condition. Study animals ranged from 1 month to 16 years in age with a median of 3 years (Table 2.3).

Table 2.3: General characteristics of the study animals participating in a survey of contagious bovine pleuropneumonia seroprevalence in the Maasai ecosystem of south-western Kenya

Variable	**Variable levels**	**Number of cattle (%)**
Animal level variables		
Age (years)	≤1	1172 (17)
	>1 and ≤2	1546 (23)
	>2 and ≤3	1176 (17)
	>3	2978 (43)
Body condition score	Good	3327 (48)
	Fair	2855 (42)
	Poor	690 (10)
CBPP outbreak risk[1]	Yes	1628 (24)
	No	5244 (76)
CBPP vaccination risk[2]	Yes	2515 (37)
	No	4357 (63)
Gender	Male	2357 (34)
	Female	4515 (66)
Herd level variables related to animals		
Bring back from market[3]	Yes	5872 (85)
	No	1000 (15)
Division	Mara	5231 (76)
	Loita	1641 (24)
Herd size	≤42 cattle	2132 (31)
	43 to 99 cattle	1975 (29)
	≥100 cattle	2765 (40)
Trypanosomosis	Yes	4037 (59)
	No	2835 (41)

1. Animal old enough to have been present at the time of most recent CBPP outbreak.
2. Animal old enough to have been present at the time of most recent CBPP vaccination.
3. Bring back (from market) expresses if animals not sold at the market are brought back home (Yes/No)

2.3.2 Herd level seroprevalence and univariable risk factor estimates based on cELISA and CFT

Competitive Enzyme Linked Immunosorbent Assay (cELISA)

Of the 175 herds, 149 (85%) had at least one animal that tested positive by cELISA. Herd seroprevalence was significantly higher in Mara than in Loita (P <0.01) and was also greater among herds that reported CBPP vaccination (90.1%) than those that did not (64.7%). A significant difference (P <0.05) was only observed between herds that had not vaccinated within the last 10 years and those that had vaccinated within the past 12 months (Table 2.4). Eighty nine percent of herds that reported a previous CBPP outbreak and ninety percent of herds that reported CBPP vaccination had at least one cELISA-positive animal. Seroprevalence estimates for herds that had vaccinated twice or more against CBPP were higher than for herds that vaccinated once but the difference was not significant. Increased time since last vaccination was associated with decreasing seroprevalence. Herd seroprevalence was higher in herds that brought in animals but the difference between these and herds that did not bring in animals was only significant in herds that brought in 10 or more animals (P < 0.10).

Complement Fixation Test (CFT)

Based on CFT, 79 of the 175 tested herds had at least one positive animal, resulting in herd-level seroprevalence of 45.7%. Herd seroprevalence in Mara was higher than in Loita, but the difference was not significant. Fifty percent of herds that reported a previous CBPP outbreak or CBPP vaccination had at least one CFT-positive animal. Point estimates for herd seroprevalence were lower for herds that did not report CBPP outbreaks or vaccination, but the difference was only significant for vaccination (P < 0.05). Point seroprevalence estimates for herds that had vaccinated twice or more against CBPP were higher than for herds that had vaccinated once but the difference was not significant. Increased time since last vaccination was associated with decreasing point estimates for herd-level seroprevalence, but differences were non-significant. No other variables were significantly associated with CFT seroprevalence (Table 2.4).

Table 2.4: Herd-level estimates of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results

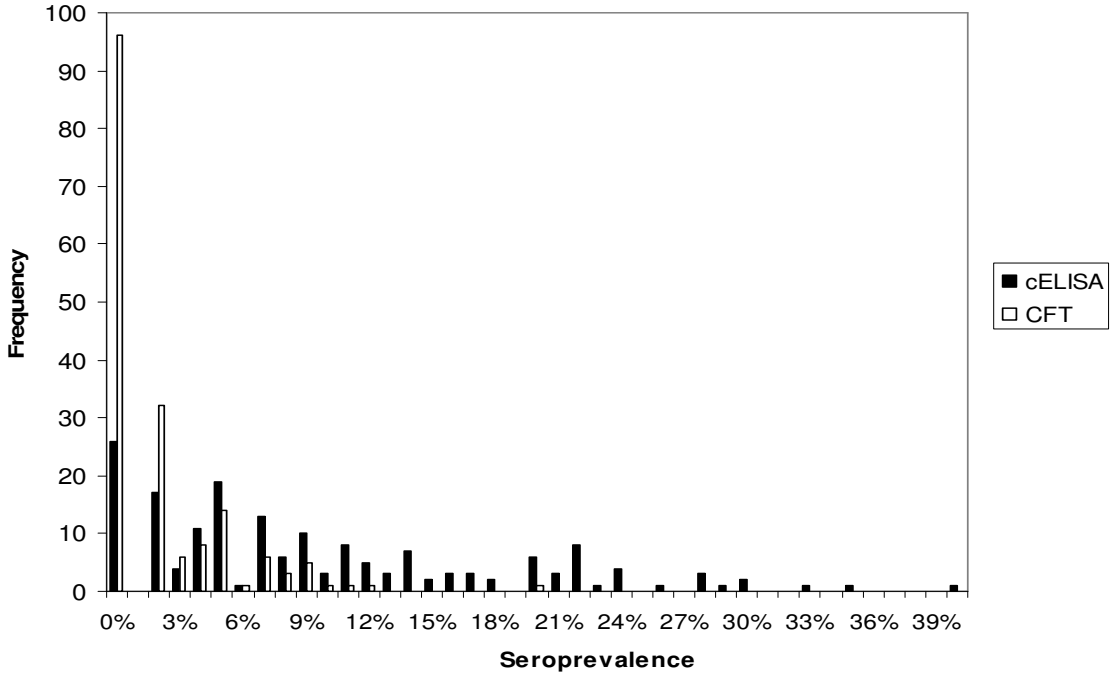
Variable	Number	cELISA Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]	CFT Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]
Herds	175	85.1 (79.8-90.5)	n/a	45.7 (38.3-53.2)	n/a
Bring back from market[2]					
Yes	49	85.9 (80.3-91.5)	1.5 (0.5-4.3)	47.0 (39.0-55.0)	1.4 (0.6-3.3)
No	26	80.8 (65.6-95.9)	Reference level	38.5 (19.8-57.2)	Reference level
Brought in (number of animals) [3]					
0	44	77.3 (64.9-89.7)	Reference level	45.5 (30.7-60.2)	Reference level
1-9	79	84.8 (76.9-92.7)	1.64 (0.64-4.2)	49.4 (38.3-60.4)	1.2 (0.6-2.5)
≥10	52	92.3 (85.1-99.6)	3.5 (1.0-12.2)*	40.4 (27.0-53.7)	0.8 (0.4-1.8)
CBPP outbreak					
Yes	74	89.2 (82.1-96.3)	1.8 (0.7-4.4)	50.0 (37.2-60.0)	1.4 (0.7-2.5)
No	101	82.2 (74.7-89.6)	Reference level	42.6 (32.9-52.2)	Reference level
CBPP outbreaks number					
0	101	82.2 (74.7-89.6)	Reference level	42.6 (32.9-52.2)	Reference level
1	63	88.9 (81.1-96.6)	1.7 (0.7-4.4)	52.3 (40.0-64.7)	1.4 (0.8-2.8)
≥2	11	90.9 (73.9-107.9)	2.2 (0.3-18.0)	36.4 (7.9-64.8)	0.8 (0.2-2.8)
CBPP vaccination					
Yes	141	90.1 (85.1-95.0)	5.0 (2.0-12.1)***	50.4 (42.1-58.6)	2.8 (1.2-6.5)**
No	34	64.7 (48.6-80.8)	Reference level	26.5 (11.6-41.3)	Reference level
CBPP vaccinations number					
0	34	64.7 (48.6-80.8)	Reference level	26.5 (11.6-41.3)	Reference level
1	108	88.9 (83.0-94.8)	4.4 (1.7-11.0)***	48.1 (38.7-57.6)	2.6 (1.1-6.0)***
≥2	33	93.9 (85.8-102.1)	8.5 (1.7-41.6)***	57.6 (40.7-74.4)	3.77 (1.4-10.5)***
Division					
Mara	132	90.9 (86.0-95.8)	4.83 (2.0-11.5)***	47.0 (38.5-55.5)	1.23 (0.6-2.5)
Loita	43	67.4 (53.4-81.4)	Reference level	41.9 (27.1-56.6)	Reference level
Herd size					
≤42 cattle;	62	82.3(72.7-91.8)	Reference level	38.7 (26.6-50.8)	Reference level
43 to 99 cattle	49	87.8 (78.6-96.9)	1.3 (0.5-3.4)	51.0 (37.0-65.0)	1.5 (0.7-3.0)
≥100 cattle	64	85.9 (77.4-94.5)	1.6 (0.5-4.5)	48.4 (36.2-60.7)	1.7 (0.8-3.5)
Mixing grazing					
<10	132	85.6 (79.6-91.6)	Reference level	46.9 (38.5-55.5)	Reference level
≥10	40	82.5 (70.7-94.3)	0.8 (0.3-2.1)	37.5 (22.5-52.5)	0.7 (0.3-1.4)

Mixing at salt lick					
≤5	111	82.9 (75.9-89.9)	Reference level	45.9 (36.6-55.2)	Reference level
>5	61	88.5 (80.5-96.5)	1.6 (0.6-4.0)	42.6 (30.2-55.0)	0.9 (0.5-1.6)
Mixing at water					
≤5	63	81.0 (71.2-90.6)	Reference level	39.7 (27.6-51.8)	Reference level
>5	108	87.0 (80.7-93.4)	1.6 (0.7-3.7)	48.1 (38.7-57.6)	1.4 (0.8-2.7)
Number of animals tested (centred)	n/a	n/a	1.1 (1.0-1.1)**	n/a	1.0 (1.0-1.0)
Time since last CBPP outbreak					
≤ 1 year	23	91.3 (79-102.8)	Reference level	47.8 (0.27-0.68)	Reference level
1 - 2 years	13	84.6 (65.0-104.2)	0.52 (0.06-4.24)	46.1 (19.5-73.3)	0.9 (0.2-3.7)
> 2 and < 10 years	38	89.5 (79.7-99.2)	0.81 (0.14-4.81)	52.6 (36.8-68.5)	1.2 (0.4-3.4)
not experienced in last 10 years	101	82.2 (74.7-89.6)	0.44 (0.09-2.04)	42.6 (32.9-52.2)	0.8 (0.3-2.0)
Time since last CBPP vaccination					
≤ 1 year	32	96.9 (90.8-102.9)	Reference level	50.0 (32.7-67.3)	Reference level
1 - 2 years	23	87.0 (73.2-100.7)	0.22 (0.02-2.21)	65.2 (45.7-84.7)	1.88 (0.62-5.65)
> 2 and < 10 years	86	88.4 (81.6-95.1)	0.25 (0.03-2.00)	46.5 (34.0-57.1)	0.87 (0.39-1.96)
not experienced in last 10 years	34	64.7 (48.6-80.8)	0.06 (0.01-0.49)**	26.5 (11.6-41.3)	0.36 (0.13-1.00)
Trypanosomosis					
Yes	98	90.8 (85.1-96.5)	2.8 (1.2-6.7)**	44.9 (35.6-57.9)	0.9 (0.5-1.7)
No	77	77.9 (68.7-87.2)	Reference level	46.8 (35.6-57.9)	Reference level

1. *P*-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$
2. Bring back (from market) express if animals not sold at the market are brought back home (Yes/No)
3. Brought in express total number of animals brought into the herd in the past 12 months through purchase, gifts and dowry

Within cELISA seropositive herds, seroprevalence ranged from 2% to 40.5% (median 8.6%). Based on CFT, seroprevalence within test positive herds ranged from 2.1 to 20% (median 4%) (Figure 2.3). For both tests, a bimodal distribution of within-herd seroprevalence was observed. A large number of herds had no or only one test-positive animal, shown as within-herd seroprevalence of 0 or 2%, respectively. The remaining herds had a wide range of within-herd seroprevalence with a mode at 5%, which would represent 2 or more seropositive animals, depending on the number of animals sampled within the herd.

Figure 2.3: Frequency distribution of within-herd seroprevalence of contagious bovine pleuropneumonia in the Maasai ecosystem of south-western Kenya based on cELISA and CFT



2.3.3 Univariable animal level risk factor estimates based on cELISA and CFT

Univariable model based on animals in cELISA positive herds

Of the 6872 animals sampled, 5963 were from cELISA-positive herds and 688 were test positive, resulting in an animal-level seroprevalence of 11.2% in seropositive herds. The proportion of cELISA-positive animals was higher among female cattle and animals from Mara than among male cattle and animals from Loita, and seroprevalence increased with increasing age (Table 2.5). When age was evaluated within gender, increasing seroprevalence was associated with increasing age for both male and female cattle. Point animal seroprevalence estimates for animals with fair or poor body condition were higher than for animals with good body condition but the difference was only significant when comparison was made between animals with good and fair body condition ($P < 0.10$). The odds of being test positive were higher for animals of herders that listed trypanosomosis among the five major diseases affecting their livestock; this was also the case for animals old enough to have been present when the herd last had a CBPP outbreak ($P < 0.01$) and for those belonging to herds that used CBPP vaccination ($P < 0.01$). Odds of being seropositive was significantly higher in animals from herds that bring back animals that fail to sell at market and for animals from herds that mix with more than 5 herds at saltlick or 10 herds at grazing ($P < 0.01$).

Univariable model based on cELISA results using all animals selected

Seroprevalence of 9.7% was estimated based on animals from all herds. The risk factor estimates based on all animals are similar to estimates based on herds with at least one positive animal (Table 2.6).

Univariable model based on animals in CFT positive herds

Based on CFT, 3170 of the 6872 animals sampled were from positive herds; of these, 133 were test-positive, resulting in an animal-level seroprevalence of 4.2% in seropositive herds. Animal-level CFT seroprevalence did not differ with division or CBPP vaccination status and was significantly higher among animals that were old

enough to have been present when the herd had last experienced a CBPP outbreak (6.0%) than for animals that were not old enough for that (3.5%). Animal seroprevalence was higher among animals in age categories 3 and 4 than in the age category 1 (Table 2.5).

Univariable model based on CFT results using all animals selected

Animal-level seroprevalence of 1.9% was estimated based on 6872 animals from all herds. The odds of being test positive was significantly higher among animals in Mara division (P <0.10), for animals old enough to have been present when the herd last had a CBPP outbreak (P <0.01), and for animals old enough to have been present at the most recent CBPP vaccination (P <0.05). Mixing at water points was significantly associated with seroprevalence based on CFT (P <0.1) (Table 2.6). Other risk factor estimates were similar to those based on animals from CFT positive herds (Tables 2.5 and 2.6).

Table 2.5: Animal-level estimates of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results (seropositive herds only)

Variable	Number	cELISA Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]	Number	CFT Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]
Animals	5963	11.2 (10.4-12.0)	n/a	3170	4.2 (3.5-4.9)	n/a
Age						
1 month to 1 year	975	3.7 (2.5-4.9)	Reference level	504	2.8 (1.3-4.2)	Reference level
>1 to 2 yrs	1354	6.4 (5.1-7.7)	1.8 (1.2-2.7)***	731	3.1 (1.9-4.4)	1.1 (0.6-2.2)
>2 to 3 yrs	1011	9.5 (7.7-11.3)	2.7 (1.9-4.1)***	547	5.7 (3.7-7.6)	2.1 (1.1-4.0)**
>3 years	2623	17.1 (15.7-18.6)	5.4 (3.8-7.6)***	1388	4.7 (3.6-5.8)	1.7 (1.0-3.1)*
Body condition						
Good	2806	10.3 (9.1-11.4)	Reference level	1519	3.6 (2.6-4.5)	Reference level
Fair	2524	12.0 (10.7-13.2)	1.2 (1.0-1.4)*	1318	4.6 (3.5-5.8)	1.3 (0.9-1.9)
Poor	633	12.3 (9.8-14.9)	1.2 (0.9-1.6)	333	5.4 (3.0-7.8)	1.7 (0.9-2.7)
Bring back from market[2]						
Yes	5123	11.6 (10.7-12.5)	1.4 (1.1-1.8)**	2771	4.3 (3.5-5.0)	1.1 (0.66-2.0)
No	840	8.7 (6.8-10.6)	Reference level	399	3.4 (1.9-5.6)	Reference level
CBPP outbreak risk						
Yes	1472	17.5 (15.5-19.5)	2.1 (1.8-2.5)***	855	6.0 (4.4-7.6)	1.7 (1.2-2.4)***
No	4491	9.1 (8.3-10.0)	Reference level	2315	3.5 (2.8-4.3)	Reference level
CBPP vaccination risk						
Yes	2348	16.5 (15.0-18.0)	2.4 (2.0-2.8)***	1398	4.5 (3.4-5.6)	1.2 (0.8-1.6)
No	3615	7.7 (6.9-8.6)	Reference level	1772	4.0 (3.0-4.9)	Reference level
Division						
Mara	4866	12.1 (11.2-13.0)	1.8 (1.4-2.3)***	2495	4.4 (3.6-5.2)	1.3 (0.8-2.1)
Loita	1097	7.2 (5.7-8.7)	Reference level	675	3.4 (2.0-4.8)	Reference level
Gender						
Male	2039	8.3 (7.3-9.7)	Reference level	1077	4.2 (3.0-5.4)	Reference level
Female	3924	12.6 (11.6-13.6)	1.54 (1.3-1.9)***	2093	4.2 (3.3-5.1)	1.01 (0.70-1.5)
Male						
1 month to 1 year	460	3.5 (1.8-5.2)	Reference level	234	3.42 (1.1-5.8)	Reference level
>1 to 2 yrs	625	5.9 (4.1-7.8)	1.7 (1.1-32)**	327	2.8 (1.0-4.5)	0.8 (0.3-2.1)
>2 to 3 yrs	486	11.3 (8.5-14.1)	3.5 (2.0-6.3)***	264	6.4 (3.5-9.4)	1.9 (0.8-4.6)
>3 years	468	14.1 (10.9-17.3)	4.6 (2.6-8.0)***	252	4.4 (1.8-6.9)	1.3 (0.5-3.3)
Female						
1 month to 1 year	515	3.9 (2.2-5.6)	Reference level	270	2.2 (0.5-4.0)	Reference level

>1 to 2 yrs	729	6.9 (5.0-8.7)	1.8 (1.1-3.1)***	404	3.5 (1.7-5.3)	1.6 (0.6-4.2)
>2 to 3 yrs	525	7.8 (5.5-10.1)	2.1 (1.2-3.6)***	283	5.0 (2.42-7.47)	2.3 (0.9-6.1)*
≥3 years	2155	17.8 (16.2-19.4)	5.4 (3.4-8.5)***	1136	4.8 (3.5-6.0)	2.2 (0.9-5.2)*
Herd size						
≤42 cattle	1781	10.2 (8.8-11.6)	Reference level	785	4.6 (3.1-6.0)	Reference level
43 to 99 cattle	1790	10.6 (9.1-12.0)	1.0 (0.8-1.3)	1057	4.3 (3.0-5.5)	0.9 (0.6-1.5)
≥100 cattle	2392	12.4 (11.1-13.7)	1.3 (1.0-1.5)**	1328	3.9 (2.9-5.0)	0.9 (0.6-1.3)
Mixing at grazing						
<10	4656	10.4 (9.5-11.2)	Reference level	2640	4.0 (3.3-4.8)	Reference level
≥10	1307	14.2 (12.3-16.0)	1.4 (1.18-1.71)***	530	5.1 (3.2-7.0)	1.3 (0.8-2.0)
Mixing at salt lick						
≤5	3725	10.3 (9.3-11.3)	Reference level	2112	4.3 (3.4-5.1)	Reference level
>5	2238	12.7 (11.4-14.1)	1.3 (1.1-1.5)***	1058	4.1 (2.9-5.3)	1.0 (0.7-1.4)
Mixing at water						
≤5	2032	10.2 (8.9-11.6)	Reference level	1047	9.5 (7.6-11.2)	Reference level
>5	3931	11.7 (10.7-12.7)	1.2 (1.0-1.4)*	2123	10.4 (9.1-11.7)	1.2 (0.9-1.8)
Trypanosomosis						
Yes	3711	12.3 (10.7-12.5)	1.6 (1.3-1.9)***	1817	4.3 (3.4-5.2)	1.1 (0.7-1.5)
No	2252	8.7 (6.8-10.6)	Reference level	1353	4.1 (3.0-5.1)	Reference level

1. *P*-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$
2. Bring back (from market) express if animals not sold at the market are brought back home (Yes/No)

Table 2.6: Animal-level estimates of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results (all herds)

Variable	Number	cELISA Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]	CFT Seroprevalence (95% CI)	Odds Ratio (95% CI)[1]
Animals	6872	9.7 (9.0-10.4)		1.9 (1.6-2.3)	
Age					
1 month to 1 year	1172	3.1 (2.1-4.1)	Reference level	1.2 (0.6-1.8)	Reference level
>1 to 2 yrs	1546	5.6 (4.5-6.8)	1.9 (1.3-2.8)***	1.5 (0.9-2.1)	1.3 (0.6-2.4)
>2 to 3 yrs;	1176	8.2 (6.6-9.7)	2.8 (1.9-4.2)***	2.6 (1.7-3.6)	2.2 (1.2-4.2)**
>3 years	2978	15.1 (13.8-16.4)	5.6 (4.0-7.9)***	2.2 (1.7-2.7)	1.9 (1.0-3.3)**
Body condition					
Good	3327	8.7 (7.7-9.6)	Reference level	1.6 (1.2-2.1)	Reference level
Fair	2855	10.6 (9.5-11.7)	1.3 (1.1-1.5)**	2.1 (1.6-2.7)	1.3 (0.9-1.9)
Poor	690	11.3 (8.9-13.7)	1.3 (1.0-1.8)**	2.6 (1.4-3.8)	1.6 (1.0-2.8)*
Bring back from market[2]					
Yes	5872	10.1 (9.4-10.9)	1.4 (1.1-1.8)**	2.0 (1.7-2.4)	1.4 (0.8-2.3)
No	1000	7.3 (5.7-8.9)	Reference level	1.5 (0.8-2.3)	
CBPP outbreak risk					
Yes	1628	15.6 (14.1-17.6)	2.2 (1.9-2.6)***	3.1 (2.3-4.0)	2.0 (1.4-2.9)***
No	5244	7.8 (7.1-8.6)	Reference level	1.6 (1.2-1.9)	Reference level
CBPP vaccination risk					
Yes	2515	15.4 (14.0-16.8)	2.7 (2.3-3.1)***	2.5 (1.9-3.1)	1.6 (1.1-2.2)**
No	4357	6.4 (5.7-7.2)	Reference level	1.6 (1.2-2.0)	Reference level
Division					
Loita	1641	4.8 (3.8-5.9)	Reference level	1.4 (0.8-2.0)	Reference level
Mara	5231	11.3 (9.9-13.7)	2.5 (2.0-3.2)***	2.1 (1.7-2.5)	1.5 (1.0-2.4)*
Gender					
Male	2357	7.4 (6.3-8.4)	Reference level	1.9 (1.4-2.5)	Reference level
Female	4515	10.9 (10.0-11.9)	1.5 (1.3-1.9)***	2.0 (1.6-2.4)	1.0 (0.7-1.47)
Male					
1 month to 1 year	561	2.9 (1.5-4.2)	Reference level	1.4 (0.4-2.4)	Reference level
>1 to 2 yrs	705	5.3 (3.6-6.9)	1.9 (1.0-3.4)**	1.3 (0.5-2.0)	0.9 (0.3-2.3)
>2 to 3 yrs	553	10.0 (7.5-12.4)	3.8 (2.1-6.7)***	3.1 (1.6-4.5)	2.2 (0.9-5.1)*
>3 years	538	12.3 (9.5-15.0)	4.8 (2.7-8.3)***	2.0 (0.9-3.2)	1.4 (0.6-3.6)
Female					
1 month to 1 year	611	3.3 (1.9-4.7)	Reference level	1.0 (0.2-1.8)	Reference level

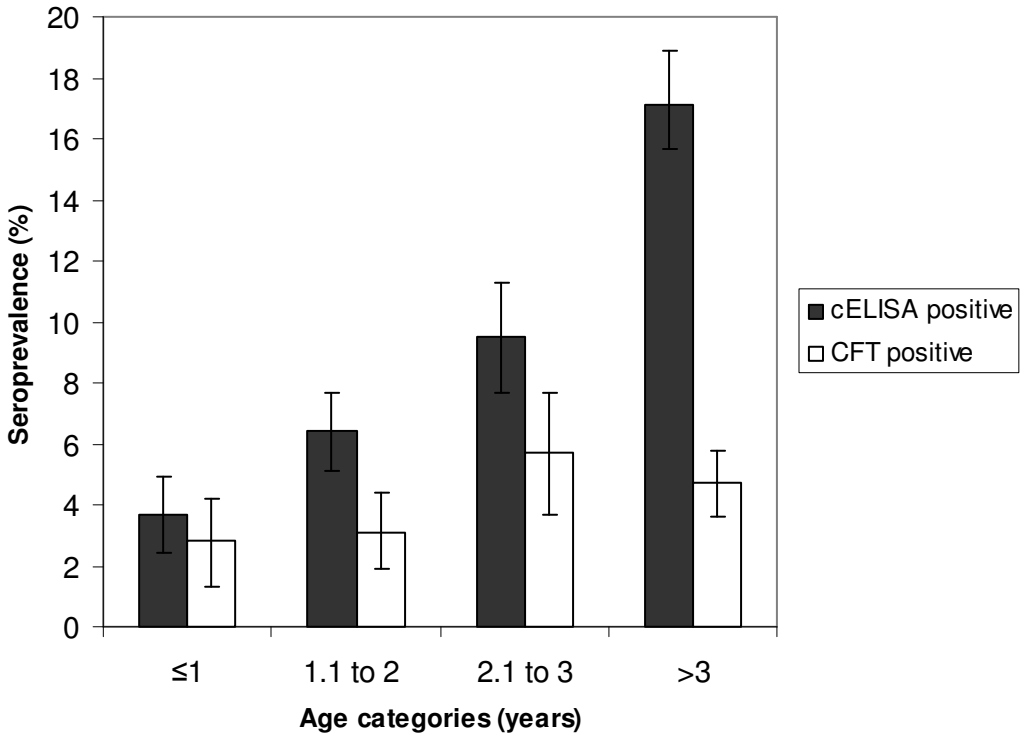
>1 to 2 yrs	841	5.9 (4.4-7.5)	1.9 (1.1-3.2)**	1.7 (6.8-2.5)	1.7 (0.7-4.5)
>2 to 3 yrs	623	6.6 (4.6-8.5)	2.1 (1.2-3.6)**	2.3 (1.1-3.4)	2.3 (0.9-6.1)*
>3 years	2440	15.7 (14.3-17.1)	5.5 (3.5-8.7)***	2.3 (1.6-2.8)	2.3 (1.0-5.3)*
Herd size					
≤42 cattle;	2132	8.5 (7.4-9.7)	Reference level	1.7 (1.1-2.2)	Reference level
43 to 99 cattle	1975	9.6 (8.3-10.9)	1.1 (0.9-1.4)	2.3 (1.6-2.9)	1.4 (0.9-2.1)
≥100 cattle	2765	10.7 (9.6-11.9)	1.3 (1.1-1.6)**	1.9 (1.4-2.4)	1.1 (0.7-1.7)
Mixing at grazing					
<10	5315	9.1 (8.3-9.9)	Reference level	2.0 (1.6-2.4)	Reference level
≥10	1557	11.9 (10.3-13.5)	1.4 (1.1-1.6)***	1.7 (1.1-2.4)	0.9 (0.6-1.3)
Mixing at salt lick					
≤5	4358	8.8 (8.0-9.6)	Reference level	2.1 (1.6-2.5)	Reference level
>5	2514	11.3 (10.1-12.6)	1.3 (1.1-1.6)***	1.7 (1.2-2.2)	0.8 (0.6-1.2)
Mixing at water					
≤5	2498	8.3 (7.2-9.4)	Reference level	1.5 (1.0-2.0)	Reference level
>5	4374	10.5 (9.6-11.4)	1.3 (1.1-1.5)***	2.2 (1.7-2.6)	1.4 (1.0-2.1)*
Trypanosomosis					
Yes	4037	11.3 (10.3-12.3)	1.6 (1.3-1.9)***	1.9 (1.5-2.4)	1.0 (0.7-1.4)
No	2835	7.5 (6.5-8.5)	Reference level	1.9 (1.4-2.4)	Reference level

1. *P*-value indicated by superscript: * P < 0.10, ** P < 0.05, *** P < 0.01
2. Bring back (from market) express if animals not sold at the market are brought back home (Yes/No)

2.3.4 Comparison of cELISA and CFT seroprevalence results

The herd-level seroprevalence based on cELISA was higher than herd-level seroprevalence based on CFT for herds found in both Loita and Mara ($P < 0.05$). At animal level, cELISA seroprevalence was also higher than that based on CFT. Female cattle had a higher seroprevalence than male cattle based on cELISA but not based on CFT. Animal-level seroprevalence based on cELISA was significantly higher in animals old enough to have been present when the herd had last been vaccinating against CBPP, whilst that based on CFT was not. Increasing age was associated with increasing animal-level seroprevalence as detected by cELISA but not CFT (Figure 2.4). Although animals from herders reporting trypanosomosis among important diseases had a higher seroprevalence based on cELISA ($P < 0.01$), the association was not significant when examined with the CFT data.

