ESTIMATING THE SEROPREVALENCE OF *T. PARVA* INFECTION AND DETERMINATION OF ASSOCIATED RISK FACTORS IN THE CATTLE POPULATION OF MBEERE DISTRICT, KENYA

John Mwangi Gachohi (BVM, Nairobi)

A thesis submitted in partial fulfillment of the requirements for the award of a degree in Master of Science in Veterinary Epidemiology and Economics, Department of Public Health, Pharmacology and Toxicology,

Faculty of Veterinary Medicine, University of Nairobi.

2009

DECLARATION

This thesis is my original work and has not been presented for an award of a degree in any other university.

John Mwangi Gachohi. B.V.M. (Nairobi)
Department of Public Health, Pharmacology and Toxicology,
Faculty of Veterinary Medicine, University of Nairobi.

This thesis has been submitted with our approval as the supervisors:

Dr. Philip M. Kitala, Ph. D.
Department of Public Health, Pharmacology and Toxicology,
Faculty of Veterinary Medicine, University of Nairobi.

Dr. Priscilla N. Ngumi, Ph. D.
Kenya Agricultural Research Institute – Veterinary Research Centre

---

Dr. Rob A. Skilton, Ph. D.
Biosciences eastern and central Africa (BecA) – International Livestock Research Institute (ILRI) Hub

DEDICATION

To my wife Judy, daughters Lorna and Linah, my dad Gachohi Ndiki and mum Ekra Gachohi

TABLE OF CONTENTS

Contents **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

^{o}C:	Degree Celsius
AEZ:	Agro-ecological zone
ANOVA:	Analysis of variance
ASALs:	Arid and semi-arid lands
CBPP:	Contagious bovine pleuropneumonia
CBS:	Central Bureau of Statistics
CI:	Confidence interval
ECF:	East Coast fever
ELISA:	Enzyme-linked immunosorbent assay
FAO:	Food and Agricultural Organization
FMD:	Foot-and-Mouth Disease
GDP:	Gross domestic product
GLMM:	Generalized linear mixed model
GoK:	Government of Kenya
IFAT:	Indirect Fluorescent Antibody Test
LM3:	Lower midlands 3 (The marginal cotton zone)
LM4:	Lower Midlands 4 (the Lower midland livestock-millet)
L5:	Lowlands 5 (Lowland livestock millet zone).
MSB:	Mean square between herds
MSW:	Mean square within herds
MEC:	Ministry of Economic planning
NDVI:	Normalized Differential Vegetation Index
NEPAD:	New Partnership for Africa's Development
P:	Proportion
PBS:	Phosphate buffered saline
PCR:	Polymerase chain reaction
PIM:	Polymorphic immunodominant molecule
PP:	Percentage positivity
SSA:	Sub-Saharan Africa
TBDs:	Tick-borne diseases
Var:	Variance
VIF:	Variance Inflation Factor

ACKNOWLEDGEMENTS

I start by thanking and praising the Almighty God for His love and for giving me the precious life, good health and strength for doing this work.

I am greatly indebted to my study supervisors, Drs. P. Kitala, P. Ngumi and R. Skilton. I especially thank them for providing technical insights, scholarly advice and guidance, and helping to shape my thoughts on this study. I appreciate that they provided different disciplinary perspectives to my thinking. I greatly thank them for allowing me to stand on their shoulders so that I could see far.

I acknowledge with gratitude all farmers from Mbeere District for availing their animals for sampling and finding time to attend to the questionnaire. The Mbeere District Veterinary Officer and his staff are highly acknowledged for laying the necessary groundwork for this study.

I am delighted to acknowledge the assistance of many supportive professional colleagues and friends for their useful comments and suggestions through the years: Drs. D. Mwangi and R. Pelle of ILRI, B. Bett, S. Thumbi, N. Githaka, G. Monda, D. Nanyende and E. Kiema. Many thanks are due to Brigid McDermott for her helpful ideas and constructive comments and suggestions on data analyses. I am also grateful to Patrick Chege of ILRI GIS/Remote Sensing Unit for providing NDVI values for Mbeere District. Thank you so much Dr John Kagira for showing me that rigorous thinking and discipline can coexist with fun and a sense of humor.

I gratefully acknowledge the financial support of University of Nairobi through award of a scholarship, Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) and Kenya Agricultural Productivity project

(KAPP).

I want to express my appreciation to BecA – ILRI Hub for providing material and human laboratory facilitation during the project period. In particular, "*hats off*" to Alice Njeri of ILRI who, with little talk and a lot of practice, guided me through the intricate technical expertise of ELISA.