Figure 2.4: Age seroprevalence profile based on cELISA and CFT for detection of contagious bovine pleuropneumonia among the Maasai ecosystem of south-western Kenya



Of the tested samples, 6103 (89%) were negative for both tests and 32 (0.47%) were positive for both tests, whilst 101 (1.47%) were positive only for CFT, and 636 (9.25%) were positive only for cELISA. The observed test-agreement (89.3%) therefore barely exceeds the test-agreement expected based on chance (88.7%), resulting in a very low value for Kappa (0.05).

2.3.5 Multivariable herd-level risk factor estimation

Based on cELISA, vaccination was significantly associated with seroprevalence. Seroprevalence was also associated with division (Mara) and number of animals tested per herd ($P < 0.05$) (Table 2.7). Although time since last vaccination was significant using the univariable model, it was not so with the multivariable model.

Herd CFT seroprevalence was also positively associated with vaccination. No other variables were significantly associated with seroprevalence (Table 2.7).

Table 2.7: Herd-level multivariable model of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results

Variable	cELISA OR (95%CI)[1]	CFT OR (95% CI)[1]
CBPP vaccination number		
0	Reference level	Reference level
1	2.6 (1.0-7.1)*	2.6 (1.1-6.2)**
≥2	3.8 (0.7-20.7)	3.8 (1.3-10.9)**
Division (Mara) [2]	3.4 (1.3-8.9)**	
Number of animals tested (centred)[3]	1.0 (1.0-1.1)**	1.0 (0.990-0.997)

1. OR = odds ratio. *P*-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$
2. Division is significantly correlated with CBPP vaccination. When the variable Division is omitted from the model then both levels of vaccination number, i.e. once or more than once, become significant ($P < 0.05$)
3. Number of animals tested was based on the difference between actual number tested from the median number of animal tested per herd

Cross-tabulation to assess interdependence between variables showed that reporting trypanosomosis as important disease and Mara division were positively correlated ($P < 0.01$), as were trypanosomosis and reporting CBPP vaccination, and reporting vaccination and outbreak (Table 2.8).

Table 2.8: Cross tabulation of herd variables included in models to predict seroprevalence of contagious bovine pleuropneumonia in the Maasai ecosystem of south-western Kenya[1]

	CBPP outbreak (Yes)	**CBPP vaccination (Yes)**	**Trypanosomosis (Yes)**	**Division (Mara)**
CBPP outbreak (Yes)		26.77	2.94	1.28
CBPP vaccination (Yes)	0.000		18.06	22.32
Trypanosomosis (Yes)	0.087	0.000		24.81
Division (Mara)	0.258	0.000	0.000	

1. Upper right half of the table shows Chi square statistic and lower left the *P*-values.

In addition, Mara division was positively correlated with higher numbers of herds mixing at salt licks and watering points and increased number of animals brought-in through purchases and gifts (Table 2.8).

Table 2.8 (continued): Cross tabulation of herd variables included in models to predict seroprevalence of contagious bovine pleuropneumonia in the Maasai ecosystem of south-western Kenya [1]

	Division (Mara)	**Mixing at salt** (>5 herds)	**Mixing at grazing** (>10 herds)	**Mixing at water** (>5 herds)	**Brought-in** (≥10 animals)	**Bring back from market** (Yes)
Division (Mara)		6.63	0.58	4.08	4.93	3.18
Mixing at salt(>5 herds)	0.01		9.27	13.12	9.70	1.87
Mixing at grazing (>10 herds)	0.44	0.002		21.63	6.41	0.22
Mixing at water(>5 herds)	0.04	0.000	0.000		5.37	0.36
Brought-in in[2](>10 animals)	0.03	0.008	0.04	0.07		2.91
Bring back from market[3](Yes)	0.08	0.17	0.07	0.55	0.23	

1. Upper right half of the table shows the Chi square statistic and lower left the p-values.
2. Brought in expresses total number of animals brought into the herd in the past 12 months through purchase, gifts and dowry
3. Bring back (from market) expresses if animals not sold at the market are brought back home (Yes/No)

2.3.6 Multivariable animal-level risk factor estimation

Multivariable model based on animals in cELISA positive herds

Based on cELISA, increasing age and being old enough to have been present during the last CBPP vaccination were positively associated with seroprevalence (Table 2.9). When trypanosomosis was dropped from the model, division of origin was significantly associated with seroprevalence, whilst trypanosomosis was significantly associated with seroprevalence when division was dropped from the model.

Multivariable model based on cELISA results using all animals selected

Increasing age, being old enough to have been present during the last CBPP vaccination and being old enough to have been present during the last CBPP outbreak were positively associated with animal-level seroprevalence based on the multivariable model

with all animals (Table 2.10). In addition, animals from herds reporting trypanosomosis and animals from herds originating from Mara were positively associated with cELISA seroprevalence.

Multivariable model based on animals in CFT positive herds

Based on CFT, being old enough to have been present during last CBPP outbreak was significantly ($P < 0.10$) associated with seroprevalence (Table 2.9). No other variable was significantly associated with animal level CFT seroprevalence.

Table 2.9: Animal-level multivariable model of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results (seropositive herds only)

Variable	cELISA OR (95% CI)	CFT OR (95% CI)
Age		
2	1.6 (1.1-2.4)** [2]	
3	2.4 (1.6-3.6)***	
4	4.1 (2.8-6.2)***	
CBPP outbreak risk (Yes)	1.4 (1.1-1.8)***	1.7 (1.2-2.5)*
CBPP vaccination risk (Yes)	1.3 (1.0-1.7)**	1.0 (1.0-1.0)
Trypanosomosis (Yes) [1]	1.3 (0.9-1.7)	
Division (Mara) [1]	1.4 (0.9-2.0)	

1. When variable trypanosomosis was dropped out of the model, division (Mara) became significant ($p<0.05$) and when division was dropped from the model then trypanosomosis became significant ($p<0.05$).
2. *P*-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$.

Multivariable model based on CFT results using all animals selected

The multivariable model based on all animals indicated that animal being old enough to have been present during last CBPP outbreak was significantly ($P < 0.05$) associated with seroprevalence. Originating from a herd mixing at salt licks with five or more herds was protective whilst mixing with five or more herds at watering points increased the risk of being seropositive (Table 2.10)

Table 2.10: Animal-level multivariable model of risk factors predicting prevalence of contagious bovine pleuropneumonia based on cELISA and CFT results (all herds included)

Variable	cELISA OR (95% CI)[1]	CFT OR (95% CI)[1]
Age		
2	1.6 (1.0-2.4)**	
3	2.3 (1.5-3.6)***	
4	4.0 (2.7-6.0)***	
CBPP outbreak risk (Yes)	1.4 (1.1-1.9)**	2.0 (1.3-3.1)
CBPP vaccination risk (Yes)	1.4 (1.1-1.8)**	1.4 (0.9-2.1)
Trypanosomosis (Yes)	1.3 (1.0-1.8)*	
Division (Mara)	2.0 (1.3-3.0)***	
Mixing at saltlick		0.7 (0.4-1.1)*
Mixing at water		1.8 (1.1-2.9)**

1. *P*-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$.

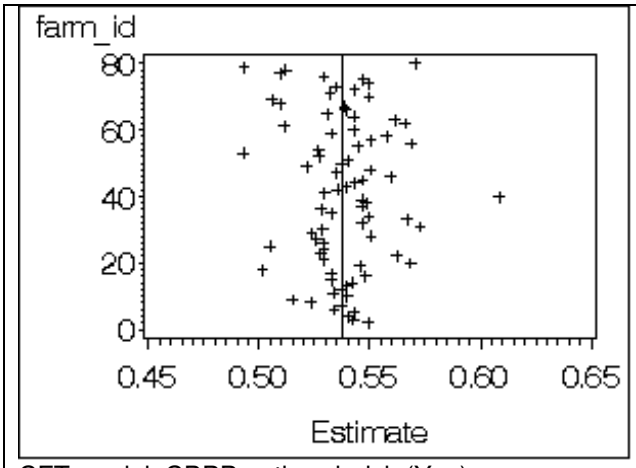
Attributable fraction

Based on animals from seropositive herds the proportion of cELISA seroprevalence that was attributable to exposure to previous vaccination and outbreaks were estimated at 30% and 24%, respectively, while for CFT seroprevalence estimates of attributable fraction were 0% and 41%, respectively. Based on all animals, the proportion of cELISA seroprevalence that was attributable to previous exposure was estimated at 29% for both vaccination and outbreaks, while those for CFT seroprevalence were 50% and 29% for vaccination and outbreaks, respectively. Values reported for AF are based on OR-values and do not take into account whether or not the risk factors were significantly associated with seropositive results in multivariable analysis.

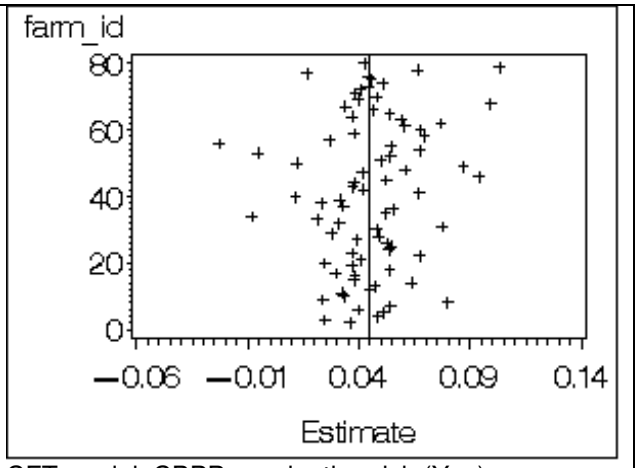
2.3.7 Model diagnostics

Changes in the herd-level estimates were observed when the model was re-run with one herd omitted at a time. A small number of herds (n = 4) from the Mara division caused considerable changes in the animal-model estimates based on cELISA results after they were deleted from the data (Figure 2.5).

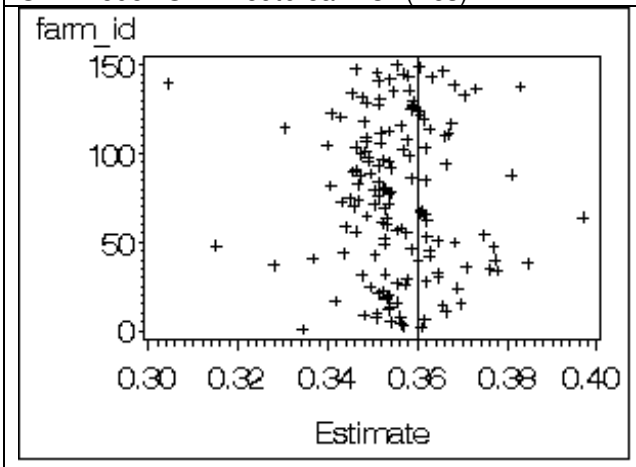
Figure 2.5: Model diagnostics indicating influential herds[1]



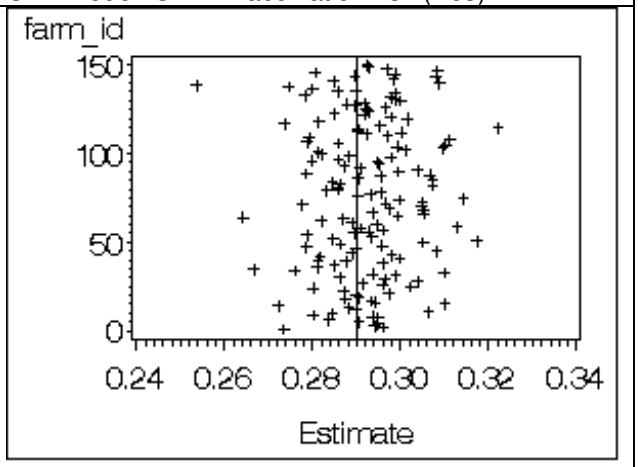
CFT model: CBPP outbreak risk (Yes)



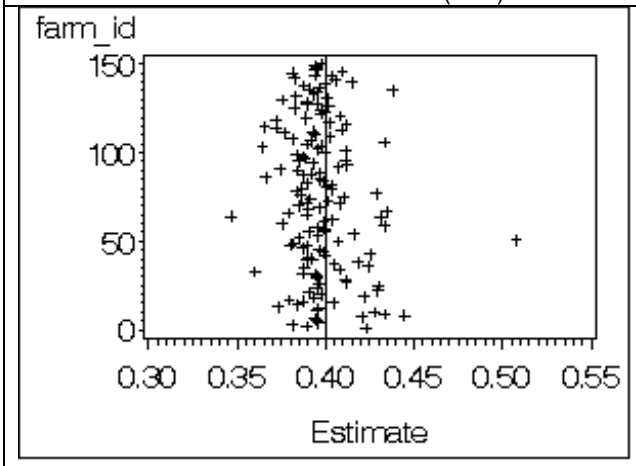
CFT model: CBPP vaccination risk (Yes)



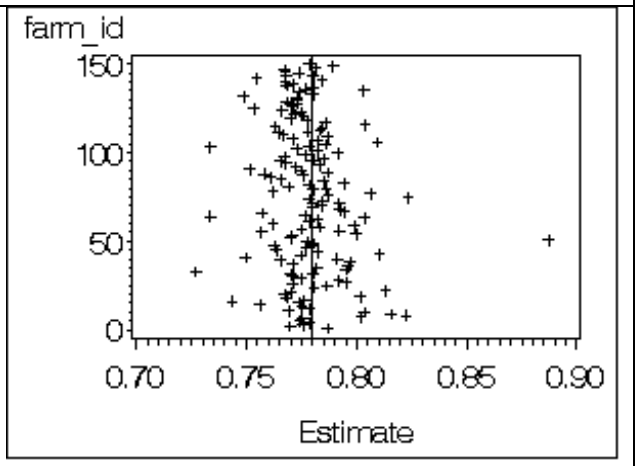
cELISA model: CBPP outbreak risk (Yes)



cELISA model: CBPP vaccination risk

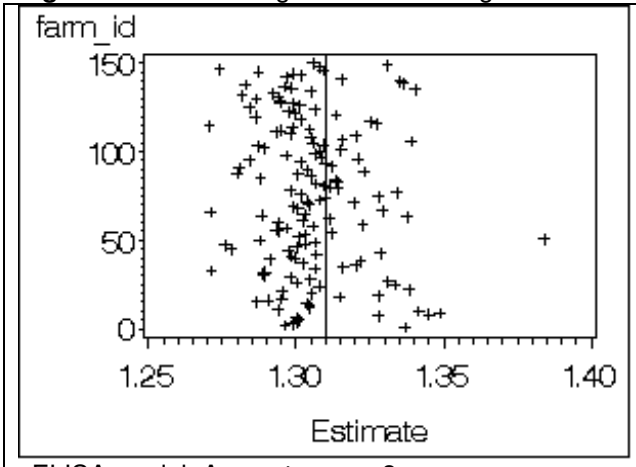


cELISA model: Age category 1.1 to 2 years

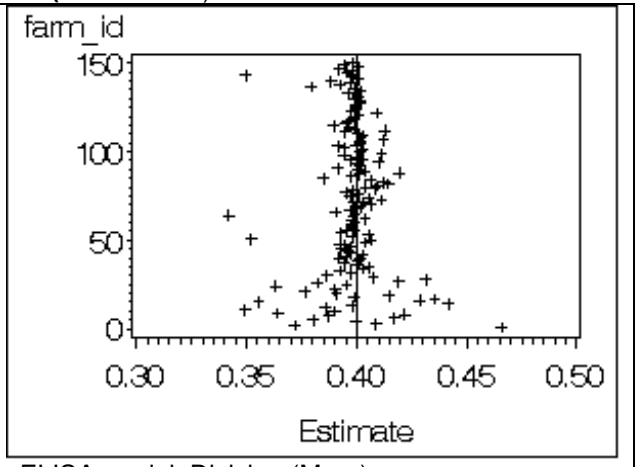


cELISA model: Age category 2.1 to 3 years

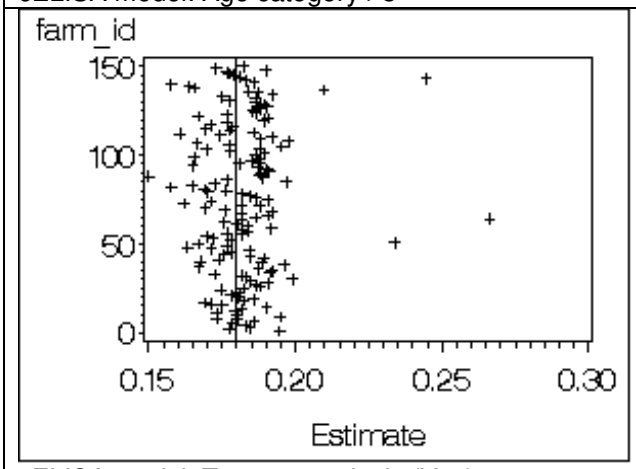
Figure 2.5: Model diagnostics indicating influential herds[1] **(**continuation)



cELISA model: Age category >3



cELISA model: Division (Mara)



cELISA model: Trypanosomiasis (Yes)

1. Each symbol shows the results of the model after one herd is deleted from the dataset. The estimate is based on the final animal-level model (Logit (p)=β_0 + Vaccination + Outbreak + Age(category 2-4) + Trypanosomosis + Division + b_j + ε and Logit (p)=β_0 + Vaccination + Outbreak + b_j + ε based on cELISA and CFT respectively.; where b_j = herd effect ; ε=error term. The diagnostics were based on the model with herds that had at least one positive animal.

2.4 Discussion

Only three cross-sectional studies have been reported to estimate CBPP seroprevalence and risk factors in pastoral systems (Bonnet *et al*., 2005; McDermott *et al.*, 1987; Zessin *et al.*, 1985), each of which focused on Ethiopia and Southern Sudan. The current cross sectional study aimed to provide an estimate of CBPP seroprevalence and associated risk factors in pastoral Maasai ecosystems of south-western Kenya.

2.4.1 Seroprevalence estimates

Reports on herd level CBPP seroprevalence based on the cELISA are limited. A cross-sectional study in the Ethiopian Highlands using cELISA found herd seroprevalence of 4.6% (Bonnet *et al*., 2005), much lower than 85% observed in the current study. However, our observed CFT seroprevalence lies within the range reported for the pastoral system of southern Sudan under circumstances where vaccination had been practiced in the four years prior to the study (Zessin *et al.*, 1985). At herd level, an association was evident between seroprevalence and previous CBPP vaccinations. The study area was classified as "recently infected" in 1998 and, in 2003, reclassified as "infected" and hence vaccination and animal movement control was being practised (Wanyoike, 1999; Wanyoike-Kairu *et al*., 2004).

Based on herds with at least one positive animal, animal level seroprevalences of 11.2% and 4.2% were estimated for cELISA and CFT, respectively. Animal seroprevalence of 9.7% and 1.9% based on cELISA and CFT, respectively, were estimated based on animals from all herds. The estimated CFT seroprevalence were lower than the 10.5% seroprevalence reported for 13,251 samples submitted to the Central Veterinary Laboratory in Kenya between 1988 and 1998 (Wanyoike, 1999). Further, CFT seroprevalences of 8.1% (sample size = 1037) and 8.3% (sample size = 7036) were reported in two separate studies carried out in southern Sudan (Zessin *et al.*, 1985; McDermott *et al.*, 1987). An abattoir serosurvey conducted in CBPP endemic areas in Nigeria estimated much higher seroprevalences of 32% and 27% based on cELISA and CFT, respectively (Aliyu *et al.*, 2003). The high animal CFT seroprevalences reported in these studies could be attributed to the non-random nature of sample selection in these

studies. For example, the higher seroprevalences observed in Kenya may be due to the fact that the samples were taken from animals suspected of CBPP (Wanyoike, 1999), and the high estimates in Nigeria could be influenced by sick animals being sent for slaughter. The CFT animal seroprevalence estimate in the current study compares well with animal infection prevalence predicted for a pastoral system by a CBPP transmission model (Mariner *et al.*, 2006). Furthermore, a seroprevalence of 4.9% was recently estimated in Kajiado, Kenya using CFT (Matua-Alumira *et al.*, 2006). The current study may have underestimated animal-level seroprevalence in the population because the age distribution of animals included in the seroprevalence study was not representative of the population as a whole. Based on the vaccine study (Chapters 4 and 5) it was observed that the population consisted of 22% young animals (≤3 years) and 78% adult animals (>3 years). However, in the seroprevalence study 56% of the sampled animals were young indicating that they were overrepresented compared to the population as a whole. Since the young animals had a lower risk of being seropositive than adult animals this may have resulted in underestimation of the seroprevalence.

Agreement between CFT and cELISA was poor indicating that the two tests might be detecting different isotypes (Amanfu *et al*., 2000). CFT is said to be detecting IgM and possibly IgG_1, which are able to fix complement, and cELISA is detecting IgG_2 and possibly IgG_1. Poor test agreement implies that serial interpretation of test results would lead to very low seroprevalence estimates. Therefore, separate models were developed for CFT and cELISA results in this study.

2.4.2 Risk factor estimation

Higher seroprevalence was associated with herds reporting previous CBPP vaccination. Vaccinated animals are expected to mount an immune response which could be detected by CFT or cELISA (Masiga and Mugera, 1973; Geiger, 2003; Thiaucourt *et al.*, 2000). Antibodies detectable by CFT have been reported as early as 5 days post vaccination and may persist for 5 months (Masiga and Mugera, 1973).

At the herd level, reported CBPP outbreaks were not significantly associated with seroprevalence. Reports of the persistence of antibodies following naturally occurring CBPP outbreaks were not observed during writing of the thesis. Nonetheless, in challenge trials, CFT positive test results have been reported from 7 days post infection (Barber et al., 1970) up to 5 months post infection (Pearson and Lloyd, 1972). Persistence of antibodies for up to 19 months post challenge has been reported based on indirect ELISA (Onoviran and Taylor-Robinson, 1979). The lack of an association of seroprevalence with previous outbreaks may arise from a masking effect of vaccination, since all herds that reported outbreaks also vaccinated. Another possible reason could be under-reporting of CBPP by the herders. This could be the case when animals experience subclinical disease, which is not noticed and reported by herders. Herders are capable of recognizing clinical signs but subclinical disease is defined by the absence of overt signs and may pass unnoticed (Zessin *et al.*, 1985; AU/IBAR, 2002).

The current study established that seroprevalence was significantly higher in Mara than in Loita. This may be attributable to differences between the two divisions in contact structure. Contact structure, herd size and seasonal herding practices have been reported to influence CBPP disease distribution patterns (Mariner *et al.*, 2006 ;Zessin *et al.*, 1985). In this study, risk factors describing aspects of contact structure (mixing at watering points and salt licks, animals brought into herds) were considered and Mara division was characterized by a greater number of herds mixing at salt lick and watering areas, a greater tendency to bring back animals that fail to sell at market and a larger number of animals brought in through purchases and gifts. In univariable analysis based on cELISA, expected high risk strata for these factors were indeed associated with increased seroprevalence, but in the multivariable analysis, which included division, they were not significant. Conversely, contact structure-associated risk factors were not significant in univariable analysis based on CFT, but some were significant in multivariable analysis.

Age was positively associated with seroprevalence as detected by both cELISA and CFT, which may be explained by the fact that increasing age is a surrogate measure of

repeated exposure (Boelaert *et al*., 2005). Similar findings were reported based on CFT analysis of samples from Sudan (Zessin *et al.*, 1985; McDermott *et al.*, 1987). However, the age variable was still highly significant in the multivariable model, where exposure as a result of vaccination and outbreaks were accounted for. In addition to exposure to vaccination and clinical outbreaks, age could be indicating cumulative exposures to subclinical disease or infection with other *Mycoplasma* species that might cross-react with *Mmm*SC (Regalla, 1995). In the current study, female gender was significant in the univariable analysis, but not when other variables were accounted for in the multivariable model, as it was highly correlated with age. Similar findings were reported in other studies (Zessin *et al.*, 1985; McDermott *et al.*, 1987).

It was also observed that seroprevalence was positively associated with reported occurrence of trypanosomosis. This was unexpected because trypanosomal infection has been reported to have an immunosuppressive effect in cattle immunized against CBPP (Ilemobade *et al.*, 1982). Trypanosomosis may be a proxy of division (Mara) or *vice versa* since the two were significantly correlated.

The slight difference observed in the models based on seropositive herds and all herds could be attributed to the difference in structure of the two "populations". The model based on cELISA seropositive herds only included a greater number of herds from Mara than Loita and thus created increased correlation between Mara and some variables that were highly reported in Mara (e.g. trypanosomosis). When all herds were included in the model, trypanosomosis and Mara division were independently associated with seroprevalence.

The fact that CBPP is an infectious disease means that a true positive herd is likely to have more than one seropositive animal unless it is at the onset of the outbreak or a seropositive animal has just been brought into a clean herd. Figure 2.3 shows a bimodal distribution, with a mode at 0% within-herd seroprevalence and a second mode at 5% within herd seroprevalence. It is possible that herds with 2% seropositive animals (i.e. a

single animal) primarily represent false positive test results, whereas the second mode primarily represents a group of true positive herds, most of which would have multiple infected animals. The second mode appears to be surrounded by a normal to right-skewed distribution starting at within-herd seroprevalence of 3%. When this value, i.e. ≥ 3% of animals within a herd tested positive, was used as cut-off to define seropositive herds the same risk factors were significant in the herd-level model, and their significance level improved (results not shown).

2.5 Conclusion and recommendations

The study has estimated herd-level CBPP seroprevalences of 85% and 45.7% based on cELISA and CFT, respectively, in the study herds in the Maasai ecosystem of Kenya. Animal seroprevalences of 11.2% and 4.2% were estimated based on cELISA and CFT respectively, which may be an underestimate because young animals were overrepresented in the sample.

The study shows that seroprevalence is significantly higher in herds reporting vaccination. This finding suggests that accurate CBPP diagnosis using serological assays is complicated in areas where vaccination is being practised. In addition, poor agreement between serological assays was observed. There is therefore a need for better diagnostic tests, specifically tests that can differentiate vaccination from infection/disease.

The fact that some important risk factors, such as previous occurrence of CBPP outbreaks were not significantly associated with seroprevalence may indicate inadequacy of data based on herders recall, correlation between CBPP outbreaks and vaccination resulting in masking of the effect of outbreaks, or false-positive results at herd level due to the fact that herd-level specificity was compromised by multiple testing for the sake of herd-level sensitivity. To reduce probability of false positive herds, the cut-off value for the number of positive animals required for a herd to be declared positive could be set at more than two animals.

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Chapter 3 - Spatial distribution of Contagious Bovine Pleuropneumonia in the Mara and Loita divisions of south-western Kenya

Abstract

A spatial analysis was conducted to investigate the difference observed in herd seroprevalence of contagious bovine pleuropneumonia (CBPP) between Mara and Loita divisions of south-western Kenya. Herds were categorized as CBPP seropositive using two criteria, i.e. presence of at least one seropositive animal in the herd based on cELISA, or positive cELISA results for at least 3% of animals tested. Irrespective of cut-off selected, a spatial scan statistic revealed CBPP clusters situated in the Mara division, a finding consistent with the increased seroprevalence based on the seroprevalence study (Chapter 2). The clusters identified based on the two diagnostic criteria at herd level showed partial overlap. Herds that were included in both clusters were found in areas identified as CBPP 'hot spots' by veterinary personnel.

Results from a logistic regression model showed that variation of seroprevalence in the two divisions was partly explained by agro-ecological zone and proximity to river with higher seroprevalence among herds located in the midland zone and near the river (water source). Additionally, the scan statistic proved to be a useful method for identifying CBPP 'hot spots'. The high correlation between vaccination and Mara makes it difficult to determine whether the hot spot reflects a centre of infection or vaccination. The results further emphasize the need for developing a DIVA vaccine and corresponding diagnostic tests.

3.1 Introduction

Spatial concepts were first developed by a Russian parasitologist, Pavlovsky in the 1930's describing what he called 'landscape epidemiology'. He stated, "Geographical variation in disease occurrence could be attributed to variations in the underlying biological or physical conditions which are required to support the pathogens, vectors and hosts and their probability of close encounter. If these biotic and abiotic factors were known and could be delineated, it could be possible to predict current and future distribution of a disease" (Pavlovsky, 1966).

"Disease maps provide a visual summary of complex geographic information and identify subtle patterns in the data that are missed in tabular presentations" (Elliot and Wartenberg, 2004). For example, mapping of CBPP outbreaks and cattle movement suggested an association between cattle movements and the maintenance and spread of the disease (Masiga *et al.*, 1996). Mapping of CBPP outbreaks between 1988 and 1989 in Kenya made it possible to report spread of disease into districts previously unaffected based on visual comparison of maps for different years (Wanyoike, 1999). Advancement of technology for remote sensing and geographical information systems (GIS) has enabled identification of areas with increased risk, formulation of hypotheses about disease aetiology and assessment of needs for health resources (Pfeiffer *et al.*, 2008). The production of attractive and informative disease maps complements spatial statistical analysis and results in larger visual impact on recipients than the accompanying statistics (Rezaeian *et al.*, 2004).

A number of clustering and cluster detection methods provide enhanced detection of areas of increased risk (Besag and Newell, 1991; Kulldorff and Nagarwalla, 1995; Openshaw *et al.*, 1987; Turnbull *et al.*, 1990). These methods can broadly be classified as "global" when clustering is assessed across the study region without identifying a specific cluster location, and as "local" when the method identifies the location and size of the cluster. The Spatial scan statistic (SaTScan), developed by Kulldorf and

Nagarwalla (1997) has gained much use in the field of spatial epidemiology. Using this tool, Fèvre and colleagues (2001) reported expansion of rhodesiense sleeping sickness into previously unaffected districts within Uganda. The outbreak was initially centred close to a cattle market and spread outwards from the initial focus. The study further described risk factors associated with the spatial distribution of the disease. In a study to investigate spatial and temporal dynamics of acute respiratory disease in cattle in Hedmark and Oppland counties in the south eastern part of Norway, the spatial scan statistic indicated that outbreaks were clustered in space and time. Primary and secondary clusters were identified in areas with higher than expected disease incidence. Some of the affected herds were not included in any of the cluster groupings; and the authors suggested that a different transmission mechanism or even a different agent might be involved. In one affected district, detection of a disease cluster was attributed to reporting bias of farmers and veterinarians (Norström *et al.*, 2000).

During a cross-sectional study of CBPP in the Maasai ecosystem of south-western Kenya, Mara was identified as having higher seroprevalence based on cELISA than Loita ($P < 0.01$) (Chapter 2). Spatial analysis was used to further investigate the observed difference between the two divisions. The expectation was that agro-ecological zones (AEZ) would influence farming systems in different areas; farming systems in turn will have effect on the herd size, seasonal herding practices and contact structure, which have been reported to influence CBPP distribution patterns (Mariner *et al.*, 2006; Zessin *et al.*, 1985). During the cross-sectional study, animal movement, contact structure and herd size were accounted for in statistical models and were significant in the univariable model but no evidence of their influence on seropositivity was observed in the multivariable model. In addition, proximity to markets was expected to be associated with increased seroprevalenve because markets have been reported to be a source of infection for some diseases (Fèvre *et al*., 2001). Finally, proximity to river was expected to be associated with increased seroprevalence because of the possibility of increased contact between animals of different herds. It was hoped that additional investigation into spatial disease patterns would assist in elucidating the risk factors

underlying seroprevalence in Mara and Loita divisions. Insight into risk factors underlying disease distributions can lead to the development of recommendations for minimizing the impact of these risk factors, hence reducing the prevalence of the disease (Graham *et al.*, 2005).

The objective of this study was therefore to describe spatial patterns of herd seropositivity observed in the Maasai ecosystem of south-western Kenya. Specifically, the study was aimed at:

i. Identifying clusters of seropositivity
ii. Identifying factors associated with the spatial distribution of herd seropositivity

3.2 Materials and methods

3.2.1 Description of study area and design

The current study made use of the data collected during a cross-sectional study carried out in Loita and Mara between July and September 2006. Details of the study design, sampling and laboratory analysis are given under "CBPP seropositivity and associated risk factor study in the Maasai ecosystem of south-western Kenya" (Chapter 2). Briefly, the number of herds to be sampled in each division was proportional to the total number of animals in each division. Within each division, administrative sub-locations were purposely selected to attain a wide geographical representation. Participants were then selected randomly from a sampling frame consisting of herders listed within selected sub-locations. Structured pre-tested questionnaires were administered to the selected farmers and simultaneously their herds were bled for estimation of herd seropositivity. All serum samples were analysed by competitive Enzyme Linked Immunosorbent Assay (cELISA) as described in detail in Chapter 2. Categorization of herds as seropositive was based on having at least one positive animal, or on a within herd seropositivity higher than 3% among animals tested. The number of animals tested ranged from 3-47 per herd.

3.2.2 Development of maps

Global positioning system (GPS) coordinates were taken for each *boma* that was visited for interview and animal bleeding. Each *boma* was assigned a unique number, which was used consistently in the analysis. In addition, GPS records were taken for market centres nearest to the visited *boma*. The coordinates taken were in latitude/longitude format and were recorded using a hand held Garmin eTrex Vista (Taiwan) receiver. The coordinates were then imported into ArcView GIS 3.2 (Environmental Systems Research Institute (ESRI), Inc., Redlands, USA) as database file (DBF3) and projected into Universal Transverse Mercator 1983 (UTM83), zone 36 S and converted into shape files for further use.

Maps for AEZs and rivers were downloaded from International Livestock Research Institute (ILRI) GIS databases (http://www.ilri.org/gis/search.asp). These maps were in geographic coordinate system: GCS_Arc_1960, datum: D_Arc_1960 and were also projected in to UTM83, zone 36 S. Agro-ecological zones were based on the Farm Management Handbook of Kenya (Jaetzold and Schmidt 1983), digitized by Kenya Soil Survey and Kenya Agricultural Research Institute (KARI). Coverage showed the AEZs of Kenya based on annual mean temperature belts, i.e. upper highland (10 to 15˚C), lower highland (15 to 18˚C), upper midland (18 to 21˚C), and lower midland (21 to 24˚C). The original aim of the map was to provide the frame-work for ecological land-use potential. The map for rivers was originally called "Klriversis" and coverage showing rivers of Kenya was provided by the Japan International Cooperation Agency.

The map with herd locations was then overlaid onto the AEZ map. AEZ information was extracted and four zones were identified (upper and lower highland, upper and lower midland) which were categorized into a single highland zone (10-18˚C) and a single midland zone (18-24˚C) for use in the model. The map with market centres was also overlaid onto the map with herd locations, and the map with herd locations was further overlaid onto the map of Kenyan rivers. Distances to the nearest market centres and rivers were extracted using the spatial analyst extension called "nearest feature" (ArcView 3.2, ESRI, USA) and used in the models.

3.2.3 Statistical analysis

First, exploratory analysis of data was performed, based on spatial cluster analysis using SaTScan version 7.0.3 (http://www.satscan.org/references.html; last accessed 14th April 2009). A detailed account of the theory behind the methodology is given elsewhere (Kulldorff *et al.*, 1997; Kulldorff and Nagarwalla, 1995). Briefly, the SaTScan test detects the most likely clusters of events in a data set and infers their significance using Monte Carlo replications. In our analysis, cluster detection was purely spatial as the study was a cross-sectional study and did not include time variables. SaTScan imposed circular windows centered on each of the coordinates located in the study area. For each coordinate, the radius of the window ranged from the smallest inter-event distance to

typically the distance that contains 50% of the population in the study area. A likelihood ratio test for each potential cluster was then calculated by comparing the alternative hypothesis of increased risk of disease inside the circle and the null hypothesis of equal risk inside and outside the circle. Non overlapping clusters were identified. Separate cluster tests were performed for the two methods of identifying CBPP test-positive herds.

The second part of the analysis was based on predictive regression models to identify risk factors associated with the spatial distribution of herd seropositivity. The dependent variable was whether the herd was classified as CBPP positive or CBPP negative. Two separate models were developed for the two methods of categorizing CBPP positive herds. Explanatory variables were limited to independent variables that were considered to influence the spatial pattern of the disease, i.e. AEZ, proximity to market centres and proximity to rivers. Independent variables that were significantly positive at all levels of variable in the univariable herd model based on cELISA results (Chapter 2) were re-evaluated in the model, because the current analysis included only 166 of the 175 herds from the original analysis. The variables included were vaccination (yes/no), number of vaccinations (none, one, more than one in the past 10 years), and trypanosomosis (yes/no) listed by the herder among the five most important diseases affecting his herd). Since the chapter was based on identifying underlying parameters that could explain the difference between the two administrative units (Mara and Loita divisions), division was not included as independent variable. Univariable analysis was performed for all variables (variables influencing the spatial pattern of the disease and taken from Chapter 2). All analyses were performed using the STATA statistical package (Intercooled 8th version; StataCorp: Texas, USA). The model was built by backward elimination with *P* value set at ≤ 0.10 for retention in the model. The general equation is given by

$$\text{logit}\,(p)=\beta_0+\sum\beta_i X_i+\varepsilon; \qquad [1]$$

where p = outcome (positive or negative for CBPP); β_0 = intercept; β_i = regression coefficient for risk factor i; X_i = value of risk factor i; ε = error term. Regression coefficients were the natural logarithm of the odds ratio (Pfeiffer *et al.*, 2008). Cross tabulation was carried out to assess correlation between independent variables based on the Chi statistic. Mean proximity to river for herders reporting/not reporting trypanosomosis among important diseases was analysed based on the t-test. Variables that were considered for inclusion in the analysis are described in Table 3.1.

Table 3.1: Description of variables included in the models for CBPP detection in Maasai herds in the south-western Kenya

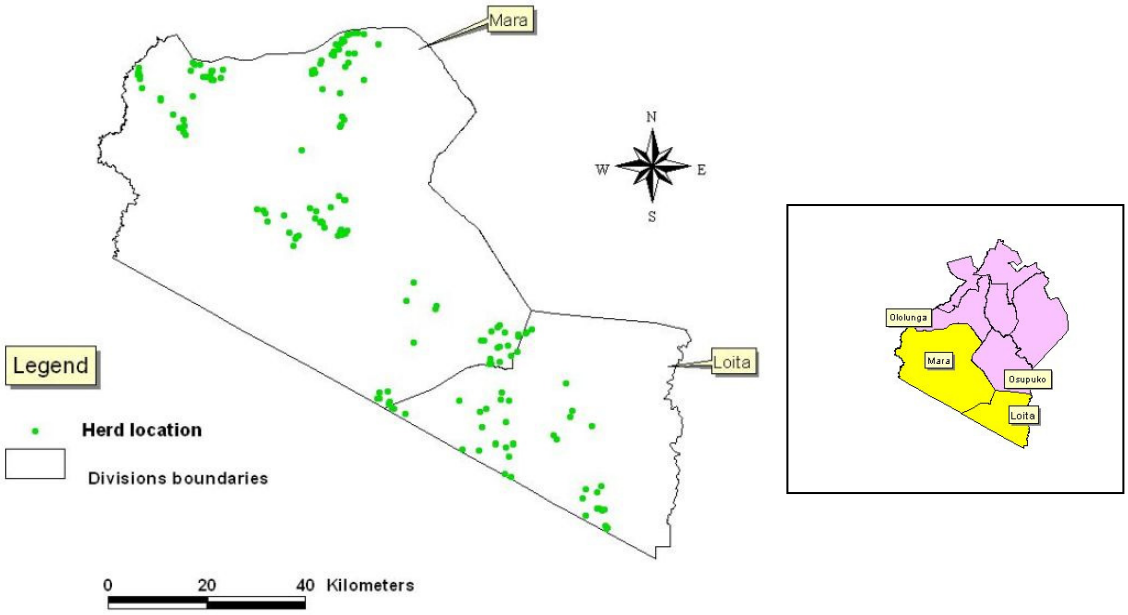
Dependent variable	**Description**
AEZ	Agro ecological zones (Highland; Midland)
Proximity to river	Distance from the river as continuous variable in km
Proximity to market	Distance from market as continuous variable in km
CBPP vaccination	CBPP vaccination carried out in the past 10 yrs (Yes/No)
CBPP vaccination number	Number of years in which CBPP vaccination was carried out over past 10 years (1=not vaccinated; 2=once; 3=twice and more)
Trypanosomosis	Trypanosomosis listed among 5 important diseases by herder (Yes /No)

3.3 Results

3.3.1 Herd characteristics

In total, 175 herders participated in the cross sectional study. For 9 herders, locations were lost upon overlay with the AEZ map of Kenya and full data was available for 166 herders, i.e. 129 from Mara and 37 from Loita (Figure 3.1).

Figure 3.1: Spatial distribution of the study herds in Mara and Loita divisions of Narok district[1] in south-western Kenya



[1] Insert is the map of Narok indicating Mara and Loita as yellow polygons

Herd size ranged from 6 to 450 cattle with a median of 70. The majority of herders (71%) were located in the midland zone; herds in Mara were predominantly located in the midland zone whilst herds in Loita were more likely to be in the upland zone ($P < 0.01$). Most herders (81%) reported vaccinating against CBPP in the past ten years (Table 3.2).

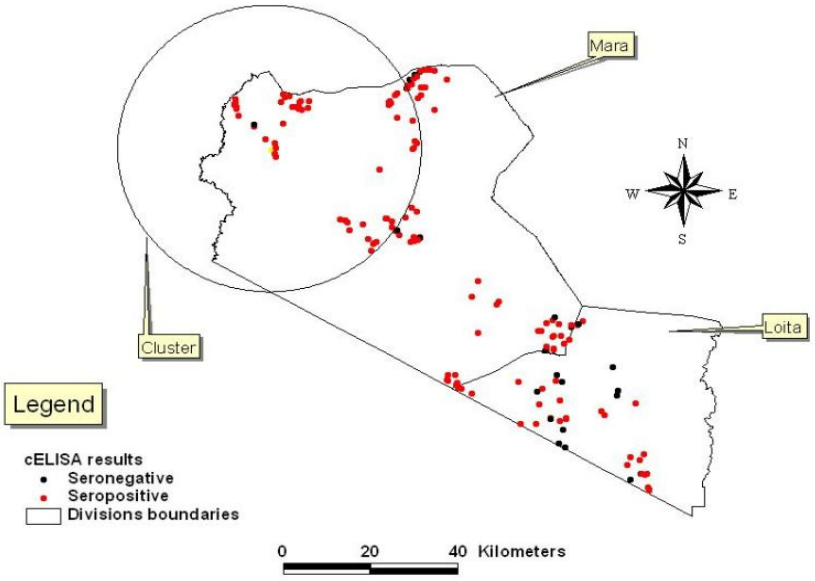
Table 3.2: General characteristics of the study herds

Variable	**Levels**	**Loita**	**Mara**
Categorical		Herds (%)	Herds (%)
Agro ecological zone (AEZ)	Highland	29 (78.4)	19 (14.7)
	Midland	8 (21.6)	110 (85.3)
CBPP Vaccination	Yes	17 (45.9)	14 (10.9)
	No	20 (54.1)	115 (89.1)
CBPP vaccination number	0	17 (46.0)	14 (10.9)
	1	18 (48.6)	85 (65.9)
	≥2	2 (5.4)	30 (23.2)
	Yes	28 (74.7)	87 (67.4)
Trypanosomosis	No	9 (24.3)	42 (32.6)
Continuous		Median (range)	Median (range)
Proximity to river (km)		2.6 (0.05-8.4)	2.0 (0.01-8.04)
Proximity to market (km)		4.0 (0.74-9.1)	7.8 (0.28-21.2)

3.3.2 Seropositivity and cluster detection

A total of 141 out of 166 herds had at least one positive animal resulting in 85% of herds being classified as CBPP positive based on this criterion. Based on this categorization of test-positive herds, one significant cluster was detected with 47 positive herds. This was significantly higher than the expected number of positive herds (n = 40), giving a relative risk of 1.3 ($P < 0.05$). The cluster was located in Mara division (Figure 3.2 and Table 3.3). A non significant secondary cluster was also identified (results not shown).

Figure 3.2: Cluster of CBPP positive herds identified based on SaTScan using model with herd categorized as positive if at least one animal is positive



Seventy three percent (73%) of herds were classified as CBPP positive based on categorization of herds as positive if within herd seropositivity was greater than 3%. The SaTScan test detected one most likely cluster with 60 seropositive herds, a significantly higher number of seropositive herds than expected (47 herds), giving a relative risk of 1.53 ($P < 0.05$) (Table 3.3).

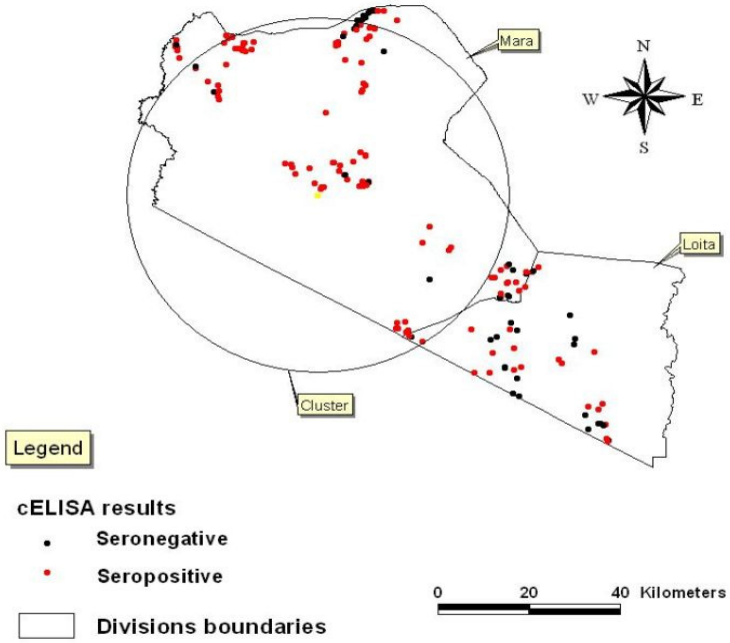
Table 3.3: Details of spatial clusters of CBPP positive herds in Mara and Loita divisions of south-western Kenya based on cELISA[1]

Variable	CBPP definition 1	CBPP definition 2
Most likely cluster location		
Latitude	737755	760627
Longitude	855581	983135
P – value	0.005	0.001
Observed/expected number of cases	1.18	1.27
Relative risk	1.27	1.53
Log likelihood	9.17	11.56
Radius (km)	34.65	42.14

1. CBPP definition 1: Herd CBPP positive if ≥ 1 animals cELISA positive; CBPP definition 2: Herd CBPP positive if >3% of animals that were tested were cELISA positive.

The cluster covered most of Mara division (Figure 3.3). A non significant secondary cluster was also identified (results not shown).

Figure 3.3: Cluster of CBPP positive herds identified based on SaTScan using model with herd categorized as positive if within herd seropositivity is >3%



3.3.3 Proximity to market centres and rivers

Proximity to market centre ranged from 0.3 km to 21.2 km with median at 6.8 km. *Boma* tended to be located close to rivers and proximity to rivers ranged from 0.01 km to 8.4 km with median of 2.1 km. The *boma* locations were connected to the nearest market centre and river (Figure 4 and 5) regardless of the actual use of the market centre or river. Two market centres within the study area were far from any of the selected herds and did not have connecting lines. For some herds, the nearest market centre was outside the divisions that were part of the study.

Figure 3.4: Location of herds (green dots) and market centres (purple dots) showing shortest herd to market distances (purple lines)

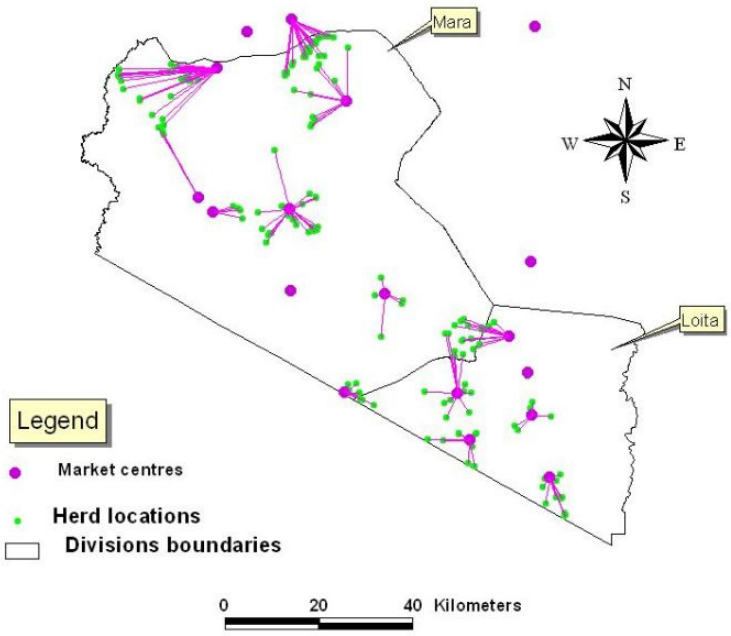
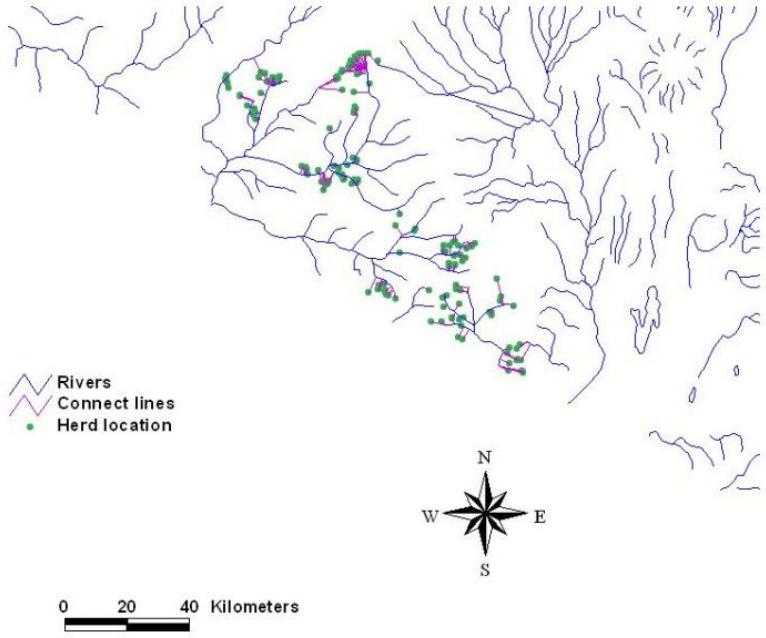


Figure 3.5: Location of herds (green) and rivers (blue), showing shortest herd to river distances (purple)



3.3.4 Statistical analysis

Based on univariable analysis, herds were more likely to be CBPP-positive if they were located in the midland zone, close to a river, or far from a market, and if they had vaccinated against CBPP in the past or listed trypanosomosis among the five most important diseases affecting their herds. These results were obtained based on both methods of classifying herds as CBPP-positive, with minor differences in estimates for odds ratios or significance level (Table 3.4).

Table 3.4: Univariable model identifying risk factors for CBPP positive herds in Mara and Loita divisions of south-western Kenya based on cELISA[1]

		CBPP definition 1		CBPP definition 2	
Variable	**Herds**	**Seropositivity (95% CI)**	**Odds ratio[2]**	**Seropositivity (95% CI)**	**Odds ratio[2]**
Herd level		84.9 (79.4-90.4)		72.9 (66.1-79.7)	
AEZ					
Highland	48	70.8 (58.0-83.7)	Reference level	56.3 (41.7-70.8)	Reference level
Midland	118	90.7 (85.4-95.9)	4.01 (1.67-9.65)***	79.7 (72.3-87.0)	3.05 (1.47-6.29)***
CBPP vaccination					
Yes	135	90.4 (84.1-94.8)	Reference level	78.5 (70.6-85.1)	Reference level
No	31	61.3 (42.2-78.2)	5.93 (2.36-14.90)***	48.4 (30.2-66.9)	3.90 (1.73-8.81)***
CBPP vaccinations number					
0	31	61.3 (42.2-78.2)	Reference level	48.4 (30.2-66.9)	Reference level
1	103	89.3 (81.7-94.5)	5.28 (2.03-13.74)**	74.8 (65.2-82.8)	3.16 (1.37-7.27)**
≥2	32	93.8 (79.2-99.2)	9.41 (1.91-47.09)**	90.6 (75.0-98.0)	10.31 (2.59-41.06)***
Trypanosomosis					
Yes	96	91.7 (84.2-96.3)	Reference level	83.3 (74.4-90.2)	Reference level
No	70	75.7 (64.0-85.2)	3.53 (1.42-8.74)**	58.6 (46.2-70.2)	3.54 (1.73-7.24)***
Proximity to river	166		0.83 (0.69-1.00)*		0.85 (0.72-0.99)**
Proximity to market centres	166		1.14 (1.01-1.29)**		1.08 (1.00-1.17)*

1. CBPP definition 1: Herd CBPP positive if ≥ 1 animals cELISA positive; CBPP definition 2: Herd CBPP positive if >3% of animals that were tested were cELISA positive.
2. *P*-value indicated by superscript: * P < 0.10, ** P < 0.05, *** P < 0.01

Based on multivariable analysis, midland zone, vaccination and proximity to rivers were associated with increased risk of being CBPP positive based on detection of at least one seropositive animal in a herd, whilst vaccination, proximity to rivers and trypanosomosis were associated with increased risk of being CBPP positive based on at least 3% seropositive animals (Table 3.5).

Table 3.5: Multivariable model identifying risk factors for CBPP positive herds in Mara and Loita divisions of south-western Kenya based on cELISA[1]

Variable	CBPP definition 1	CBPP definition 2
	Odds ratio (95% CI)[2]	**Odds ratio (95% CI)[2]**
AEZ (Midland)	3.40 (1.20-9.62)**	2.78(1.22-6.32)**
Vaccination (Yes)	3.32 (1.19-9.22)**	2.36 (0.96-5.78)*
Proximity to river	0.80 (0.64-1.01)*	0.82 (0.69-0.99)**
Trypanosomosis (Yes) [3]	3.03(1.08-8.53)**	3.56 (1.59-8.00)***

1. CBPP definition 1: Herd CBPP positive if ≥ 1 animals cELISA positive; CBPP definition 2: Herd CBPP positive if >3% of animals that were tested were cELISA positive.
2. *P*-value indicated by superscript: * P < 0.10, ** P < 0.05, *** P < 0.01
3. AEZ is significantly correlated with trypanosomosis. When the variable AEZ is omitted from the model then trypanosomosis, become significant (*P* < 0.05 and *P* < 0.01 for definition 1 and 2 respectively)

Four variables were highly correlated, i.e. Midland was positively correlated to Mara division, CBPP vaccination and reporting trypanosomosis among important diseases (Table 3.6). Based on the t-test, mean proximity to river among herders reporting trypanosomosis as important disease was not different from herders not reporting it as important disease (km 2.84 (2.38-3.29)) and (2.28 (1.85-2.71)) (*P* = 0.96).

Table 3.6: Cross tabulation of independent variables associated with the risk of CBPP in the Maasai ecosystem of south-western Kenya[1]

	Vaccination (Yes)	**AEZ (Midland)**	**Trypanosomosis (Yes)**	**Division (Mara)**
Vaccination (Yes)		19.44	16.03	23.32
AEZ (Midland)	0.00		56.90	56.67
Trypanosomosis (Yes)	0.00	0.00		21.92
Division (Mara)	0.00	0.00	0.00	

1. Upper right half of the table shows the Chi square statistic and lower left the *P*-values

3.4 Discussions

This is the first report on CBPP spatial clusters and associated risk factors identified based on cELISA. A significant cluster of CBPP positive herds was identified with each of two separate Bernoulli models using different cut-offs to classify herds as CBPP positive. Both clusters were located in Mara. The cluster based on herds classified as positive if at least one animal was positive tended to be smaller than the cluster based on classification of herds as CBPP positive if more than 3% of the animals that were tested within the herd were seropositive. Despite the difference in size and location, 42 herds were included in both clusters. These herds fall within areas such as Sekenani, Talek, Olesere and Aitong, which are among areas marked as CBPP "hot spots" in the district (Mark Lemein (2009), personal communication). Such areas might act as a centre of endemicity and may be a source of infection to other parts of the country through animal movements and tribal exchanges. However, the high correlation between vaccination and Mara where the clusters are based makes it difficult to determine whether the 'hot spots' reflect centres of infection or vaccination. The results further emphasize the need for developing DIVA vaccines and corresponding diagnostic tests to make differentiation of infected and vaccinated animals possible.

Based on the regression model, midland zone and CBPP vaccination were positively associated with classification of a herd as CBPP positive. Based on a Chi-square statistic, Mara and Loita were significantly different with regards to AEZ, with herds in Mara predominantly located in the midland zone and herds in Loita in the highland zone. Agro-ecological zones were hypothesized to predict farming systems practiced in the area, which in turn could have impact on the CBPP distribution. AEZ could play a potential role in maintaining CBPP in the study area through its influence on farming system (herd sizes, animal movements and contact structures). Indeed, herd sizes, animal movements and contact structures were significant in the univariable model based on the cross-sectional studies. They were highly correlated with division and other independent variables and dropped out of the multivariable model (Chapter 2).

In univariate and multivariate models, herds located near the river edge had increased risk of being classified as CBPP positive compared to herds located far from the river edge, regardless of classification criteria. Proximity to water sources has been reported to be associated with increased risk of trypanosomosis (Michel *et al.*, 2002). Trypanosomosis was reported among important diseases prevalent in the area. The disease causes immunosuppression, which may make animals more susceptible to disease or unable to mount a good immune response upon vaccination (Ilemobade *et al.*, 1982). Mean proximity to river was not different for herders reporting and not reporting trypanosomosis among important diseases, so the effect of proximity to river cannot be attributed to trypanosomosis. Fifty percent of herders resided within 2 km from the river edge. This suggests that animals located near the river edge may be in close contact, which could increase the probability of disease spread among them. Proximity to livestock market centres was expected to be a risk factor for seroprevalence as observed for rhodesiense sleeping sickness reported in Uganda (Fèvre *et al.*, 2001). This was not observed in the current study, possibly because not all nearest market centres engaged in livestock trade and even if they did, they were not necessarily the herders' preferred markets. Another explanation could be that herders staying far from the market are at increasing risk because animals purchased from the market had to be trekked over a long distance, increasing their possibility of contracting disease and introducing into the herd.

3.5 Challenges/limitations

The current study used the data obtained during a cross-sectional study. Participating herders were randomly selected and the selection was fit for that purpose. However, this type of selection may not be fit for all types of studies. For spatial analysis, spatial systematic sampling or stratified random sampling based on knowledge of spatial structure would have been more appropriate (Longley *et al.*, 2005). For example, AEZs could have been used as strata and random selection could have been carried out within strata.

Locations of herders were condensed into single points. The benefit of using single points includes ease of data storage and manipulation and simple visual output (Carpenter, 2001). For farmed animals, herd locations are not points but areas, represented by polygons. For the Maasai pastoral system, a farm area would not provide additional information since animals are not confined to farms. In our study, coordinates were taken at an arbitrary point close to the *boma*. The use of a standardized method to choose the point for which GIS data were collected would have been preferable (Pfeiffer *et al.*, 2008; Durr and Froggatt, 2002).

Spatial data are often obtained from various sources; for instance, the data used in the current study were obtained from field recordings (with precision of ≤ 8 m) and the ILRI-GIS database (http://www.ilri.org/gis/search.asp; accessed on 18th February 2009). The data were in different geographical projections and had to be converted to UTM83 zone 36 S. According to Monmonier (1996), map projections distort geographical relationships and therefore may results into errors.

3.6 Conclusion

The current study identified clusters of CBPP-positive herds, which had been identified using cELISA test results. Based on the spatial scan statistic, two clusters located in Mara were identified. Some herds were included in both clusters and were located in areas identified as CBPP 'hot spots'. The high correlation between vaccination and Mara makes it difficult to determine whether the identified 'hot spots' reflects a centre of infection or vaccination. Results from a logistic regression model showed that seropositivity in the two divisions was partly explained by agro-ecological zone difference and proximity to river. Higher seropositivity was associated with herds located in the midland zone and near the river (water source). Additionally, vaccination and reporting trypanosomosis were highly associated with seropositivity. The results further emphasize the need for developing DIVA vaccine and corresponding diagnostic tests, which will make differentiation of infected and vaccinated animals possible.

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Chapter 4 - A comparative CBPP vaccine safety study in the Maasai ecosystem of south-western Kenya

Abstract

A double-blind CBPP vaccine trial was carried out including 79,959 cattle in 458 herds in the Loita, Mara and Osupuko divisions of Narok District, Kenya, between February and July 2007. A standard vaccine (Contavax®) currently used in Kenya for control of CBPP and a buffered vaccine formulated as suggested by March (2004) were compared. The aim of this study was to identify and quantify adverse events *post*-vaccination and to compare incidences of adverse events between vaccine types. Adverse events observed within 100 days *post*-vaccination were classified into specific (swollen tail, tail slough, wounds or swelling in the perineal or gluteal region), non-specific (weakness, lameness, abortion and mortality) adverse events and antimicrobial treatments.

486 adverse events were reported among all vaccinates of which 326 (4.1 per 1000) were specific and 160 (2.1 per 1000) were non-specific. A subset of animals was subjected to detailed follow-up (n = 2038 animals in 58 herds); the incidence of adverse events in the subset was 2 to 3 times higher than in the complete study population. The highest recorded incidence was for 'swollen tail', whilst 'wounds in the perineal region' were rare in both populations. The incidence of antimicrobial treatments per 1000 animals was 12.7% in all vaccinates and 16.2% in the subset.

The effect of vaccine on the odds of a reported adverse event (specific, non-specific, or antimicrobial treatment) was analysed using generalized linear mixed models. The study shows that the buffered vaccine was associated with increased odds of specific adverse events when compared to the standard vaccine. The effect of the vaccines on specific adverse events was modified by age, gender and division. Adult and female animals vaccinated with the buffered vaccine had higher odds of specific adverse events compared to those vaccinated with the standard vaccine, whilst no difference between vaccines was observed in young and male animals. In all three divisions, animals vaccinated with the buffered vaccine had an increased odds of specific adverse events compared to those vaccinated with the standard vaccine, and animals from Osupuko had increased odds of specific adverse events compared to animals from both other divisions. No effect of vaccine type was observed on the odds of non-specific adverse events. However modification effect of division was observed. Vaccination with buffered vaccine was associated with increased odds of antimicrobial use and this effect was not modified by age, gender or division.

The current study shows that the odds of specific adverse events was higher for animals vaccinated with the buffered vaccine compared to animals that were vaccinated with the standard vaccine. Modification of the vaccine effect by age, gender and origin suggests that naïve populations (young cattle and cattle in Osupuko, where farmers were less likely to have reported outbreaks and/or vaccinations within the past 10 years) were at higher odds of adverse events than populations with prior exposure to CBPP or CBPP vaccine. Vaccine safety should be given due consideration when developing more efficacious CBPP vaccines.

4.1 Introduction

Contagious bovine pleuropneumonia (CBPP) *post*-vaccination adverse events were first documented in 1849 when Louis Willems demonstrated that inoculation of serous fluid from CBPP infected lungs into healthy cattle caused pronounced local reactions. This crude method resulted in a high percentage of severe *post* vaccine reactions (10%) and deaths (1%) which prompted a search for safer vaccines (Huygelen, 1997). Broth vaccines based on T_1 and KH_3J strains of *Mycoplasma mycoides* subsp. *mycoides (Mmm*SC) have been developed and used widely for CBPP control in endemic African countries in the 1960s and early 1970s. These vaccines were fragile and freeze-dried formulations with improved viability were later developed and used for the control of disease. In the 1980s a streptomycin resistant strain of T_1 and KH_3J ($T_{1/sr}$ and KH_3J/sr respectively) were also developed for use in the combined Rinderpest-CBPP vaccination campaigns (Lubroth *et al*., 2007).

The vaccines are administered subcutaneously at the tail-tip or behind the shoulder (Masiga and Windsor 1974; Gilbert *et al*., 1970; Lindley, 1967; Davies *et al*., 1968). The KH_3J vaccine was reported to cause no adverse events but was less immunogenic and hence withdrawn (Mowat and Rweyemamu, 1997). Tail tip administration of T_1/44 vaccine was associated with adverse events (Daleel, 1971; Revell, 1973). A general description of the adverse events *post*-vaccination was given by Provost *et al*., (1987) and adverse events were characterized by erythema and swelling extending over part of the tail. In some animals swelling extended to the whole tail and into the gluteal and perineal regions, ending in fatality. Vaccination behind the shoulder resulted in development of a transient local oedematous swelling resulting in necrosis in some animals.

Detailed field studies on adverse events *post* CBPP vaccination are limited. An incidence of 0.8% was reported based on a retrospective study where 296,248 animals were vaccinated against CBPP using freeze dried T_1/44 vaccine in Ethiopia (Sori,

2005). The study also reported 0.17% mortality. The author suggests that contamination, intramuscular injection and poor body condition were among factors contributing to the incidence of adverse events *post*-vaccination. Revell (1973) reported tail-tip slough in 12% of 6,673 animals vaccinated with freeze-dried T_1/44 vaccine in an 'infected' area in Zambia. The same author reported tail-tip slough in 0.17% out of 1,162 vaccinated animals from an 'uninfected' area bordering the infected area. Two out of 86,035 vaccinated animals experienced swelling at the vaccination site based on a T_1 broth vaccine in Sudan (Daleel, 1971). In addition to poor safety, the T_1/44 CBPP vaccine has been reported to have poor efficacy (Thiaucourt *et al*., 2000; Karst, 1972). Low vaccine titres attained during production were reported as a major factor behind this poor efficacy (Rweyemamu *et al*., 1995).

Two vaccine types were used for the current study. One vaccine was manufactured based on the standard method used for production of the vaccine that is currently used for control of the disease in Kenya. The other vaccine was manufactured based on a buffering formulation suggested to maintain stable vaccine titre during production and use (March, 2004; Waite and March, 2001). It was hypothesized that maintenance of a higher vaccine titre by buffered vaccine would be associated with higher immunogenicity and, possibly, more reactogenicity. Based on a limited vaccine trial carried out at Kenya Agriculture Research Institute (KARI) in Muguga, in advance of the work reported here, mild swelling at the site of inoculation was observed between 3 and 20 days *post*-vaccination using standard or a buffered vaccine (Wesonga H. and Schnier C. 2008, personal communication).

This study was undertaken to compare the safety of the standard freeze-dried T_1/44 CBPP vaccine with the buffered formulation under field conditions. The specific objectives of the study were to:

i. Identify and quantify adverse events *post*-vaccination
ii. Compare incidence of adverse events between vaccine types

4.2 Materials and methods

4.2.1 Description of the study area and selection of crushes

A CBPP vaccine field trial was carried out between February and July 2007 in Narok South district in Kenya. The district borders Kajiado on the east, Transmara on the west, Narok North on the north and Loliondo district of Republic of Tanzania on the south. CBPP vaccination involved cattle from three divisions within Narok district; namely Osupuko, Mara and Loita. The divisions are inhabited by Maasai communities whose livelihood depends mostly on pastoral and agro-pastoral systems. Vaccination was carried out at 19 crushes selected from 38 listed in the three divisions. Selection of the crushes was without any formal randomization and was based on the working condition of the crushes. Also, for logistical reasons selected crushes had at least 2,000 unvaccinated animals living within the villages or clusters served by the crush, a number that was supplied by the District Veterinary Office in collaboration with Naroosura Agro-Vet Services. Of the 19 crushes, 7 were in Mara, 6 were in Loita and 6 were in Osupuko. Allocation of crushes to two vaccinating teams was carried out in such a way that two neighbouring crushes were allocated to either of the two teams.

4.2.2 Type, source, reconstitution fluid and storage of the CBPP vaccine

Two CBPP vaccines, manufactured in January 2007 by Kenya Agriculture Research Institute - Veterinary Vaccine Production Centre (KARI-VVPC) based in Nairobi, were used. The vaccines were presented in 5 ml glass vials as a freeze-dried material with batch number 01/07. One vaccine was the standard (Contavax®) vaccine based on freeze dried live attenuated *MmmSC* T_1/44 vaccine cultured in un-buffered growth media with 10% glucose, and was reconstituted with normal saline. The standard vaccine is currently used in Kenya and other African countries for control of CBPP. The buffered vaccine was a freeze-dried live attenuated *MmmSC* T_1/44 vaccine, cultured on N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered culture media (March 2004; Waite and March 2001). The buffering system prevents a drop in pH hence maintaining optimal titre during vaccine production. The same authors suggested

the use of phosphate-buffered saline (PBS) as a reconstitution fluid to offer additional buffering effect. Both vaccines had the same batch number and were packed in similar containers; it was therefore decided to label one with red marker and the same labelling was used for the respective reconstitution fluid. Reconstitution fluids (PBS and normal saline) were packed in 50 ml plastic bottles. Reconstituted vaccine vials had approximately 100 vaccine doses. Vaccines and accompanying reconstitution fluids were collected from the KARI-VVPC two days prior to start of vaccination. Vaccines were transported in dry ice to Narok where they were kept frozen at -20°C. During fieldwork, both vaccines were kept in a freezer compartment of a refrigerator located at a central point under supervision of the author. Sufficient vaccine doses for a particular crush were assigned to team leaders each time the teams moved to a new crush.

4.2.3 Composition of the teams and pre testing of protocols

Vaccines were administered by two teams, which were named Narok and Naroosura. Each team had a veterinarian, animal health assistants and a community animal health worker. The Narok team was composed of employees from District Veterinary Office, the office responsible for planning and regulating veterinary services in the district. The Naroosura team was composed of staff from Naroosura Agro-Vet Services; a privately owned veterinary centre that provides agro-vet inputs and animal health services in Osupuko and Loita divisions. Private veterinary facilities are regulated by state veterinarians based in the district veterinary office, which implies that they work in a similar manner. Prior to the vaccination exercise, two days of training (11th -13th January 2007) were carried out to impart team members with common understanding of the vaccination and monitoring protocols. In addition, pre-testing of protocols was carried out in order to harmonize vaccination, bleeding and recording methods.

Sensitization of the community leaders and society at large was carried out from 16th to 30th January 2007 using a pre-designed message detailing how and by whom the vaccine would be administered, how the monitoring would be carried out and how farmers would benefit from participation (Appendix ii).

4.2.4 Vaccination protocols

The trial was double-blind in that neither vaccinators nor personnel monitoring vaccinated animals were aware of the type of the vaccine that had been administered to the animal. Vaccination was carried out such that all animals in a full crush (20-30 animals) were vaccinated with one type of vaccine. The crush was then emptied and filled with another lot, all of which received the other type of vaccine. Animals were injected with 0.5 ml of the reconstituted vaccine subcutaneously at the base of the tail switch using hypodermic needles (17G x ½", Hauptner, Germany) and automatic syringes (10 ml; Hauptner, Germany). The route is generally referred to as "tail tip" but practically vaccine is not injected at the tip since injecting 0.5 ml at the tip of the tail will often require pressure to empty the syringe and may result in the needles being forced off the nozzle. Vaccination needles were replaced for every fresh reconstitution of vaccine, which were approximately 100 doses. Reconstituted vaccines were placed inside cool boxes packed with ice blocks; each type in a separate cool box. Two data loggers (Tinytag, UK) were programmed to record temperature every 10 minutes and each was placed inside the cool boxes on arbitrary days. Time from vaccine reconstitution to end of use ('Vaccine use time') was additionally recorded on the same days as the cool box temperatures were monitored.

On average, 1000 animals were vaccinated per day by each team. At herd level, effort was made to have half of the animals vaccinated with each of the vaccine types. All vaccinated animals were ear tagged; animals vaccinated with red marked vaccine were right ear-tagged and animals vaccinated with unmarked vaccine were left ear-tagged. This method of ear tagging was designed to ease identification of animals during subsequent vaccinations. It was thought that this would not bias follow-up studies because personnel involved in vaccination and monitoring had no idea of vaccine type associated with either right or left ear tagging. Background information such as age, gender, breed and body condition score were recorded in a pre-designed form (Appendix iii).

4.2.5 Study population and selection and use of subset

It was planned to include approximately 100,000 animals from 500 herds in the study. This was based on the assumption that very few vaccinated herds (approximately 5%) would break down with clinical CBPP within one year of follow-up. Given the low expected incidence, large numbers of animals were included in order to find a small improvement in the vaccine's ability to prevent clinical disease.

Twelve out of 19 crushes were purposely selected, and from each crush, 5 herders were selected to participate in the detailed safety study, which involved tail measurement and bleeding *pre*- and *post*-vaccination. The results from bleedings are presented in the vaccine efficacy study (Chapter 5). Inclusion of herders was based on their willingness to comply with bleeding schedules and regular visits as per study protocol. Sample size calculations are detailed in the vaccine efficacy study (Chapter 5). Briefly, a sample size of 2400 animals was calculated to estimate 5% seroconversion difference between animals vaccinated by either vaccine. Animals were drawn from 60 herds, each contributing 40 conveniently selected animals (20 animals vaccinated with each vaccine type). The tail diameters of selected animals were measured using vernier callipers *pre*- and *post*-vaccination to assess the change in tail diameter and the measurements were taken at the base of the tail switch (inoculation site). Pre-vaccination tail measurements were carried out at the crush site (Appendix iv).

4.2.6 *Post*-vaccination monitoring

All vaccinated animals, regardless of inclusion in the subset, were actively monitored by trained community animal health workers visiting weekly for the first month *post*-vaccination and thereafter monthly for the purpose of recording adverse events. Adverse events observed within 100 days *post*-vaccination were recorded in the specific monitoring forms (Appendix v). Tail measurements were carried out approximately one month and three months *post*-vaccination. The follow-up measurements were carried out at herders' homesteads. Information on prior CBPP exposure through outbreak and vaccination was also collected during the follow-up visits (Appendix iv).

4.2.7 Statistical analysis

Adverse events were classified into specific events (swollen tail, tail-tip slough, and wounds and/or swellings in the perineal or gluteal region), non-specific events (weakness, lameness, abortion and mortality) and antimicrobial treatments. Descriptive statistics, graphics and logistic regression modelling were carried out using statistical package STATA (Inter cooled 8th version; StataCorp, Texas, USA). Logistic regression models were developed for each outcome; specific, non-specific and antimicrobial treatments, and models were based on all vaccinates. The final models were based on generalized linear mixed models (GLMM) using GenStat (Release 10.2; Copyright 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) based on the general equation [1]

$$\text{logit}\,(p)=\beta_0+\beta_1\text{vaccine type}_{ij}+\beta_2X_{ij}+\beta_3\,(\text{vaccine type}_{ij}*X_{ij})+(bj)_k+\varepsilon; \qquad [1]$$

where, β_0 = intercept; β_1 = regression coefficient for vaccine type; β_2 regression coefficient for independent variable; β_3 = regression coefficient for interaction between vaccine type and independent variable; X_i = value for independent variable (age, gender, team, body condition score, and animal origin); b_j = random effect for herd (j) in crush (k); ε = error term. Vaccine type was the only independent variable that was kept in all models. 'Crush' and 'herd' were included as random effects. A detailed description of variables included in the results is provided in Table 4.1.

Table 4.1: Description of variables included in the models

Variable	Description
Adverse events	Adverse events that were reported within 100 days *post*-vaccination
Types	**Specific adverse events**: swollen tail or swollen tail base; tail-tip slough off; involvement of gluteal muscles and wounds at the vaccination site and/or near tail base; with or without non-specific adverse events (No; Yes). **Non-specific adverse events**: abortion; lameness; weakness; mortality (No; Yes) **Antimicrobial treatments**: Antimicrobial (tetracyclines 10% and 20% brands) use with or without recording of a specific or non-specific adverse event (No; Yes)
Age	Age in years (Young: ≤3 yrs; Adult: >3 yrs)
Herd	Group of animals vaccinated under one herder name
Body condition	Body condition score (Good; Fair)
Crush	Vaccination site **Loita**: Enkoseremai, Entasekera, Morijo, Nkopon, Olmesutie and Olorte. **Mara**: Sekenani, Oldisare, Oloonchora, Ololturoto, Olpusimoru, Orkinyei and Talek. **Osupuko**: Elang'ata Enterit, Naroosura, Nkimpa, Ntuka, Olepariata and Oloigeruno.
Division	Administrative unit (Loita; Mara; Osupuko)
Gender	Animal gender (Male; Female)
Tail change	Tail diameter *post*-vaccination less diameter *pre*-vaccination in cm.
Temperature	Cool box temperature (℃) calculated from the data logger records
Use time	End time use minus time of reconstitution (minutes).
Team	Vaccine delivery team for an individual animal (Narok team; Naroosura team)
Vaccine type	Vaccine type administered to an individual animal (standard; buffered)

4.3 Results

4.3.1 Description of the general study population

In total 79,959 animals from 458 herds were vaccinated against CBPP. Of these, 49.1% were administered the standard vaccine and 50.9% the buffered vaccine; 52.6% of the animals were vaccinated by the Narok team and 47.4% by the Naroosura team. Most animals (99.4%) were zebu in good body condition (Table 4.2).

A subset of 60 herders (20 herders from each division) participated in the detailed CBPP vaccine safety study. Two herds were lost at the first *post*-vaccination follow up because the herders refused to have their animals bled and examined according to study protocol. A total of 2414 animals were recruited, complete data was available for only 2038. Incomplete data was due to difficulty in identifying the animals during follow up visits because the cattle marker that was used had faded. The composition of animals and distribution of teams and vaccines in the subset were similar to the complete study population (Table 4.2).

4.3.2 Vaccine handling

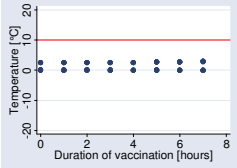
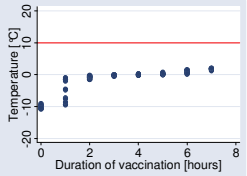
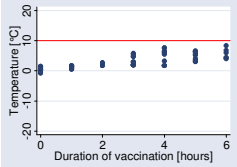
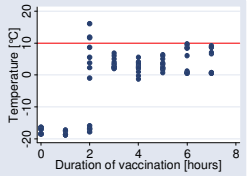
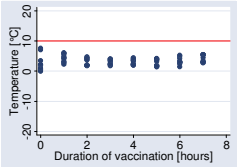
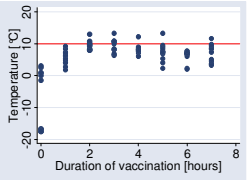
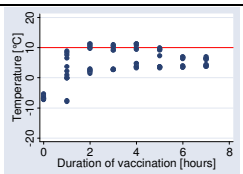
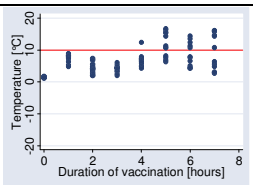
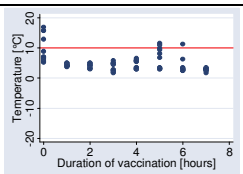
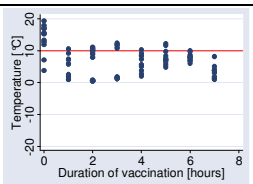
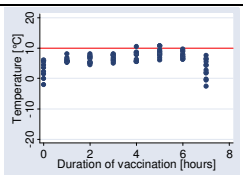
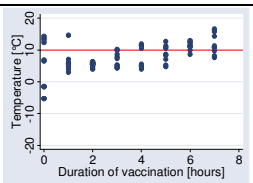
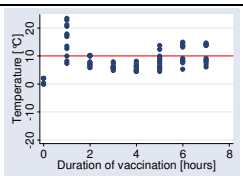
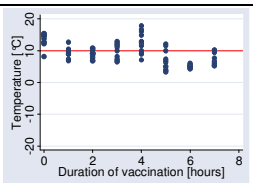
Cool box temperatures were generally kept below 10˚C, although there was variation, with the standard vaccine having been kept at temperatures ranging from -18.8˚C to 23.5˚C with median at 5.5˚C and the buffered vaccine kept at median temperature of 4.5˚C (-17.6˚C to 22.8˚C). A graphic presentation of the cool box temperatures maintained by the teams is provided (Figure 4.1).

Table 4.2: General characteristics of the study animals

Variable	Variable levels	Number of cattle (%)		Number of cattle (%)	
		All vaccinates			Subset[1]
Division	Loita	26,632	(33.3)	685	(33.6)
	Mara	27,731	(34.7)	681	(33.4)
	Osupuko	25,596	(32.0)	672	(33.0)
Body condition score	Good	73,923	(92.4)	1,825	(89.6)
	Fair	6,036	(7.6)	213	(10.4)
Gender	Male	25,521	(31.9)	592	(29.1)
	Female	54,425	(68.1)	1,446	(71.0)
Age	Young ≤3 years	17,802	(22.3)	679	(33.3)
	Adult ≥4 years	62,131	(77.7)	1,359	(66.7)
Breed	Cross	469	(0.6)	22	(1.1)
	Zebu	79,490	(99.4)	2,016	(98.9)
Crush					
	Elang'ata enterit	7,282	(9.1)	-	-
	Enkoseremai	1,805	(2.3)	126	(6.2)
	Entasekera	4,636	(5.8)	187	(9.2)
	Morijo	3,012	(3.8)	-	-
	Naroosura	3,861	(4.8)	-	-
	Nkimpa	4,892	(6.1)	185	(9.1)
	Nkopon	4,152	(5.2)	-	-
	Ntuka	3,896	(4.9)	154	(7.6)
	Oldisare	4,902	(6.1)	137	(6.7)
	Olepariata	3,937	(4.9)	149	(7.3)
	Olmesutie	3,564	(4.5)	200	(9.8)
	Oloigeruno	1,723	(2.2)	184	(9.0)
	Ololturoto	1,419	(1.8)	40	(2.0)
	Oloonchora	3,958	(5.0)	123	(6.0)
	Olorte	9,779	(12.2)	174	(8.5)
	Olpusimoru	8,771	(11.0)	200	(9.8)
	Orkinyei	1,883	(2.4)	-	-
	Sekenani	4,584	(5.7)	-	-
	Talek	1,903	(2.4)	179	(8.8)
Team	Narok team	42,087	(52.6)	903	(44.3)
	Naroosura team	37,872	(47.4)	1,135	(55.7)
Vaccine type	Standard	39,234	(49.1)	1,028	(50.4)
	Buffered	40,725	(50.9)	1,010	(49.6)

1. Subset was selected from vaccinated animals for detailed examination

Figure 4.1: Cool box temperatures for the two teams sorted by increasing median temperatures. The red line indicates a temperature of 10°C.

Narok team	Naroosura team
 8th March 2007	 12th March 2007
 3rd March 2007	 13th March 2007
 22nd March 2007	 20th March 2007
 2nd March 2007	 6th March 2007
 1st March 2007	 15th March 2007
 23rd March 2007	 16th March 2007
 9th March 2007	 5th March 2007

Vaccination procedure and vaccine cool box used are shown (Figure 4.2a and 4.2b). Vaccines were used within 60 to 130 minutes *post* reconstitution with median at 95.5 minutes for the standard vaccine and between 65 and 142 minutes with median of 95 minutes for the buffered vaccine (Table 4.3).

Table 4.3: Cool box temperature and vaccine use time

Variable	Median (Range)
Temperature (℃)	
Narok team	3.8 (-7.8 to 23.5)
Naroosura team	6.6 (-18.8 to 19.3)
Standard	5.5 (-18.8 to 23.5)
Buffered	4.5 (-17.6 to 22.8)
Use time (minutes)	
Narok team	91.5 (69 to 142)
Naroosura team	95.0 (60 to 130)
Standard	95.5 (60 to 130)
Buffered	95.0 (65 to 142)

4.3.3 Description of adverse events

In all vaccinates, 326 specific adverse events were reported, corresponding to a reporting rate of 4.1 cases per 1000 vaccinates within 100 days *post*-vaccination. 'Swollen tail' had the highest recorded incidence, corresponding to 3 cases per 1000 vaccinates whilst 'wounds at the perineal region' was a rare adverse event corresponding to only 7 adverse events during the study period. In the subset, 18 specific adverse events were reported corresponding to 8.8 cases per 1000 vaccinates within 100 days *post*-vaccination. Again 'swollen tail' had the highest recorded incidence, corresponding to 5.4 cases per 1000 vaccinates. Some of specific adverse events observed *post* vaccination are shown (Figure 4.2c-2i).

Figure 4.2: Vaccination, vaccine storage and adverse events

a. the vaccination site; **b.** cool box with ice blocks, vaccine, syringe for vaccine reconstituting and data logger (yellow instrument); **c.** swollen tail; **d.** mild lesion at vaccination site; **e.** and **f.** necrotic and infected wound which may end up with slough-off of tail tip; **g.** tail-tip slough off; **h.** and **i.** Perineal involvement

Non-specific adverse events such as weakness, lameness, abortion and mortality were also recorded. A total of 160 non-specific adverse events were reported in all vaccinates, corresponding to a reporting rate of 2.1 cases per 1000 vaccinates within 100 days *post*-vaccination. 'Mortality' had the highest incidence recorded, corresponding to 1 case per 1000 based on all vaccinates. In the subset, 14 non-specific adverse events were reported corresponding to 7 cases per 1000 vaccinates within 100 days *post*-vaccination. 'Abortion' had the highest incidence recorded for the subset, corresponding to 3.4 cases per 1000 vaccinates (Table 4.4).

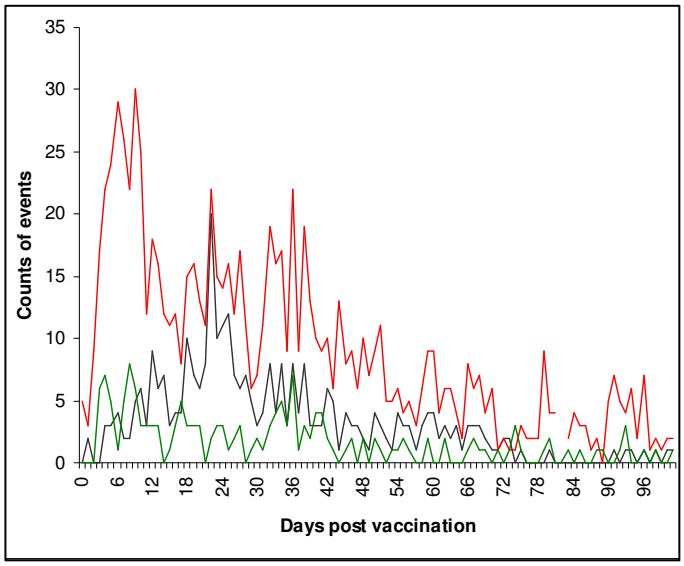
Table 4.4: Incidence of adverse events post-vaccination

Adverse event type	**All vaccinates Number (incidence per 1000)**		**Subset Number (incidence per 1000)**	
i. Specific events				
Swollen tail only	228	(2.9)	11	(5.4)
Tail slough only	81	(1.0)	4	(1.96)
Swollen tail and mortality	1	(0.01)	1	(0.49)
Swollen tail and lameness	2	(0.01)	0	(0)
Swollen tail and weakness	1	(0.01)	0	(0)
Swollen tail and tail slough	6	(0.1)	2	(0.98)
Perineal wound	7	(0.1)	0	(0)
Subtotal	*326*	*(4.1)*	*18*	*(8.8)*
ii. Non specific events				
Mortality only	79	(1.0)	3	(1.5)
Abortion only	32	(0.4)	7	(3.4)
Lameness only	30	(0.4)	1	(0.5)
Weakness only	22	(0.3)	3	(1.5)
Subtotal	*160*	*(2.1)*	*14*	*(6.9)*
Total (i+ii)	*486*	*(6.1)*	*32*	*(15.7)*
iii. Antimicrobial treatments	1018	(12.7)	33	(16.2)

A total of 12.7 and 16.2 antimicrobial treatments per 1000 animals were recorded in all vaccinates and in the subset, respectively. Plotting the number of adverse events against time *post*-vaccination revealed a first peak of specific, non-specific and antimicrobial treatments within the first two weeks *post*-vaccination. The second peak of adverse events involving antimicrobial treatments and specific adverse events occurred between three and four weeks *post*-vaccination. A third peak involving specific, non-specific

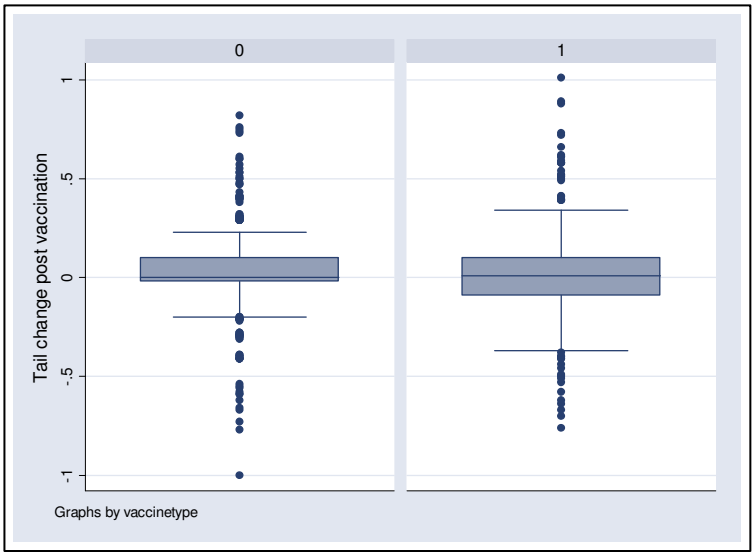
adverse events and antimicrobial treatments was observed between 32-38 days *post*-vaccination (Figure 4.3).

Figure 4.3: Reported specific (black) and non-specific (green) adverse events and antimicrobial treatments (red) *post*-vaccination



Tail diameter change was graphically presented and no difference in tail change was observed between vaccine types pre- and *post*-vaccination. Animals' tail change for either vaccine type was normally distributed around zero (Figure 4.4).

Figure 4.4: Tail change (diameter one month post-vaccination minus diameter pre-vaccination in cm) by vaccine type (0 = standard vaccine; 1 = buffered vaccine)



4.3.4 Univariable analysis of risk factors for specific and non-specific adverse events and of antimicrobial treatments

Animals vaccinated with the buffered vaccine had higher odds of specific adverse events compared to animals vaccinated with the standard vaccine ($P < 0.05$). Female animals and animals vaccinated by the Naroosura team had significantly higher odds of specific adverse events compared to male animals and animals vaccinated by the Narok team ($P < 0.05$). Animals from Osupuko had significantly more reports of specific adverse events compared to animals from Loita ($P < 0.01$) (Table 4.5a).

Table 4.5a: Univariable model based on specific adverse events

Variable	Number[1]	Adverse events incidence (95% CI)[2]	Odds Ratio (95% CI)[2]
Vaccinating team			
Narok team	42,087	0.35 (0.30-0.42)	Reference level
Naroosura team	37,872	0.46 (0.40-0.54)	1.31 (1.06-1.63)**
Vaccine type			
Standard	39,234	0.33 (0.27-0.39)	Reference level
Buffered	40,725	0.48 (0.42-0.56)	1.47 (1.18-1.84)***
Age (years)			
≤3	17,802	0.46 (0.36-0.56)	Reference level
>3	62,131	0.39 (0.35-0.45)	0.87 (0.67-1.11)
Body condition			
Good	73,923	0.42 (0.37-0.46)	Reference level
Fair	6,036	0.27 (0.15-0.43)	0.63 (0.38-1.05)*
Division			
Loita	26,632	0.26 (0.20-0.33)	Reference level
Mara	27,731	0.25 (0.20-0.32)	0.96 (069-1.34)
Osupuko	25,596	0.73 (0.63-0.84)	2.78 (2.11-3.66)***
Gender			
Male	25,521	0.33 (0.26-0.41)	Reference level
Female	54,425	0.44 (0.39-0.50)	1.35 (1.05-1.72)**

1. number of animals monitored
2. *P*-value indicated by superscript: ** $P < 0.10$ ** $P < 0.05$ *** $P < 0.01$.

The odds of non-specific adverse events in animals vaccinated with the standard and the buffered vaccine were not significantly different; similar results were observed in animals vaccinated by Narok and Naroosura teams. Young animals and those in fair body condition had significantly higher odds of non-specific adverse events than adults and animals in good body condition ($P < 0.01$) (Table 4.5b).

Table 4.5b: Univariable model based on non-specific adverse events

Variable	Number[1]	Adverse events incidence (95% CI)[2]	Odds Ratio (95% CI)[2]
Vaccinating team			
Narok team	42,087	0.23 (0.18-0.28)	Reference level
Naroosura team	37,872	0.19 (0.15-0.24)	0.83 (0.61-1.13)
Vaccine type			
Standard	39,234	0.22 (0.17-0.27)	Reference level
Buffered	40,725	0.20 (0.16-0.25)	0.94 (0.69-1.27)
Age (years)			
≤3	17,802	0.31 (0.24-0.41)	Reference level
>3	62,131	0.18 (0.15-0.22)	0.57 (0.42-0.79)***
Body condition			
Good	73,923	0.19 (0.16-0.23)	Reference level
Fair	6,036	0.41 (0.27-0.61)	2.15 (1.40-3.28)***
Division			
Loita	26,632	0.13 (0.09-0.18)	Reference level
Mara	27,731	0.32 (0.26-0.39)	2.52 (1.70-3.74)***
Osupuko	25,596	0.18 (0.13-0.24)	1.38 (0.86-2.15)
Gender			
Male	25,521	0.19 (0.14-0.25)	Reference level
Female	54,425	0.22 (0.18-0.26)	1.17 (0.84-1.62)

1. number of animals monitored
2. *P*-value indicated by superscript: ** $P < 0.10$ ** $P < 0.05$ *** $P < 0.01$.

Animals vaccinated by the Naroosura team had higher odds of antimicrobial treatments than animals vaccinated by the Narok team ($P < 0.01$). Young animals ($P < 0.05$) and animals from Osupuko ($P < 0.01$) had significantly more antimicrobial treatments than adult animals and animals from Loita (Table 4.5c).

Table 4.5c: Univariable model based on antimicrobial treatment

Variable	Number[1]	Adverse events incidence (95% CI)[2]	Odds Ratio (95% CI)[2]
Vaccinating team			
Narok team	42,087	1.06 (0.97-1.17)	Reference level
Naroosura team	37,872	1.51 (1.38-1.63)	1.42 (1.25-1.61)***
Vaccine type			
Standard	39,234	1.21 (1.10-1.32)	Reference level
Buffered	40,725	1.34 (1.23-1.45)	1.11 (0.98-1.26)
Age (years)			
≤3	17,802	1.46 (1.29-1.65)	Reference level
>3	62,131	1.22 (1.14-1.31)	0.83 (0.72-0.96)**
Body condition			
Good	73,923	1.27 (1.19-1.35)	Reference level
Fair	6,036	1.29 (1.02-1.61)	1.02 (0.81-1.28)
Division			
Loita	26,632	1.22 (1.09-1.36)	Reference level
Mara	27,731	1.08 (0.96-1.21)	0.89 (0.76-1.04)
Osupuko	25,596	1.54 (1.40-1.70)	1.27 (1.10-1.48)***
Gender			
Male	25,521	1.25 (1.11-1.40)	Reference level
Female	54,425	1.28 (1.19-1.38)	1.02 (0.89-1.17)

1. number of animals monitored
2. *P*-value indicated by superscript: ** $P < 0.10$ ** $P < 0.05$ *** $P < 0.01$.

Cross tabulation of independent variables revealed that Naroosura team was positively correlated with adult, female gender and negatively correlated with fair body condition score. Also, adult positively correlated to female and negatively correlated to fair body condition score' (Table 4.6).

Table 4.6: Cross tabulation of independent variables [1]

	Vaccine type (Buffered)	Team (Naroosura)	Age (Adult)	Gender (Female)	Body condition (Fair)
Vaccine type(Buffered)		2.05	15.40	2.56	2.73
Team (Naroosura)	0.15		327.52	92.11	3.5e+03
Age (Adult)	0.00	0.00		1.2e+03	3.7e+03
Gender (Female)	0.11	0.00	0.00		7.07
Body condition (Fair)	0.10	0.00	0.00	0.01	

[1] Upper right half of the table shows Chi square statistic and lower left the p-values

4.3.5 Multivariable analysis of risk factors for specific and non-specific adverse events and of antimicrobial treatments post-vaccination

Multivariable analysis of specific adverse events

Animals vaccinated with the buffered vaccine had higher odds of specific adverse events compared to animals vaccinated with the standard vaccine. The effect of vaccine on the odds of specific adverse events was modified by 'age', 'vaccinating team', 'gender' and 'origin of animals' ($P < 0.01$). The odds of specific adverse events in young animals vaccinated with either vaccine was similar whilst adults vaccinated with the buffered vaccine had increased odds of specific adverse events compared to adults vaccinated with the standard vaccine. The odds of specific adverse events in animals vaccinated with the buffered vaccine by the Narok team were higher than the odds of specific adverse events in animals vaccinated with the standard vaccine by the same team. The odds of specific adverse events were modified by gender. Odds of specific adverse events in male animals vaccinated with either vaccine were similar, whilst female animals vaccinated with the buffered vaccine had higher odds than female animals vaccinated with the standard vaccine. The odds of specific adverse events in animals from Osupuko vaccinated with the buffered and standard vaccine were higher than the odds ratio for animals originating from Loita and Mara vaccinated with either vaccine. Animals from Mara vaccinated with the standard vaccine had the lowest odds (Table 4.7; model output in Appendix vi.). There was no evidence that the effect of the vaccine type on specific adverse events was modified by body condition score (results not shown).

Multivariable analysis of non-specific adverse events

The effect of the vaccine type on the odds of non-specific adverse events was significantly modified by the origin of animals ($P < 0.10$). Compared to animals originating from Osupuko that were vaccinated with the standard vaccine, animals originating from Osupuko that were vaccinated with the buffered vaccine had lower odds of non-specific adverse events. Compared to animals originating from Loita that were vaccinated with the standard vaccine, animals originating from Loita that were

vaccinated with the buffered vaccine had higher odds of non-specific adverse events (Table 4.7; model details in Appendix vii). The odds of non-specific adverse events in animals vaccinated with either vaccine were higher in animals originating from Mara than in animals from Loita and Osupuko.

Multivariable analysis of antimicrobial treatments

The effect of the vaccine on the odds of antimicrobial treatments was significantly modified by age ($P < 0.10$). In young animals, odds of antimicrobial treatments were higher for animals vaccinated with the standard vaccine than in animals vaccinated with the buffered vaccine. In adult animals, no effect of the vaccine on the odds of antimicrobial treatments was observed. Compared to young animals, adult animals vaccinated with either vaccine had lower odds of antimicrobial treatment (Table 4.7; and detailed in Appendix viii). The effect of the vaccine on the odds of antimicrobial treatments was not modified by body condition score, gender, vaccinating team and animal origin (results not shown).

Table 4.7: Multivariable linear logistic mixed model based on specific, non-specific and antimicrobial treatments adverse events

Variables	Standard vaccine (Odds)	Buffered vaccine (Odds)
Specific adverse events		
Vaccine type	0.0019	0.0028
Young animals	0.0027	0.0027
Adult animals	0.0017	0.0029
Na**rok**	0.0015	0.0028
Naroo**sura**	0.0022	0.0027
Male	0.0017	0.0019
Female	0.0020	0.0032
Loita	0.0011	0.0026
Mara	0.0010	0.0020
Osupuko	0.0047	0.0051
Non-specific adverse events		
Vaccine type	0.001	0.001
Loita	0.0007	0.0008
Mara	0.0014	0.0014
Osupuko	0.0010	0.0007
Antimicrobial treatment		
Vaccine type	0.009	0.010
Young animals	0.0117	0.0109
Adult animals	0.0081	0.0094

4.4 Discussion and conclusion

4.4.1 Discussion

A vaccine safety trial based on T_1/44 buffered vaccine has not been reported before. Limited safety trials have been reported based on the standard vaccine. This is the first study in which adverse events *post* CBPP vaccination have been systematically monitored and classified as specific and non-specific. Generally, herders in the study area did not seem to worry much about adverse events *post*-vaccination. During the cross-sectional study (Chapter 2), it was revealed that herders were aware of *post*-vaccination adverse events (both specific and non-specific) but they valued vaccination as a major control method against the disease. Additionally, herders indicated that *post*-vaccination adverse events were successfully managed by the use of antimicrobials.

The incidence of adverse events in the current study is similar to that reported by Sori (2005). However, Daleel (1971) reported very low incidence with only two cases out of 86,035 vaccinates in Sudan. The lower incidence reported in the Sudan trial might be due to a difference in the vaccine used. The T_1/44 broth vaccine was used in the trial carried out in Sudan; this particular vaccine has been reported to have low incidence of adverse event *post*-vaccination (Karst, 1971). The incidence reported in our study is lower than reported in Zambia (Revell, 1973). Revell (1973) reported tail-tip slough off incidence of 12% (corresponding to 120 cases per 1000 vaccinates) based on 6,673 animals vaccinated from 'infected' areas which was higher than the incidence of 0.17% (corresponding to 2 cases per 1000) reported in "uninfected" areas. The author suggested a possibility of biased attendance of herders with animals that had experienced tail-tip loss, because of the promise of free treatments for any adverse reaction following second vaccination.

Changes in tail diameter measurements were used as a tool in assessing tail swelling *post*-vaccination. The tail changes for animals vaccinated with either vaccine were normally distributed around the mean zero indicating no difference. The same tool was

used in the trial carried out in KARI, Muguga and it proved to be useful when tail measurements were recorded daily for the first 30 days *post*-vaccination (Wesonga, H. and Schnier, C. (2009) personal communication). Tail swelling was mostly observed in the first two weeks *post* vaccination in the trial at KARI. Failure to find a difference in tail swelling in my field trial may be due to a long interval between readings but measurement error could also play a role.

A number of non-specific adverse events (death, abortion and lameness) were reported by herders to be associated with CBPP vaccination. In our study, 1 death per 1000 vaccinates was observed based on all vaccinates and 1.5 per 1000 vaccinates based on the subset. Mortality reported in our study was similar to mortality reported by Sori (2005). Much higher incidence of 1% corresponding to 10 adverse events per 1000 vaccinates based on a crude vaccine was reported by Louis Willems, which prompted a search for safer vaccines (Huygelen, 1997).

Three peaks of adverse events (specific, non-specific and antimicrobial treatments) were observed based on the subset. In the first peak, specific, non-specific and antimicrobial treatments were all recorded at high incidence, although antimicrobial treatments had the highest incidence. This could be related to increased reports of diseases such as ephemeral fever coinciding with vaccination. The second peak involved specific adverse events and antimicrobial treatments; the third peak of adverse events was observed between 32-38 days *post*-vaccination and all adverse events (specific, non-specific and antimicrobial treatments) were at high incidence. The third peak of events coincided with the follow up visit by a bleeding team which suggest a possibly of reporting bias.

Field vaccine handling has been reported as an important aspect in assuring viability of the live vaccines. In our study, vaccine handling was assessed based on the maintenance of cool box temperatures below 10˚C and use of the reconstituted vaccine within 2 hours. The 10˚C temperature cut off was selected because it was thought to be within the range of recommended vaccine storage temperature of 4˚C and 23˚C which also

revealed good maintenance of vaccine viability in a trial (Ngbede *et al*., 1994). Generally, the cool box temperatures were maintained below 10˚C and median vaccine use time was within the recommended time. However, there were several occasions when the temperature and use time conditions were not met. This suggests that there is a need for vaccination teams to have a plan of assessing if the guidelines on the use of vaccine are being adhered to. Vaccine titres were not evaluated during use and it is unknown whether prolonged use or high cool box temperatures actually affected viability of the vaccine.

The result shows that the buffered vaccine was associated with increased odds of specific adverse events when compared to the standard vaccine. The effect of vaccine on specific adverse events was modified by age (age was also correlated to gender and body condition) of the animal. The results show increased odds of specific adverse events in young animals vaccinated with either vaccine. Increased specific adverse events in young animals was also reported in a study carried out in Zambia based on the standard vaccine (Revell, 1973). The results also indicate that adult animals vaccinated with the buffered vaccine had increased odds of specific adverse events compared to adults vaccinated with the standard vaccine. Lower odds observed in adults vaccinated with the standard vaccine could suggest that the animals had previously been exposed to the same vaccine type. Age could be a proxy for accumulated exposure to CBPP vaccination or outbreak. It is possible that a previously exposed animal will have antibodies against Mycoplasma that neutralizes the live vaccine stain, resulting in low reactivity (Siegrist, 2007). Revell (1973) observed that adverse events were significantly reduced *post* second vaccination.

The result of the study shows that the odds of specific adverse events in young animals vaccinated with either vaccine is similar. This is consistent with the findings of the safety trial carried out at KARI, Muguga to compare the standard with the buffered vaccine. For the KARI trial, young and unexposed animals were recruited and no significant differences in tail swelling (specific adverse events) between the two

vaccines were observed (Schnier C. (2009) personal communication). The lack of evidence for a vaccine effect could also be attributed to short vaccine use time *post* reconstitution and small sample size in the KARI trial. If vaccine is used within a short time, as in the KARI trial, there would not be enough time for an effect of the buffer on vaccine survival to become manifest.

The effect of the vaccine on the odds of specific adverse events was modified by the origin of animals, with animals from Osupuko developing more specific adverse events than animals from Loita and Mara. The cross sectional study (Chapter 2) revealed that herders in Mara reported a higher frequency of both CBPP outbreaks and vaccinations than herders from Loita. Based on the vaccination survey, herders from Osupuko that participated in the safety study did not report CBPP outbreaks or vaccinations in the past ten years. It is therefore possible that animals from Osupuko experienced a higher incidence of specific adverse events compared to animals from Mara and Loita because they had no previous exposure to the organism.

Despite the effect of vaccine depending on age, gender and animal origin, the buffered vaccine was consistently associated with a higher incidence of specific adverse events than the standard vaccine. The reasons for specific adverse events *post*-vaccination have not been elucidated. Sori (2005) concluded that adverse events were a consequence of improper vaccine handling and injection technique. However, according to Revell (1973), it was found that changing the vaccination needle after every 10 animals did not result in a significant change in the incidence of adverse events. In the controlled vaccine trial conducted in KARI it was observed that animals vaccinated with either standard or the buffered CBPP vaccine had significantly increased tail swelling compared to control animals (animals inoculated with PBS or normal saline alone) (Wesonga, H. and Schnier C. (2008) personal communication). Possibly, CBPP *post*-vaccination local adverse events are associated with *Mmm*SC residual pathogenicity since the vaccines are based on live organisms. In a different study, *Mycoplasma* T_1 vaccine strain was isolated from a skin lesion *post*-vaccination and identified based on PCR (Wesonga and Thiaucourt 2000), demonstrating that the vaccine strain could be

associated with specific adverse events. Therefore, the increase in incidence of specific adverse events could be associated with a stable vaccine titre in the buffered formulation as compared to the standard vaccine. However, vaccination tires were not evaluated in my study. The withdrawal of KH_3J on the basis of being less immunogenic and without *post*-vaccination adverse events (Rweyemamu *et al*., 1995) matches the belief by scientists in the 1940's that local reaction was a proof of vaccine viability (Mowat and Rweyemamu, 1997).

Based on the multivariable model, occurrence of non-specific adverse events did not depend on the age or body condition of an animal. However, the effect of vaccine on the odds of non-specific adverse event was significantly modified by origin of animals. Non-specific adverse events could be related to other factors or diseases specific to the origin. It was not clear how area specific factors could influence vaccine effect. Sori (2005) observed difference in *post*-vaccination adverse events (specific and non-specific) in the zones studied. Poor body condition of vaccinated animals in one zone was suggested to be associated with increased incidence of adverse events.

Based on the multivariable model, young animals vaccinated with either vaccine had higher odds of antimicrobial treatments than adult animals. This finding may suggest that antimicrobials were used in the susceptible group to treat both specific and non-specific adverse events. Antimicrobial treatments have been recommended in treating local adverse events *post* CBPP vaccination (Mowat and Rweyemamu, 1997). Increased antimicrobial treatments in animals with adverse events were reported in Ethiopia, where timely treatments were responsible for decreased case-specific mortality (Sori, 2005). However, our study showed higher incidence of antimicrobial treatments than of specific and of non-specific adverse events combined. In our study population, antimicrobial treatments were very common and we suspect that antimicrobial treatments *post*-vaccination were not restricted to treating adverse events *post*-vaccination but also included treatments for other bacterial and secondary bacterial infections following protozoan or viral diseases prevalent in the area. Indeed, during the

follow up period diseases such as ephemeral fever, trypanosomosis, East Coast Fever, anaplasmosis, babesiosis and Foot and Mouth Disease were reported

4.4.2 Conclusion

The current study shows that both the standard and the buffered vaccines gave rise to adverse events *post*-vaccination. However, the buffered vaccine was associated with an increased risk, which appeared to be influenced by previous exposure as expressed by age and origin of the animal. The difference between the two vaccines was significant when the vaccines were used over a longer period. The new vaccine was developed based on the expectation that the buffer system would help to maintain a high vaccine titre during use. The observed difference, combined with the underlying theory, thus suggests that the amount viable *Mmm*SC may have a role to play in the post-vaccination adverse events. Since increased odds of specific adverse events might be related to enhanced efficacy, which is also thought to be due to increased viability of *Mmm*SC, it is emphasized that when developing more efficacious CBPP vaccines, vaccine safety should be given due consideration.

4.5 References

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Chapter 5 - A comparative CBPP vaccine efficacy estimation based on a field trial carried out in south-western Kenya

Abstract

A double-blind CBPP vaccine trial was carried out among 79,959 cattle in 458 herds in Loita, Mara and Osupuko divisions of Narok District, Kenya, between February 2007 and March 2008. The objective of this study was to compare period prevalence of clinical signs suggestive of CBPP (coughing and laboured breathing) and seroconversion *post* vaccination in cattle vaccinated with either a standard CBPP-vaccine (Contavax®), currently used in Kenya for control of CBPP or a buffered vaccine manufactured according to suggestions of March (2004).

A total of 164 animals out of 79,959 (0.21%) were reported showing clinical signs suggestive of CBPP, while 32% of cattle in a subset of 1827 cattle seroconverted within 3 months *post* vaccination. Compared to animals vaccinated with the buffered vaccine, animals vaccinated with the standard vaccine had increased odds of showing clinical signs and lower odds of seroconversion *post* vaccination. The effect of the vaccine on the odds of showing clinical signs was modified by age, gender and origin of animals.

Interpretation of the results of our study is not straightforward because it is not known whether the animals that seroconverted *post* vaccination were the same animals that were protected against developing respiratory signs suggestive of CBPP positive. Since the buffered vaccine was additionally associated with increased odds of specific adverse events (Chapter 3), there is a need to develop safer CBPP vaccines with enhanced protection.

5.1 Introduction

CBPP control has been based on vaccination and animal movement control. Vaccination initially was based on broth T_1 (1920's to early 1970's) vaccine (Davies *et al*., 1968; Gilbert *et al*., 1970) which was later replaced by freeze dried live attenuated *Mmm*SC vaccine T_1/44 vaccine (OIE, 2008). A streptomycin variant (T_1sr) was developed and used in combination with Rinderpest vaccine (Rweyemamu *et al.,* 2000). Observations from CBPP outbreaks in the 1990s indicated that the T_1sr vaccine had failed to protect cattle against the disease in Botswana (Amanfu *et al*, 1998; Thiaucourt *et al*, 2000). Failure to protect was attributed to sub-optimal titres of viable *Mmm*SC in the vaccine. According to OIE, CBPP vaccine should have at least 10^8 CFU per dose (OIE, 2004). Previous studies have indicated that lower titres (10^5 CFU per dose) were not protective but vaccine titres from 10^7 and above were protective (Gilbert and Windsor, 1971).

Low vaccine titres have been attributed to low viability of the vaccine culture caused by metabolism of glucose in standard Mycoplasma growth media (Gourlay's) (Windsor, 1978), which results in a drop in pH to values below the optimum of 7.4 (Gourlay and MacLeod, 1966). To prevent the drop in pH and thereby increase viability and vaccine efficacy, inclusion of a buffer system based on 4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) to standard Gourlay's culture media has been suggested. In the experiment reported by Waite and March, 2001 it was observed that the pH of standard media (unbuffered) dropped from 7.4 to 5.5 during growth, and this decline was mirrored by a rapid drop in titre from 10^{10} per ml to completely inactivated within 2 days at 37°C. However, the pH of HEPES-buffered media dropped from 8.0 to 7.1 during growth, and both the final bacterial titre and culture survival were increased compared to standard media; maximum titre was at least one log10 higher and maintained above 10^8 after a month kept at 37°C. To give additionally buffering effect, reconstitution with phosphate buffered saline was suggested (March, 2004).

CBPP vaccine efficacy under field conditions has not been evaluated in a pre-planned and systematic manner. The current study compared two vaccines, a standard vaccine (Contavax®) currently used in Kenya for control of CBPP and a buffered vaccine

manufactured according to the suggestions of March (2004). It was hypothesized that inclusion of a buffer-system to both growing media and diluent would result in increased survival of *Mmm*SC, and thereby higher vaccine protection. The aim of this study was to compare

i. Prevalence of clinical signs suggestive of CBPP in cattle vaccinated with either of the vaccines within a year *post* vaccination
ii. Seroconversion in cattle vaccinated with either of the vaccines within 3 months *post* vaccination

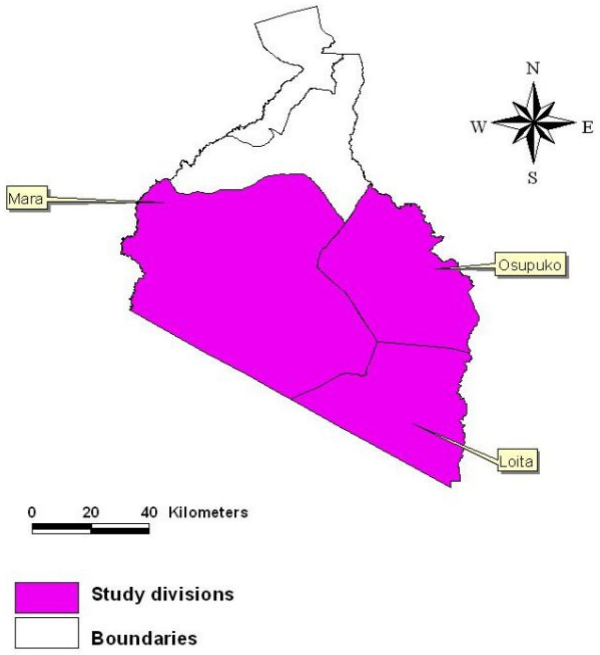
In addition, detailed outbreak investigations were carried out upon receipt of a report of the death of an animal with clinical signs and *post mortem* lung lesions suggestive of CBPP. Clinical diagnosis was presumptive and no confirmatory tests were carried out.

5.2 Materials and methods

5.2.1 Study area and design

A cohort study designed to compare efficacy of the standard vaccine with the buffered vaccine was carried out between February 2007 and February 2008. The study involved animals from Loita, Mara and Osupuko divisions of Narok south district, Kenya (Figure 5.1). A detailed account of the study area and design is given in Chapter 4.

Figure 5.1: Study area located in the district of Narok, south-western Kenya



5.2.2 Vaccines and vaccination

Both vaccines were based on freeze dried live attenuated *Mmm*SC T_1/44 and were manufactured by the Kenya Agriculture Research Institute - Veterinary Vaccine Production Centre (KARI-VVPC) in Nairobi. They differed in that the standard vaccine

was cultured in un-buffered growth media and was reconstituted with normal saline while the buffered vaccine was cultured in HEPES-buffered growth media and reconstituted with phosphate-buffered saline (PBS).

Vaccination was carried out at 19 crushes selected from 38 listed in the three divisions. Two teams, one from the Narok District Veterinary Office and one from the Naroosura Agro-Vet Services, were involved in the vaccination. At the crush, group of 20-30 animals were assigned alternately to either standard or buffered vaccine and all vaccinates were ear-tagged to enable identification during follow-up visits. All animals within a herd were vaccinated and effort was made to vaccinate 50% with each vaccine. Unvaccinated animals and animals with unknown vaccine status could enter the herd during the follow-up period due to purchases, tribal exchanges and births.

5.2.3 Monitoring for clinical signs

Monitoring was carried out by trained community animal health workers for a period of one year *post* vaccination. To record all diseases, animals were monitored weekly during the first month *post* vaccination and then monthly thereafter (Appendix 4). Clinical signs suggestive of CBPP, which herders were capable of recognizing (Zessin *et al.,* 1985), were 'laboured breathing' and 'coughing'. Because cattle could have been showing any of those clinical signs even before vaccination, a period prevalence rather than an incidence of clinical signs was estimated In addition to recording clinical signs, detailed outbreak investigations were carried out upon receipt of a report of the death of an animal with clinical signs and *post mortem* lung lesions suggestive of CBPP (a firm discoloured lung with marbled appearance and a chest cavity filled with yellowish fluid; (FAO, 2002)). Outbreak investigations were carried out by the author and/or the district veterinary officers within a week after receipt of the report and involved clinical examination and bleeding of all animals that shared the same *boma*. Serum samples were processed and tested using the cELISA kit developed by CIRAD-EMTV (detailed in Chapter 2.

5.2.4 Selection of subset monitored for seroconversion

To compare the effect of the vaccines on seroconversion, 2400 animals were recruited as a subset from the study population. The sample size of the subset was calculated using equation [1]

$$n = \frac{\left\{M_{\alpha}\sqrt{2\rho(1-\rho)} + M_{\beta}\sqrt{\rho_1(1-\rho_1)+\rho_2(1-\rho_2)}\right\}^2}{(\rho_2-\rho_1)^2} \qquad [1]$$

where n = sample size for each group; ρ_1 = seroconversion in standard group (0.20); ρ_2 = seroconversion in buffered group (0.25); $\rho = (\rho_1 + \rho_2)/2 = 0.225$; $M_{\alpha/2}$ = multiplier associated with 95% CI (1.96); M_{β} = multiplier associated with β probability of type II error (0.84) (Thrusfield, 2005).

The estimate for the seroconversion in cattle vaccinated with the standard vaccine was based on a study in an endemic area (Provost *et al.,* 1987). A 5% difference in seroconversion between the two vaccines was chosen to detect a relatively small improvement in vaccine efficacy. The formula indicated a sample number of 1092 animals per vaccine; however, the number was increased to 1200 animals per vaccine to compensate for any withdrawals and losses to follow-up. Animals were recruited from 60 herds selected on the basis of the herders' willingness to comply with bleeding schedules and regular visits as per protocol of the study. From each herd, 40 animals were selected purposefully to include 20 animals per vaccine type and to include cattle of all sex and age groups. Animals were bled *pre*-vaccination and approximately one month and three months *post* vaccination. Bleeding and sera storage was carried out as detailed for the cross sectional study (Chapter 2). Serum samples were tested using a commercially available competitive ELISA (CIRAD-EMTV) test. An animal was regarded to have seroconverted if it was seronegative (percent inhibition (PI) <50) pre-vaccination and seropositive (PI≥50) at either or both of the *post* vaccination bleeding.

5.2.5 Statistics

Descriptive statistics were carried out using statistical package STATA (Intercooled 8th version; StataCorp, Texas, USA). Outcome variables were clinical signs suggestive of CBPP (Yes/No) based on all vaccinates; seroconversion *post* vaccination (Yes/No) based on the subset and testing positive in a cELISA (Yes/No) and clinical signs suggestive of CBPP (Yes/No) based on the detailed outbreak investigation. Separate models were developed for each outcome. Clinical signs and seroconversion were analysed using generalized linear mixed models (GLMM) with 'herd' and 'crush' included as random effects. Testing positive in a cELISA and clinical signs suggestive of CBPP *post* 'outbreak' were analysed using GLMM with *boma* included as a random effect. Data were analysed using GenStat Release 10.2 (Lawes Agricultural Trust, Rothamsted Experimental Station).

Because the objective of the analysis was to compare the two vaccines, the variable relating to the type of vaccine was the only independent variable maintained in all models. To test whether the effect of the vaccine was modified by other variables, interaction terms were added. A detailed description of variables included in the analysis is given in Table 5.1.

Table 5.1: Description of variables included in the models

Dependable variable	Description
Age	Age in years categorized (Young: ≤3 yrs; Adult: >3 yrs)
Herd	Group of animals vaccinated under one herder name
Body condition	Body condition score (Good; Fair)
Crush	**Loita**: Enkoseremai, Entasekera, Morijo, Nkopon, Olmesutie and Olorte. **Mara**: Sekenani, Oldisare, Oloonchora, Ololturoto, Olpusimoru, Orkinyei and Talek. **Osupuko**: Elang'ata Enterit, Naroosura, Nkimpa, Ntuka, Olepariata and Oloigeruno.
Division	Administrative unit (Loita; Mara; Osupuko)
Gender	Animal gender (Male; Female)
Team	Vaccine delivery team for an individual animal (Narok; Naroosura)
Vaccine type	Vaccine type administered to an individual animal (Standard; Buffered)

5.3 Results

5.3.1 General description of study animals

In total, 79,959 animals from 458 herds were vaccinated against CBPP; 49.1% were administered with the standard vaccine and 50.9% with the buffered vaccine. In both cohorts, most animals were Zebu type and in good body condition (Table 5.2).

In the subset, 2038 animals were included, of which 211 tested cELISA positive pre-vaccination and were therefore omitted from analysis. Of the 1827 remaining animals, 949 (51.9%) were vaccinated with the standard vaccine and 878 (48.1%) with the buffered vaccine (Table 5.2).

A total of 293 animals belonging to four *bomas* with suspected outbreaks of CBPP were included in the outbreak investigations. Of these, 141 were vaccinated with the standard vaccine and 152 with the buffered vaccine (Table 5.2). Several animals with unknown vaccine history were excluded from analysis. All *bomas* included in the outbreak investigation were from Mara division and had been vaccinated by the Narok team.

Table 5.2: General characteristics of the study animals

Variable	Variable levels	Number of cattle (%) All vaccinates		Number of cattle (%) Subset		Number of cattle (%) Outbreak subset	
Division	Loita	26,632	(33.3)	614	(33.6)		
	Mara	27,731	(34.7)	599	(32.8)	293	(100.0)
	Osupuko	25,596	(32.0)	614	(33.6)		
Body condition score	Good	73,923	(92.4)	1,632	(89.3)	214	(73.0)
	Fair	6,036	(7.6)	195	(10.7)	79	(27.0)
Gender	Male	25,521	(31.9)	538		66	(22.5)
	Female	54,425	(68.1)	1,289	(29.4) (70.6)	227	(77.5)
Age	Young ≤3 years	17,802	(22.3)	626	(34.3)	91	(31.1)
	Adult ≥4 years	62,131	(77.7)	1,201	(65.7)	202	(68.9)
Breed	Zebu crosses	469	(0.6)	19	(1.0)		
	Zebu	79,490	(99.4)	1,808	(99.0)	293	(100.0)
Team	Narok team	42,087	(52.6)	822	(45.0)	293	(100.0)
	Naroosura team	37,872	(47.4)	1,005	(55.0)		
Vaccine type	Standard	39,234	(49.1)	949	(51.9)	141	(48.12
	Buffered	40,725	(50.9)	878	(48.1)	152	(51.9)

5.3.2 Univariable analysis based on clinical signs suggestive of CBPP (all vaccinates)[1]

A total of 164 animals out of 79,959 (0.21%) were reported showing clinical signs of coughing and laboured breathing during the follow-up period of one year. The period prevalence of clinical signs suggestive of CBPP was not significantly different between the two vaccines (OR of buffered vaccine vs. standard = 0.81). Compared to animals from Loita, animals from Mara had significantly higher period prevalence (P <0.01) whilst those from Osupuko had a significantly lower period prevalence. Compared to animals vaccinated by the Narok team, animals vaccinated by the Naroosura team had significantly higher prevalence (P <0.01) (Table 5.3).

[1] Vaccine used in the current study were produced based on Standard Operation Procedures for quatity production of CBPP vaccines and OIE guidelines and Author was adviced that both vaccine had titre that met minimum standard specified in the OIE manual (OIE, 2004). Vaccine stability was extrapolated based on the previous studies (Waite and March, 2001).

Table 5.3: Univariable analysis of period prevalence based on all vaccinates

Variable	Number[1]	Signs incidence (95%CI)[2]	Odds Ratio (95% CI)[2]
Clinical signs	79,959	0.21 (0.17-0.24)	
Age			
Young	17,802	0.24 (0.17-0.33)	Reference level
Adult	62,131	0.19 (0.16-0.23)	0.81 (0.57-1.14)
Body condition			
Good	73,923	0.21 (0.18-0.24)	Reference level
Fair	6,036	0.17 (0.08-0.30)	0.79 (0.42-1.51)
Division			
Loita	26,632	0.17 (0.13-0.23)	Reference level
Mara	27,731	0.37 (0.30-0.45)	2.15 (1.52-3.05)***
Osupuko	25,596	0.06 (0.03-0.10)	0.34 (0.19-0.61)***
Gender			
Male	25,521	0.20 (0.15-0.26)	Reference level
Female	54,425	0.21 (0.17-0.25)	1.04 (0.75-1.45)
Team			
Narok	42,087	0.15 (0.11-0.19)	Reference level
Naroosura	37,872	0.27 (0.22-0.33)	1.83 (1.33-2.51)***
Vaccine type			
Standard	39,234	0.22 (0.17-0.27)	Reference level
Buffered	40,725	0.19 (0.15-0.24)	0.90 (0.66-1.22)

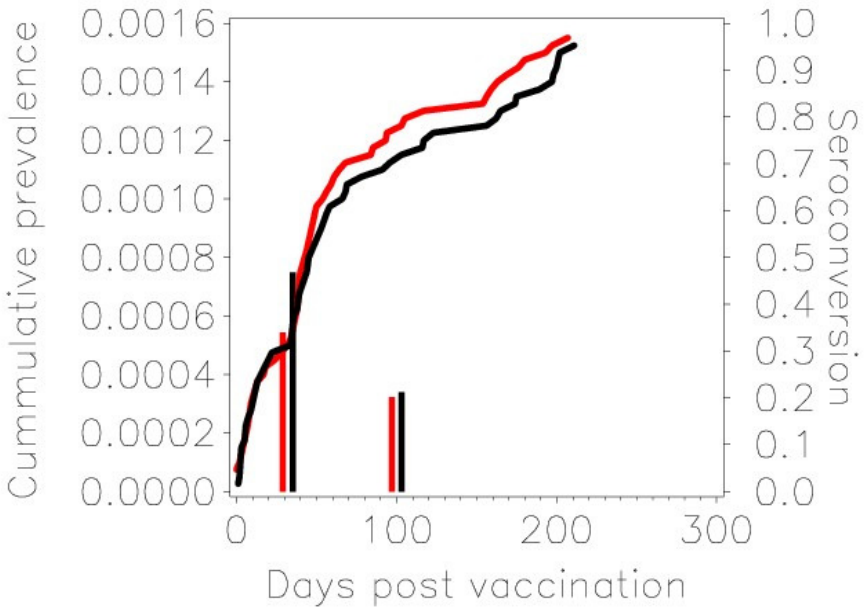
1. number of animals monitored
2. *P*-value indicated by superscript: ** $P < 0.10$ ** $P < 0.05$ *** $P < 0.01$.

In the first month *post* vaccination, the cumulative period prevalence was similar between the two vaccine types. Thereafter, animals vaccinated with the standard vaccine had higher prevalence compared to animals vaccinated with the buffered vaccine (Figure 5.2).

5.3.3 Univariable analysis based on seroconversion *post*-vaccination

In the subset, 32% of vaccinated animals seroconverted within the first 3 months *post* vaccination. Seroconversion of animals vaccinated with the standard vaccine was 28%, which was significantly lower than the 36% seroconversion of animals vaccinated with the buffered vaccine ($P < 0.01$). Compared to animals vaccinated with the standard vaccine, more animals vaccinated with the buffered vaccine had seroconverted one month *post* vaccination. At three months *post* vaccination seroconversion was similar between the vaccine types but lower than one month *post* vaccination (Figure 5.2).

Figure 5.2: Cumulative period prevalence of clinical signs suggestive of CBPP (lines) and seroconversion (bars) post vaccination with buffer (black) and standard (red) vaccine



Compared to animals that were vaccinated by the Naroosura team, significantly more animals vaccinated by the Narok team seroconverted (P <0.01). Animals from Mara had significantly higher seroconversion compared with animals from Loita, (P <0.05) (Table 5.4).

Cross tabulation of independent variables revealed that Naroosura team was positively correlated with adult, female gender and negatively correlated with fair body condition score. Also, adult positively correlated to female and negatively correlated to fair body condition score'

Table 5.4: Univariable analysis of seroconversion based on vaccination subset[1]

Variable	Number	Seroconversion[2] (95%CI)	Odds Ratio (95% CI)[3]
Overall seroconversion	1,827	31.96 (29.82-34.11)	
Vaccine type			
Standard	949	27.92 (25.09-30.90)	Reference level
Buffered	878	36.33 (33.14-39.61)	1.47 (1.21-1.79)***
Team			
Narok	822	36.13 (32.84-39.52)	Reference level
Naroosura	1,005	28.56 (25.78-31.46)	0.70 (0.58-0.86)***
Age			
Young	626	31.47 (27.85-35.27)	Reference level
Adult	1,201	32.22 (29.58-34.95)	1.04 (0.84-1.27)
Body condition			
Good	1,632	32.29 (30.03-34.62)	Reference level
Fair	195	29.23 (22.95-36.15)	0.87 (0.63-1.20)
Gender			
Male	538	29.55 (25.73-33.61)	Reference level
Female	1,289	32.97 (30.41-35.61)	1.17 (0.94-1.46)
Division			
Loita	614	30.62 (26.99-34.43)	Reference level
Mara	599	37.90 (34.00-41.92)	1.38 (1.09-1.75)**
Osupuko	614	27.52 (24.03-31.24)	0.86 (0.67-1.10)

1. animals were selected from the vaccinates for bleeding
2. animals that were negative at baseline but positive at first or second bleeding *post* vaccination
3. P-value indicated by superscript: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$.

5.3.4 Univariable analysis based on detailed outbreak investigation subset

In herds suspected of having an outbreak of CBPP, 17% of cattle tested positive using the cELISA. Compared to animals vaccinated with buffered vaccine, animals vaccinated with the standard vaccine had a higher seroprevalence; however, this difference was not significant. In the same herds, approximately 6% of vaccinated animals were observed with clinical signs suggestive of CBPP. Compared to animals vaccinated with the buffered vaccine, animals vaccinated with the standard vaccine had higher prevalence of clinical signs, but again the difference was not significant (Table 5.5).

Table 5.5: Univariable analysis of cELISA and clinical signs based on outbreak subset

Variable	**Number**	**Seroprevalence (95%CI)** cELISA	**Odds Ratio (95% CI)**	**% Signs (95%CI)** Clinical signs	**Odds Ratio (95% CI)**
Animals	293	17.41 (13.04-21.77)		5.80 (3.11-8.49)	
Age					
Young	91	13.19 (7.00-21.90)	Reference level	7.69 (3.15-15.21)	Reference level
Adult	202	19.31 (14.10-25.43)	1.58 (0.78-3.17)	4.95 (2.40-8.92)	0.61 (0.23-1.70)
Body condition					
Good	214	21.03 (16.77-27.11)	Reference level	6.54 (3.62-10.73)	Reference level
Fair	79	7.59 (2.84-15.80)	0.31 (0.13-0.76)**	3.80 (0.79-10.70)	0.56 (0.16-2.02)
Gender					
Male	66	18.18 (9.76-29.61)	Reference level	12.12 (5.38-22.49)	Reference level
Female	227	17.18 (12.51-22.73)	0.93 (0.46-1.91)	3.96 (1.82-7.39)	0.30 (0.11-0.81)**
Vaccine type					
Standard	141	20.57 (14.23-28.18)	Reference level	6.38 (2.96-11.77)	Reference level
Buffered	152	14.47 (9.30-21.09)	0.65 (0.36-1.20)	5.26 (2.30-10.11)	0.81 (0.31-2.17)

5.3.5 Multivariable model based clinical signs suggestive of CBPP (all vaccinates)

Compared to animals vaccinated with the buffered vaccine, those vaccinated with the standard vaccine had a higher prevalence of clinical signs suggestive of CBPP. The effect of the vaccine on the prevalence was modified by the age, gender, body condition and origin of vaccinates. In the young group, cattle vaccinated with the standard vaccine had a higher prevalence of clinical signs compared to cattle vaccinated with the buffered vaccine; in the adult group, the prevalences were not different. Male cattle vaccinated with the standard vaccine had a higher prevalence of clinical signs when compared to cattle vaccinated with buffered vaccine; in female animals, the prevalences were not different. Finally, animals from Mara vaccinated with either vaccine had a higher prevalence of clinical signs when compared to cattle vaccinated with either vaccine from Osupuko and Loita. Animals from Osupuko vaccinated with the buffered vaccine had the lowest prevalence of clinical signs. There was no evidence that the effect of vaccine on the prevalence of clinical signs was modified by the vaccinating team (Table 5.6; detailed in Appendix ix.).

5.3.6 Multivariable model based on seroconversion *post* vaccination

Compared to animals vaccinated with standard vaccine, those vaccinated with the buffered vaccine had increased odds of seroconversion *post* vaccination ($P < 0.01$). There was no evidence that the effect of vaccine on seroconversion was modified by vaccinating team, age, gender or body condition of the animal (Table 5.6: detailed in Appendix x).

Table 5.6: Multivariable linear logistic mixed model based on all vaccinates and subset

Variables	Standard vaccine Odds (10^{-3})	Buffered vaccine Odds (10^{-3})	Standard vaccine Odds	Buffered vaccine Odds
	Clinical signs based on all vaccinates		Seroconversion based on subset	
Vaccine type	0.41	0.31	0.27	0.36
Young animals	0.60	0.28	0.26	0.32
Adult animals	0.34	0.31	0.28	0.38
Male	0.45	0.19	0.24	0.32
Female	0.38	0.36	0.29	0.38
Good	0.39	0.31	0.28	0.38
Fair	0.49	0.15	0.24	0.24
Loita	0.51	0.38	0.26	0.37
Mara	0.90	0.95	0.35	0.40
Osupuko	0.17	0.08	0.22	0.31
Narok	0.30	0.24	0.30	0.42
Naroosura	0.54	0.56	0.25	0.31

5.3.7 Multivariable model based on detailed outbreak investigation

After correcting for the effect of 'enclosure', the model showed that, compared to animals vaccinated with the buffered vaccine, animals vaccinated with standard vaccine had significantly increased odds of testing cELISA-positive in herds suspected of having an outbreak of CBPP (P <0.10). There was no evidence that the effect of vaccine on the odds of testing cELISA-positive was modified by age, gender or body condition of the animal (Table 5.7; detailed in Appendix 3a). There was no evidence that the effect of vaccine on clinical signs was dependent on vaccine type, age, gender and body condition (Table 5.7; detailed in Appendix xi).

Table 5.7: Multivariable linear mixed model based on detailed outbreak investigation

Variables	Standard vaccine Odds	Buffered vaccine Odds	Standard vaccine Odds	Buffered vaccine Odds
	Seroprevalence based on cELISA		Clinical signs	
Vaccine type	0.22	0.13	0.05	0.04
Young animals	0.19	0.05	0.08	0.04
Adult animals	0.23	0.18	0.04	0.04
Male	0.22	0.11	0.14	0.06
Female	0.21	0.14	0.03	0.03
Good	0.25	0.16	0.06	0.04
Fair	0.12	0.05	0.03	0.05

5.4 Discussion and conclusion

5.4.1 Discussion

Vaccine efficacy studies have traditionally been carried out under controlled conditions (Thiaucourt *et al.*, 2000; Masiga and Windsor 1974; Hudson and Turner, 1963). Field trials designed to assess CBPP vaccine efficacy are rare but analogous studies have been reported for other *Mycoplasma* diseases. For example, the efficacy of a single-dose of *Mycoplasma hyopneumoniae* vaccine was evaluated in 3 to 5 week old pigs. Vaccination was associated with significantly lower percentage of lung lesion scores (Dawson *et al.*, 2002) compared to the controls. My field study on CBPP vaccine efficacy is based on period prevalence of clinical signs suggestive of CBPP and seroconversion *post* vaccination.

The results from my study suggests greater clinical protection of cattle vaccinated with the buffered vaccine, with animals vaccinated with the standard vaccine showing a higher prevalence of clinical signs suggestive of CBPP. Improved clinical protection by the buffered vaccine could be related to stable viability of *Mmm*SC resulting from the use of buffering systems at vaccine production and field application stages (March, 2004; Waite and March). However this is extrapolation from results from Waite and March (2001) because vaccine titre evaluation during use was not carried out. The vaccine effect on clinical signs was modified by age, gender and origin of animals. As buffering the vaccine may enhance vaccine titre, this observation suggests that unexposed animals (younger animals and animals from Loita and Osupuko) may require a relatively higher titre of vaccine to mount a protective immune response compared to animals that have had chance of being exposed to CBPP e.g. older animals and those from Mara. Therefore, by maintaining the vaccine titre, improvements in vaccine efficacy would be more evident in naive than in exposed animals.

The current study suggests that vaccination with the buffered vaccine was associated with reduced clinical signs suggestive of CBPP. The use of respiratory signs as indicator for clinical CBPP may have been aspecific, because coughing and difficult breathing are

not related to CBPP only but also to other diseases endemic in the study area, such as East Coast Fever and Ephemeral fever. Detailed information on differential diagnosis of CBPP is provided elsewhere (FAO, 2002).

Higher odds of seroconversion in animals vaccinated with the buffered vaccine compared to animals vaccinated with the standard vaccine was observed and suggested that the vaccine effect was not modified by vaccinating team, age, body condition, gender or origin of the animals. These results conflict with those of a controlled trial carried out at KARI-Muguga, which showed no evidence of any effect of the type of vaccine on seroconversion (Wesonga, H. and Schnier, C. personal communication). The difference between these two studies could result from variation in the time between reconstitution of the vaccine and vaccination. In the controlled trial, the vaccine was used within 20 minutes *post* reconstitution (Wesonga, H. personal communication), which was too short for the effect of the buffer to be observed. In the field trial, vaccines were used within an average of 95 minutes *post* reconstitution. In addition, 30 animals per group were used in experimental trial at KARI, which might not have been enough to detect small vaccine improvements.

The results from the current study indicate that increased odds of seroconversion *post* vaccination for cattle vaccinated with the buffered vaccine were only seen one month *post* vaccination; the difference was very small 3 months *post* vaccination. Interestingly, reduced prevalence of clinical signs suggestive of CBPP for cattle vaccinated with the buffered vaccine were only seen later than one month *post* vaccination. The higher seroconversion associated with buffered vaccine preceded the reduced clinical signs. This might be taken as evidence that seroconversion enhanced the difference in reporting of clinical signs between the vaccines.

Based on the detailed outbreak investigations, 17% of cattle tested cELISA-positive in herds suspected of having an outbreak of CBPP. Compared to animals vaccinated with the buffered vaccine, animals vaccinated with the standard vaccine had increased odds

of testing positive. However, because it is not clear whether testing positive indicates vaccine success (seroconversion due to vaccination) or failure (seroconversion due to infection despite vaccination), interpretation of these results is difficult. This finding re-enforces the view that serodiagnosis is a challenge in areas where vaccination is being practiced (Chapter 2). It also highlights the importance of DIVA vaccines.

The current study shows that vaccination with the buffered vaccine was associated with increased seroconversion, reduced clinical signs and reduced seroprevalence *post* outbreak. However, interpretation of this result should be guarded, because it is not clear whether the animals that seroconverted *post* vaccination were the same animals that were protected against developing respiratory signs suggestive of CBPP and against testing positive *post* 'outbreak'. Therefore care should be taken to avoid ecological bias, which occurs when an association observed at a group level does not necessarily represent an association that exists at an individual level.

5.4.2 Conclusion

Generally, the current study shows that the buffered vaccine was associated with a reduction in clinical signs suggestive of CBPP and an increase in seroconversion *post* vaccination. Our findings are consistent with the hypothesis that buffering results in increased survival of the *Mmm*SC, contributing to increased seroconversion, decreased clinical cases *post* vaccination and low seroprevalence *post* 'outbreak'.

Development of a DIVA vaccine might be an option to enhance differentiation of vaccinated from infected animals. In addition, since improved vaccine viability might have been associated with increased adverse events *post* vaccination; there is a need for safer vaccines with enhanced protection.

5.5 References

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Chapter 6 – Discussion and outlook

6.1 General discussion

CBPP control in Europe, the USA and Japan was possible through stamping out and strict control of animal movement (Masiga *et al.*, 1996; Rweyemamu and Benkirane, 1996; Provost *et al.*, 1987). Among African countries that reported CBPP in the 1990s, only Botswana managed to eradicate CBPP, based on a stamping out policy (Amanfu *et al.*, 1998). CBPP control based on stamping out is an expensive policy to implement and not many African countries can afford it because of economic constraints. Most African countries allocate less than 10% of national budgetary resources to agriculture and rural development and only part of those resources is allocated specifically for animal disease control programmes (ftp://ftp.fao.org/unfao/bodies/arc/23arc/; last accessed on 16th April 2009). In other countries, such as Portugal, test and cull was practiced in addition to strict cattle movement control (FAO, 2006). A testing-based policy, however, would be challenging to implement in CBPP-endemic countries of Africa where vaccination has been practiced for the past twenty years or more. It was observed that CBPP vaccination was partly responsible for seropositivity (Chapter 2), suggesting that the OIE-recommended serological tests were unable to differentiate vaccinated and infected animals. Therefore, implementation of a testing-based policy would require improved diagnostic tests that can differentiate vaccinated from infected animals. CBPP control by vaccination also requires strict control of animal movement (FAO-2002). In Namibia, it was observed that vaccination and movement control resulted in drop of both morbidity and mortality (Bamhare and Kohrs 2000). Combination of strategies is important because vaccine efficacy is less than 100%. Combination of strategies would limit the risk of disease introduction as well as the risk of disease spread if introduction happened. However, animal movement control is difficult to enforce in pastoral systems because transhumance is a coping strategy practiced during the dry season. Most of the respondents in the cross-sectional study had their animals mixing with other herds during grazing and watering and at salt lick points. More than 75% of respondents brought in animals through gifts, dowry or purchases (Chapter 2). These activities are

part of socio-cultural practices in the Maasai community and make control of animal movement very difficult to impose.

Knowledge exchange forms an important part of animal disease control programmes. Herders in the study area were aware of clinical signs of CBPP and considered vaccination to be the major control method. However, only 27% of herders (48 out of 175) knew that protection offered by the vaccine lasted for a year at most and only 57% (100 out of 175) would like their animals to be vaccinated annually. Therefore, there is knowledge gap in herder's understanding of the duration of protection offered by the vaccine and number of vaccinations required to induce "herd immunity". Although this has not been worked out specifically for CBPP, it is recommended that 100% of animals should be vaccinated 3-5 years consecutively (FAO-EMPRES, 1995). Herders understanding of the control options (e.g. combination of vaccination and control of movement) and their full participation in disease control efforts is therefore important. On the other hand, governments should assure or enable availability of affordable vaccines and maintenance of surveillance programmes.

Studies on the spatial distribution of infectious diseases such as bovine tuberculosis (bTB) have been carried out to understand mechanisms involved in the spread of the disease. For infectious diseases, short distance spread is a result of contagion to adjacent or nearby farms. This may happen by direct contact and results in local spatial clustering (Gilbert *et al*., 2005). The CBPP cluster identified based on the scan statistic is an example of short distance spread of the disease (Chapter 3). Short distance spread could have happened for example in herds located near to the water source. Location of herds close to the water source was associated with increased risk of being classified as CBPP positive (Chapter 3). Hot spots observed during the current study were observed at a single point in time. Hot spots may persist or recur or disappear over time as observed with bTB data collected over a period between 1986 and 1997 in Great Britain (Pfeiffer *et al*., 2008). Since the hot spots areas are areas of increased risk, it means that efforts could be directed to interrupt disease transmission through targeted disease control

programmes. This could be carried out in the form of ring vaccination to create a buffer zone. According to OIE regulations, a buffer zone is established to protect animals in a free zone/country from those of different health status. This can be carried out through measures such as vaccination, quarantine, movement control and surveillance (OIE, 2008). Unfortunately, due to the inability of the tests to differentiate infected from diseased animals, it was not clear if the hot spots in my study were centres of disease or centres of vaccination

Control of CBPP based on antibiotic treatment is controversial. There is a suspicion that its use could facilitate the developments of sequestra, carrier animals, and resistant strains, and that it could mask the occurrence of clinical disease (Provost *et al.*, 1987). In addition, antibiotics were commonly used by herders for a number of conditions (Chapter 4). Herders participating in our study shared the opinion that animals treated with antibiotic seem to recover but fall sick again after few months. Studies to assess the usefulness of antibiotics in the control of CBPP have been reported (Hübschle *et al.*, 2006; Twinamasiko *et al.*, 2004; Yaya *et al.*, 2004; Ayling *et al.*, 2000). Findings based on animal trials indicated decreased *Mmm*SC transmission to susceptible animals and decreased mortality compared to untreated animals. In its fourth meeting, the consultative group on CBPP in Africa emphasized the need for further science-based evidence on the impact of antibiotic use (FAO, 2006), and reiterated that the use of antibiotics is currently not recommended for CBPP control as also stated in the OIE Terrestrial Animal Health Code (Amanfu, 2006).

Vaccination currently remains a better option for CBPP control in pastoral communities of East Africa, but it should be used in combination with movement control and surveillance. In our study, vaccination was not compared with other control options but the majority of herders participating in the study recognized it as the only acceptable control method. Rider Haggard in his novel King Solomon's Mines gives the earliest description of field vaccination against CBPP "As of the lung sick which is a dreadful form of pneumonia, very prevalent in this country, they had all been inoculated against

it. This is done by cutting a slit in the tail of an ox, and binding a piece of the diseased lung of an animal which has died of the sickness. The result is that the ox sickens, takes the disease in a mild form, which causes its tail to drop off. It seems cruel to rob an animal of his tail especially in a country where there are so many flies, but it is better to sacrifice the tail and keep the ox than to lose both tail and ox" (Windsor and Masiga, 1977). The Maasai herders that participated in our study were aware of the possibility of post-vaccination adverse events and indicated that they were successfully managed by the use of antibiotics (Chapter 4).

The present PhD work was founded on the findings that use of buffer systems in vaccine production and reconstitution improved and prolonged viability of the live *Mmm*SC vaccine (Waite and March, 2001). It was hypothesized that enhanced viability will improve vaccine efficacy and possibly result in increased occurrence of adverse events. The overall aim of the project was to compare the standard CBPP vaccine and the buffered vaccine under field conditions of south-western Kenya. Results of the vaccine cohort study show that compared to animals vaccinated with the standard vaccine, animals vaccinated with the buffered vaccine had higher odds of developing specific adverse events (Chapter 4). The adverse events concentrated in a few herds and a lack of previous exposure (CBPP vaccination or outbreak) was observed to be positively associated with increased specific events. Additionally, vaccination with buffered vaccine was observed to be associated with increased seroconversion; lower odds of clinical signs post-vaccination and post 'outbreak'; and lower seropositivity post 'outbreak' (Chapter 5). Because different outcome parameters were measured in different subpopulations, it was not clear that specific events post-vaccination, seroconversion or clinical signs of CBPP occurred in the same animals and it is unknown whether seroconversion was associated with decreased risk of clinical disease. Vaccination with the buffered vaccine was associated with an increase in specific adverse event by 15 animals per 10,000; increased seroconversion by 800 animals per 10,000 vaccinates and a decrease in clinical signs suggestive of CBPP by 3 animals per 10,000 vaccinates (Chapters 4 and 5). The extent of increase in seroconversion was far

greater than the increased adverse events and reduced clinical disease. During this study, animals vaccinated with either vaccine were accommodated together and increased protection by one vaccine type would have resulted in reduction of disease spread in the other group, hence modifying the overall protection difference.

The possibility of vaccination being responsible for the observed clinical signs suggestive of CBPP was ruled out because clinical signs were first observed in unvaccinated animals that were introduced into the vaccinated herds. CBPP vaccines have not been observed to revert to virulence (Wesonga *et al*., 2004). Clinical disease has been observed when vaccine strain was introduced into the lung (Mbulu *et al*., 2004). During the current study, vaccine strain was introduced subcutaneously in the tail tip (Chapters 4 and 5). Recruitment of an unvaccinated control group was not possible due to ethical considerations. An unvaccinated control group would have given baseline results for comparison with either vaccine.

6.2 Outlook and future studies

CBPP vaccines currently used in African countries were developed in the 1950's and used as a whole cell vaccine. The information on the specific antigen involved in the protection post-vaccination is limited. For example, a study to investigate vaccine potential of *Mmm*SC capsular polysaccharide (CPS) has been reported (Loiselet, 2002). The results showed that only a quarter of CBPP vaccinated animals had immune responses against CPS. The immune response was of the IgM type even after second exposure to the pathogen. The lack of an immune response was linked to cross reaction of CPS with bovine lung antigens. This finding could possibly explain the small percentage of animals that seroconverted post vaccination. Recent studies to characterize *Mmm*SC- specific humoral responses with sera and bronchial lavage fluid (BAL) taken from experimentally infected animals indicated that IgA levels were high both in sera and BAL samples and tended to persist in subacute and chronic forms of disease (Niang

et al., 2006). The IgA proteins offer the potential for developing a mucosal vaccine. "Mucosal vaccine could offer the benefit from the existence of a 'common mucosal immune system' where immunization at some mucosal inductive sites can activate mucosal B-and T-cells to migrate from these sites and home to mucosal effector cells" (Dedieu-Engelmann, 2008). Another study was conducted to investigate vaccine delivery efficiency using immunostimulating complex (ISCOM). Use of the vaccine in combination with this adjuvant showed reduced mortality post challenge compared to unvaccinated controls (Hübschle *et al.*, 2003). The studies cited here and the results from my study indicate that progress has been made but there is a need to identify specific antigens responsible for protection post-vaccination and possibly for improvement of the vaccine delivery efficiency. Such studies may open the way to the development of DIVA vaccines. Development of a DIVA vaccine has also been recommended by the third meeting of the Consultative group on CBPP in Africa (FAO, 2004). Development of a DIVA vaccine needs to be accompanied by the improvement of diagnostic tests. Proteomic studies have indicated a number of immunogens recognized by pooled sera from experimentally infected animals. These proteins may have potential for the development of CBPP diagnostic tests (Jores *et al*., 2009).

Maasai herders were aware of the poor safety associated with CBPP vaccines and the outcome of the current study was no exception. As part of the Wellcome Trust funded project on CBPP, a scientific evaluation of perceptions and costs and benefits of use of buffered vaccine is underway based on socioeconomic data analysis. Our study has given an indication that vaccination with the buffered vaccine offered better protection but poorer safety than vaccination with the standard vaccine. The finding stresses a need to give vaccine safety due consideration when developing more efficacious CBPP vaccines.

Vaccine handling under field conditions has been suggested to influence the vaccination outcome (safety and efficacy) (Karimuribo *et al.*, 1997). In our study, vaccine handling under field conditions was monitored through vaccine use time and cool box

temperature and it was observed that reconstituted vaccines were sometimes kept beyond recommended time and temperature. There is a need therefore to carry out quantitative analysis of vaccine titre for vials kept in various temperature conditions post reconstitution. This analysis would allow an exploration of whether buffering provides a longer 'window of use' under field conditions, where infrastructure does not allow vaccine to be used within 2 hours and to be kept at ≤4˚C. Evaluation of Xerovac process for preparation of heat tolerant CBPP vaccine has been reported (Litamoi *et al*., 2005). The process involves dehydration of vaccine via careful maintenance of high pressure at the product surface. Further studies were suggested to optimize vaccine culture procedures and the actual xerovac dehydration technique.

The Narok and Naroosura teams used in the current study represented public and private teams respectively. Comparison of public and private teams was not given priority because each partner was represented by only one team. Generally, it was observed that the teams worked very similar and, as a parameter, team had no modification effect on the vaccine efficacy. Decentralization of veterinary services compounded by limited extension services and cost recovery approaches has resulted into poor access to vaccines by rural communities of developing countries (Lubroth *et al*., 2007). The use of private partnership in CBPP vaccine delivery could be assessed and complement vaccine delivery by government teams in the rural areas.

The current trial was carried out in Maasai ecosystem of south-western Kenya, which might be representative of the Maasai ecosystem of Serengeti and Ngorongoro areas of the United Republic of Tanzania. However because of variation in cattle breeds and management systems in pastoral systems of Africa there is a need to explore further their influence. Also, there is need to explore further the role of trypanosomosis in the control of infectious diseases such as CBPP.

The scan statistic test proved to be a useful method for identifying CBPP 'hot spots'. Disease hot spot detection may have great value in disease control therefore a need to

carry out pilot studies to identify and asses the usefulness of targeted CBPP control strategies in CBPP 'hot spots'.

In summary therefore, my study reinforces the need for better tools for CBPP control. Availability of safe and efficacious vaccines is of urgent importance. Vaccination should be carried out in combination with other strategies such as movement control and surveillance. Surveillance will require diagnostic tests that can confirm presence of the disease (increased sensitivity) or rule out the disease (increased specificity), even when vaccination is used in the population that is surveyed. Educating herders about the access, use and limitation of CBPP vaccines and their shared responsibility in CBPP control should be part of integrated approach.

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Appendices

Appendix i: Cross sectional study questionnaire

SERIAL NUMBER …….. Enumerator code----------Date of interview---------/-------/ 2006 Boma ID ----------
START TIME ……....END TIME……....

SECTION A: GENERAL

A1 Name of household Head *surname* ……*other name*……

A2 Respondent's name *surname* ……*other name*……

A3 Sex of respondent: ……. A4 Age set of respondent…… (yrs) A5 Division: ……

A6 Location……..A7 Sub location……

A8 Village name……

A9 GPS reading:

Boma code ……	**Altitude** ……
Latitude ……	
Longitude ……	

A10 Number of households in the boma……

SECTION B: CATTLE DISEASES

B1 What are the cattle diseases that you normally experience in your herd?

1=…….2=…….3=…….4=…….5=…….6=…….7=…….8=…….9=…….10=…… 11=……12…….

B2 Rank the 5 most important cattle diseases/conditions and give the criteria for ranking.

(1 is most important and 5 is least important)

	Disease	Criteria
1		
2		
3		
4		
5		

B3 Do you know CBPP? Y=Yes N=No (*ask this question only if CBPP is not mentioned in B1, otherwise go to B5 if No proceed to section* ***C)***

B4 *W*hat are the clinical symptoms and post mortem lesions of CBPP? (*can mention at least 6 of each,* ***PLEASE DO NOT PROMPT****)*

	Clinical signs	Post-mortem lesions
1		
2		
3		
4		
5		
6		

B5 In which year (s) did you have CBPP in your herd?, how many were affected, died or recovered, what was the duration of the clinical disease, cause of disease and how many animals did you have then, what did you do (interventions) and what was the cost?

	Disease present Y=Yes, N=No	Duration of clinical disease	No. affected	N o. died	No. recovere d	Reasons for recovery	Cattle populatio n then	Interventi ons	Cost of interventi ons	Other costs and	Causes of disease
2005		[ ]				[ ]		[ ]		[ ]	
2004		[ ]				[ ]		[ ]		[ ]	
2003		[ ]				[ ]		[ ]		[ ]	
2002		[ ]				[ ]		[ ]		[ ]	
2001		[ ]				[ ]		[ ]		[ ]	
2000		[ ]				[ ]		[ ]		[ ]	
1999		[ ]				[ ]		[ ]		[ ]	

Duration of clinical disease
1=<3 months
2=3-6 months
3=7-12 months
4=>12 months

5= I don't know

Reasons for recovery
1=treatment
2=vaccination
3=other

Interventions
1=treatment
2=vaccination
3=traditional quarantine
4=government quarantine
5=slaughter
6=report to vet office
7=other (specify)

Other costs
1=additional labour
2=time
3=travel costs
4=other (specify)

B6 **Are there cattle that have suffered the disease more than once in your herd? Y=YesN=No** (*ask farmers that had experienced CBPP*)

B7 **What was the estimated time between any two episodes?**

1=up to 6months
2=between 7-12 months
3=more than 13 months

B8 **Are there neighbours that had experienced CBPP in their herds? Y=Yes N=No**

B9 **From which villages? 1……..2……..3……..**

B10 **Do you treat animals suffering from CBPP?** Y=Yes N=No (*if No go to B18*)

B11 **What type of medicine do you use, route of administration, dosage, duration of treatment and rate of recovery?**

Drug	route of administration	dosage	duration of treatment	rate of recovery (out of 10)

Drug
1=terramycin 10%
2= terramycin 20%
3=terramycin 30%
4=Olchani oiborr
5=Other (specify)

route of administration
1=intramuscular
2=intrapleural
3=Other (specify)

B12 **Is the treatment effective?** Y=Yes N=No

Explain.....

B13 **Do the animals recovered with treatment become sick of CBPP again?** Y=Yes *N=No*

B14 **How long does it take for treated animal to fall sick again?**

1=up to2 months
2=3 – 6 months
3= 7-12 months
4=>12 months

B15 **Are there animals that recover without treatment?** *Y=Yes N=No*

B16 **Do the animals recovered without treatment become sick of CBPP again?** Y=Yes *N=No*

B17 Do you have your cattle vaccinated? Y=Yes N=No *(Please indicate cattle vaccinations for the last 12 months)*

Vaccine Against?	Who vaccinated	When vaccinated (month)	Vaccine source	Age group vaccinated (numbers)			Cost per animal
				≤1 year	1-3 years	≥3 years	
	[ ]		[ ]				
	[ ]		[ ]				
	[ ]		[ ]				

Who vaccinated
1=self
2=government vets
3=private vet

Vaccine source
1=Private vet
2= Narok Veterinary Office
3=Kabete
4=NGO
5=Other (specify)

B18 If CBPP not mentioned in B20, Ask:Do you have your cattle vaccinated against CBPP Y=Yes; N=No

B19 In which year did you have your animals vaccinated against CBPP, why, how many, which ages, at what total cost?
(*Enter for all Years, Yes or No and record reasons)*

Year (months)	Y=Yes;N=No	Reasons for vaccinating/ not vaccinating	How many	Ages	Total cost
2006	[ ]	[ ]		[ ]	
2005	[ ]	[ ]		[ ]	
2004	[ ]	[ ]		[ ]	
2003	[ ]	[ ]		[ ]	
2002	[ ]	[ ]		[ ]	
2001	[ ]	[ ]		[ ]	
2000	[ ]	[ ]		[ ]	
1999	[ ]	[ ]		[ ]	

Reasons for vaccinating
1=routine
2=rumour
3=outbreak within herd
4=ring vaccination
5=other (specify)

Reasons for not vaccinating
1= fear of severe reactions
2=expensive
3=fear reduced productivity
4=preferred to do something else
5=vaccine not available
6=other *specify)*……..

Ages vaccinated
1=<1year
2=1-3years
3=>3years
4=All

B20 Are there vaccinated animals that had CBPP? Y=Yes N=No
Which year and how many?

Age	**2006**	**2005**	**2004**	**2003**	**2002**	**2001**	**2000**	**1999**
	No. sick	No. sick	No. sick	No. sick	No. sick	No. sick	No. sick	No. sick
<1 year								
1-3 years								
>3 years								

B21 How long is the protection offered by the vaccine

1= ≤3months
2= 4-7months
3=8-12months
4=> 12 months

B22 How often would you like your cattle vaccinated against CBPP?

1=once a year why?.....
2=twice per year why?.....
3=other, specify why?.....

B23 Did cattle have any problems associated with the vaccination? Y= Yes N=No
If Yes; what kind of problems and how many out of 10

Year	Type of reaction	No. out of 10						
		1	2	3	4	5	6	7
2006	[, ,]							
2005	[, ,]							
2004	[, ,]							
2003	[, ,]							
2002	[, ,]							
2001	[, ,]							
2000	[, ,]							
1999	[, ,]							

Type of reaction
1= Slough off tail
2= Swelling at injection site
3=Death
4= Abortions
5= Weakening of animals
6= Drop of milk production
7=Others *(specify)*……

B24 Do the neighbours vaccinate against CBPP? Y=Yes N=No

SECTION C: HERD DATA

C1. Indicate herd sizes in the table below

Type of animal	**Herd size**
Cattle	
Sheep	
Goat	
Donkey	
Poultry	

C2 What are the cattle herd sizes in the other households within the boma?

Name of household head	**No. of cattle**

C3 Among the cattle, what have been the changes over the last 12 months?
(*Could be more than one reason*)

Cattle category	No. Of Births	No. Of Deaths	Reason	No. slaughtered	Reason	No. bought	Reason	No. sold	Reason	Gifts in	Reason	Gifts out	Reason	Loans in	Reason	Loans out	Reason
< 1year																	
1-3years																	
>3 years																	

Reasons for deaths	**Reasons for slaughter**	**Reasons for purchases**	**Reasons for sales**	**Reasons for loans**	**Reasons for gifts**
1=Disease	1=Disease	1=Restocking	1=cash	1=ploughing	1=Friendship
2=Age	2=Age	2=Improve breed	2=poor performance	2=breeding	2=ceremony
3=still birth	3=household use	3=build herd	3=disease (specify)	3=milk	3=dowry
4=predation	4=ceremony	4=for fattening	4=Other (Specify)	4=restocking	4=Other (specify)
5=Unknown	5=Injury	5=Other (specify)		5=Other (specify)	5=Other (specify)
6=Drought	6=Other (specify)				
7=Other (specify)					

C4 Where did animals go for watering, grazing and salt licks in the last 12 months?

(*ask the farmers to mention maximum of three places including close and far places)*

	Watering points	**Grazing places**	**Salt lick points**
Dry season	Village name ……	Village name ……	Village name ……
	Village name ……	Village name ……	Village name ……
	Village name ……	Village name ……	Village name ……
Wet season	Village name ……	Village name ……	Village name ……
	Village name ……	Village name ……	Village name ……
	Village name ……	Village name ……	Village name ……

C5 Did your animals mix with others at watering points? Y=Yes N=No; **Grazing** Y=Yes N=No; **Saltlicks** Y=Yes N=No

C6 What is the level of mixing at the common watering, Salt licks and grazing points?

	Watering points	**Grazing places**	**Salt lick points**
Dry season	Level of mixing ……	Level of mixing ……	Level of mixing ……
Wet season	Level of mixing ……	Level of mixing ……	Level of mixing ……

Fill in 1=completely mixed
2=No mixing at all
3=Partially

C7 Estimate number of herds that mix with at water, grazing and salt lick points.

(*ask the farmers to give an estimate of minimum and maximum number of herds)*

	Watering points	**Grazing places**	**Salt lick points**
Dry season	Number of herds ……...	Number of herds ……...	Number of herds ……...
Wet season	Number of herds ……...	Number of herds ……...	Number of herds ……...

C8 Where do herds that mix with at water, grazing and salt lick points come from?

(*ask the farmers to mention maximum of three places including close and far places)*

	Watering points	**Grazing places**	**Salt lick points**
Dry season	Village name ……...	Village name ……...	Village name ……...
	Village name ……...	Village name ……...	Village name ……...
	Village name ……...	Village name ……...	Village name ……...
Wet season	Village name ……...	Village name ……...	Village name ……...
	Village name ……...	Village name ……...	Village name ……...
	Village name ……...	Village name ……...	Village name ……...

C9 At which markets do you normally sell animals?.....

Market	Why this market	Origin of animals at the market (close and far places)
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**
	[, ,]	**…… …… ……**

C10 Do you bring back animals that did not fetch market? Y=Yes N=No
C11 From which markets do you bring back animals?.....,……,……

C12 How are the animals accommodated during the night, how many herds and where from?

	Number of herds	**Where from**
Dry season	……..	Village name …….....
	……..	Village name …….....
	……..	Village name …….....
Wet season	……..	Village name …….....
	……..	Village name …….....
	……..	Village name …….....

Appendix ii: Message detailing vaccination programme to herders

WELLCOME TRUST PROGRAM FOR CBPP VACCINATION IN OSUPUKO, LOITA AND MARA DIVISIONS OF NAROK DISTRICT

- **There will be a free vaccination campaign against CBPP which is a common problem among our cattle from 5th January – 26th March 2007.**

- **The Moredun Research Institute (UK) has developed a new vaccine which we hope will offer protection longer than the current CBPP vaccine so both vaccines will be used concurrently.**

- **This vaccination campaign is being funded by Wellcome Trust (UK) and coordinated by DVO-Narok and VSF-Germany. VSF-Germany has also introduced the well known E.C.F vaccine.**

- **After vaccination the herds will be monitored by Research Scientists from ILRI and KARI together with yourselves and our Community-based animal health workers for 1 year.**

- **We urge you to present your cattle of over one month of age to be vaccinated so as to get rid of this disease to allow us to sell our cattle to KMC and other livestock markets locally and internationally.**

- **DVO-Narok and Naroosura Veterinary services will be implementing this exercise.**

Thank you.

Appendix iii: Contagious bovine pleuropneumonia vaccination form

Herder's Name……Village/Cluster (s) .....Crush name.....
sub location .....location .....Division.....Date.....
Vaccination Team Narok team /Naroosura team

	Animal ID **(Ear tag No)** **Other identifier**	**Sex** **F/M**	**Breed** **Z=zebu** **C=cross**	**Age**	**Body condition**	**Batch No**	**Time**
1					Good/Fair/poor		
2					Good/Fair/poor		
3					Good/Fair/poor		
4					Good/Fair/poor		
5					Good/Fair/poor		
…					Good/Fair/poor		

Appendix iv: Contagious bovine pleuropneumonia bleeding form

Date:
Farmer's Name…… Year of last CBPP Vaccination.....
Year of last outbreak.....
Geo-reference

Longitude	**Altitude**
Latitude	

	Animal ID **(Ear tag No)** **Other identifier**	**Tail tip** **measure (cm)**	**other treatment /vaccination/remarks**
1.			OTC, Veriben, Levamisole,…….
2.			OTC, Veriben, Levamisole,…….
3.			OTC, Veriben, Levamisole,…….
4.			OTC, Veriben, Levamisole,…….
5.			OTC, Veriben, Levamisole,…….
6.			OTC, Veriben, Levamisole,…….

Appendix v: Contagious bovine pleuropneumonia monthly monitoring form

Olkipiei Vaccine Programme

Olkipiei Vaccine Programme

Herder's name…… ….Cluster/village.....Crush name.....Month.....
Name of Household monitor/CBAHW.....

S/N	Animal ID Ear tag no	Type of event (s)	Date of event (s)	PM lesions	Samples submitted	Disease Signs (if died then signs prior to death)	Drug (s) or vaccine administered and dose
1.							
2.							
3.							
4.							
5.							
…							

Type of events

1=Died	2=Sold	3=Gifts in/out	4=Diseased	5=Loans in/out	6=purchased	7=Births	8= Other …… eg. abortion
Reasons for deaths	**Reasons for sales**	**Reasons for gifts**	**Disease**	**Reasons for loans**	**Reasons for purchases**		**Reason**
1=Disease	1=cash	1=Friendship	1=Olkipiei	1=ploughing	1=Restocking		……
2=Age	2=poor performance	2=ceremony	2=Ntorobo	2=breeding	2=Improve breed		……
3= predation	3=disease (specify)	3=dowry	3=Olkirobi	3=milk	3=build herd		……
4=Unknown	4=Other (Specify)	4=Other (specify)	4=Oltikana	4=restocking	4=for fattening		……
5=other (specify)	……	……	5=Other (specify	5=Other (specify)	5=Other (specify)		……
……	……	……	……	……			……

Appendix vi: Multivariable linear logistic mixed model based on specific adverse events

a) Logit (p)=β0+ Vaccinetype**B**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	24.26	1	24.26	79908.3	<0.001

Standard	0.001902
Buffered	0.002781

b) Logit (p)=β0+ Vaccinetype**B**+Age**A**+ Vaccinetype**B** *Age**A**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	21.78	1	21.78	79890.6	<0.001
Agecat	3.00	1	3.00	78030.8	0.083
Vaccinetype**B***Age	8.39	1	8.39	79906.1	0.004

	Young animals	**A**dult animals
Standard	0.002686	0.001716
Buffered	0.002657	0.002845

b_j Random term (crush and herd)
ε Error term

c) Logit (p)=β0+ Vaccinetype**B** +Team**sura** + Vaccinetype**B** *Team**sura**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	23.50	1	23.50	79904.6	<0.001
Team**sura**	0.07	1	0.07	17.2	0.793
Vaccinetype* Team**sura**	6.99	1	6.99	79906.5	0.008

	Na**rok**	Naroo**sura**
Standard	0.001544	0.002233
Buffered	0.002835	0.002715

d) Logit (p)=β_0+ Vaccinetype**B** + Gender **F** + Vaccinetype**B** * Gender **F** + b_j + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	26.47	1	26.47	79890.8	<0.001
Gender **F**	16.56	1	16.56	79927.3	<0.001
Vaccinetype*Gender	4.58	1	4.58	79762.1	0.032

	Male	**F**emale
Standard	0.001717	0.001986
Buffered	0.001899	0.003183

e) Logit (p)= β_0+ Vaccinetype**B** +Division**MO** + Vaccinetype**B** * Division**MO** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	20.63	1	20.63	79904.4	<0.001
Division**MO**	6.79	2	3.40	20.5	0.053
Vaccinetype*Division	18.18	2	9.09	79916.2	<0.001

	Loita	**M**ara	**O**supuko
Standard	0.001063	0.001020	0.004578
Buffered	0.002616	0.001989	0.005069

Appendix vii: Multivariable linear logistic mixed model based non-specific adverse events

a) Logit (p)=β_0+ Vaccinetype**B**+ b_j + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.13	1	0.13	79895.6	0.716

Standard	0.0010022
Buffered	0.0009692

b) Logit (p)=β_0+ Vaccinetype**B**+Age**A**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.14	1	0.14	79901.7	0.707
Age**A**dult	34.26	1	34.26	77731.7	<0.001

Standard	0.001153
Buffered	0.001115
Young animals	0.001524
Adult animals	0.000844

c) Logit (p)=β_0+ Vaccinetype**B** + Body**F**air+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.13	1	0.13	79899.1	0.714
Body**F**air	9.38	1	9.38	61492.7	0.002

Standard	0.001205
Buffered	0.001167
Body**G**ood	0.000951
Body**F**air	0.001478

d) Logit (p)= β_0+ Vaccinetype**B** +Division**MO** + Vaccinetype**B** * Division**MO** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.11	1	0.11	79884.4	0.737
division	2.76	2	1.38	16.9	0.278
Vaccinetype*Division	5.53	2	2.77	79917.0	0.063

	Loita	**M**ara	**O**supuko
Standard	0.0006818	0.0013544	0.0009863
Buffered	0.0008149	0.0014410	0.0006668

Appendix viii: Multivariable linear logistic mixed model based antimicrobial treatment

a) Logit $(p)=\beta_0+$ Vaccinetype**B**$+ b_j + \varepsilon$

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	3.57	1	3.57	79888.7	0.059

Standard	0.008815
Buffered	0.009723

b) Logit $(p)=\beta_0+$ Vaccinetype**B**+Age**A**$+$ bj $+ \varepsilon$

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	2.93	1	2.93	79873.5	0.087
Age**A**dult	17.05	1	17.05	78318.1	<0.001
Vaccinetype*Age	3.39	1	3.39	79878.4	0.066

	Young animals	**A**dult animals
Standard	0.01167	0.00809
Buffered	0.01088	0.00940

c) Logit $(p)=\beta_0+$ Vaccinetype**B** + Body**F**air$+$ bj $+ \varepsilon$

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	3.57	1	3.57	79889.0	0.059
Body**F**air	6.33	1	6.33	74021.3	0.012

Standard	0.00988
Buffered	0.01089
Body**G**ood	0.00907
Body**F**air	0.01187

Appendix ix: Detailed outbreak investigation: multivariable linear logistic mixed model based on clinical signs

a) Logit (p)= β_0+ Vaccinetype**B**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.44	1	0.44	288.1	0.505

Standard	0.05368
Buffered	0.03976

b) Logit (p)=β0+ Vaccinetype**B**+Age**A**+ Vaccinetype**B** *Age**A**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.52	1	0.52	286.3	0.473
Age**A**	0.45	1	0.45	287.4	0.503
Vaccinetype**B***Age**A**	0.51	1	0.51	287.0	0.474

	Young animals	**A**dult animals
Standard	0.08012	0.04261
Buffered	0.03871	0.04064

c) Logit (p)= β_0+ Vaccinetype**B** + Gender **F** + Vaccinetype**B** * Gender **F** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.95	1	0.95	286.4	0.330
Gender **F**	4.96	1	4.96	288.0	0.027
Vaccinetype**B***Gender **F**	1.01	1	1.01	286.2	0.316

	Male	**F**emale
Standard	0.14310	0.03095
Buffered	0.06085	0.03385

d) Logit (p)= β_0+ Vaccinetype**B** + Body**F**air+ Vaccinetype**B***Body**F**air+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	0.52	1	0.52	286.1	0.471
Body**F**air	0.06	1	0.06	288.4	0.804
Vaccinetype**B***Body**F**air	0.78	1	0.78	286.1	0.378

	Good	**F**air
Standard	0.06276	0.02731
Buffered	0.03822	0.04942

Appendix x: Vaccination subset: Multivariable linear logistic mixed model based on seroconversion data

a) Logit (p)= β_0+ Vaccinetype**B**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	15.73	1	15.73	1797.2	<0.001

Standard	0.2738
Buffered	0.3616

b) Logit (p)= β_0+ Vaccinetype**B**+Age**A**+ Vaccinetype**B** *Age**A**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	15.80	1	15.80	1795.9	<0.001
Age**A**	2.20	1	2.20	1780.1	0.138
VaccinetypeB *Age**A**	0.72	1	0.72	1797.8	0.398

	Young animals	**A**dult animals
Standard	0.2637	0.2791
Buffered	0.3236	0.3834

c) Logit (p)= β_0+ Vaccinetype**B** + Gender **F** + Vaccinetype**B** * Gender **F** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	15.59	1	15.59	1795.1	<0.001
Gender **F**	5.55	1	5.55	1810.4	0.019
Vaccinetype**B** * Gender **F**	0.00	1	0.00	1791.0	0.948

	Male	**F**emale
Standard	0.2353	0.2894
Buffered	0.3208	0.3811

d) Logit (p)= β_0+ Vaccinetype**B** + Body**F**air+ Vaccinetype**B***Body**F**air+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	16.04	1	16.04	1795.9	<0.001
Body**F**air	6.05	1	6.05	1522.4	0.014
Vaccinetype**B***Body**F**air	1.96	1	1.96	1807.5	0.162

	Good	**F**air
Standard	0.2787	0.2360
Buffered	0.3818	0.2352

e) Logit (p)= β_0+ Vaccinetype**B** +Division**MO** + Vaccinetype**B** * Division**MO** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	15.40	1	15.40	1794.8	<0.001
Division**MO**	3.33	2	1.66	9.9	0.238
Vaccinetype**B** * Division**MO**	1.61	2	0.81	1794.4	0.446

vaccinetype	**L**OITA	**M**ARA	**O**SUPUKO
Standard	0.2507	0.3483	0.2213
Buffered	0.3653	0.4048	0.3127

f) Logit (p)= β_0+ Vaccinetype**B** +Team**sura** + Vaccinetype**B** *Team**sura**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	15.59	1	15.59	1796.2	<0.001
Team**sura**	3.13	1	3.13	11.0	0.104
Vaccinetype**B** *Team**sura**	0.68	1	0.68	1796.6	0.411

	Na**rok**	Naroo**sura**
Standard	0.3038	0.2454
Buffered	0.4179	0.3111

Appendix xi: Detailed outbreak investigation: Multivariable linear logistic mixed model based on seroprevalence

f) Logit (p)=β_0+ Vaccinetype**B**+ b_j + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	2.90	1	2.90	288.9	0.090

Standard	0.2161
Buffered	0.1338

g) Logit (p)= β_0+ Vaccinetype**B**+Age**A**+ Vaccinetype**B** *Age**A**+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	2.03	1	2.03	286.6	0.155
Age**A**	3.02	1	3.02	287.1	0.083
Vaccinetype**B***Age**A**	1.74	1	1.74	286.9	0.188

	Young animals	**A**dult animals
Standard	0.1853	0.2308
Buffered	0.0547	0.1819

h) Logit (p)= β_0+ Vaccinetype**B** + Gender **F** + Vaccinetype**B** * Gender **F** + bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	2.83	1	2.83	286.8	0.093
Gender **F**	0.04	1	0.04	287.1	0.846
Vaccinetype**B***Gender **F**	0.13	1	0.13	286.1	0.714

	Male	**F**emale
Standard	0.2232	0.2138
Buffered	0.1142	0.1408

i) Logit (p)= β_0+ Vaccinetype**B** + Body**F**air+ Vaccinetype**B***Body**F**air+ bj + ε

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Vaccinetype**B**	2.54	1	2.54	286.9	0.112
Body**F**air	4.26	1	4.26	287.3	0.040
Vaccinetype**B***Body**F**air	0.12	1	0.12	286.2	0.726

	Good	**F**air
Standard	0.2486	0.1223
Buffered	0.1605	0.0539