Many thanks to the Director, Kenya Agricultural Research Institute (KARI) for granting me study leave and his continued support during the study period. A special thank you goes to Dr Grace Murilla, Center Director KARI-TRC, for relentless and constructive suggestions that set me on the right path throughout the study. Particular thanks also go to the Chairman, PHPT Department, for guidance and administrative support.

Kind regards to my extended family particularly my parents, brothers and sisters and all in-laws, for even if they did not exactly understand what I was doing, they kept on urging me to soldier on. Undoubtedly, I would not have gotten through this effort at all without the support and understanding of my daughters, Lorna and Linah and my wife, Judy, who really made it all worthwhile.

Everybody not mentioned here personally who contributed in one way or another to the success of this work is also gratefully acknowledged.

ABSTRACT

The most important tick-borne disease (TBD) of cattle in Kenya is East Coast Fever (ECF) caused by *Theileria parva,* and transmitted by the tick *Rhipicephalus appendiculatus.* A study was carried out in Mbeere District, Kenya to estimate the seroprevalence of *T. parva* infection and determine the associated risk factors. Previously, no well-structured observational tick-borne diseases' study have been conducted in a mixed crop-livestock system such as that found in marginal semi-arid rangeland in Mbeere District. Tick-borne diseases are among the important livestock production constraints in Mbeere District. The objectives of this study were therefore to identify potential livestock production constraints, estimate *T. parva* seroprevalence, and to identify and assess risk factors associated with *T. parva* seropositivity in Mbeere District in a cross-sectional study. As the study data exhibited a nested hierarchy, further objectives included evaluating and quantifying variance components corresponding to three levels of cattle population organization (herd, sub-location and division) and using the variance components to identify levels in which there could be substantial opportunities for targeted interventions.

The farms were selected by a multistage random sampling method. All the four administrative divisions (Siakago, Gachoka, Evurore and Mwea) in the district were included in the study. The 39 sub-locations in the district were listed according to divisions and two sub-locations from each division were randomly selected giving a total of 8 sub-locations. In collaboration with the Assistant-chief and village elders from each selected sub-location, a list of all households owning cattle was compiled

and ten of them from each sub-location were randomly sampled using random number tables to end up with eighty farms. Sampling of individual animals in the farm used proportional allocation approach. A constant 50% proportion of animals in each herd were selected using systematic random sampling approach. All animals in a herd were, however, sampled if the herd size was 6 or less. A total of 440 animals were sampled for this study.

Risk factor information on the farming system data and on animal and farm level management practices was gathered using a standardized questionnaire. Approximately 5 ml of blood were collected from the selected animals for serum preparation. Serum samples were assayed for antibodies to *T. parva* by Enzyme-linked immunosorbent assay (ELISA). Potential livestock production constraints were analyzed descriptively by proportions and rankings. The relationship between *T. parva* seropositivity and risk factors was assessed by multivariable logistic regression models. To investigate the contribution of the levels of organization to the total variance of *T. parva* seropositivity, this study applied multilevel models using Schall's algorithm.

The 8 sub-locations selected represented 20% of all sub-locations in the district (n=39) whereas the 80 farms selected represented approximately 1.7% of all farms in the sampling frame (n=4783). The 440 animals selected comprised of 66% of all animals in the selected farms.

Ninety-four percent (94%) of the households depended on both crops and livestock as the main farm activity while the rest depended on livestock only. Sixty-three percent (63%) of the households depended on both crops and livestock for household cash income. Overall, 90% of the households kept only indigenous cattle breeds and their crosses reared under open grazing management systems. Reasons advanced for keeping cattle in order of importance were milk, homestead "security" banks, traction and manure. Feed and water availability and diseases were ranked as the most important farm constraints in all divisions. East Coast fever was ranked first only in Mwea division.

The overall ECF seroprevalence was 19.3% (range: 3.9% to 48% across divisions) in the district [95% CI: 13.7%, 24.9%]. Regression analysis found four major factors (fixed effects) associated with seropositivity: presence of the vector tick on the farm (odds ratio (OR) = 3.8), frequency (number of times) of calf tick control before 6 months of age (for frequency of >5 times, OR = 3.9 relative to frequency of $\leq$5 times), herd size (OR for herd size category '6-10 cattle' = 2.7; OR for over herd size category 'over 10 cattle' = 0.95 relative to herd size category '1-5 cattle') and division (ORs for Siakago, Gachoka and Mwea Divisions = 0.3, 0.21 and 5.1 respectively relative to Evurore Division).

There were distinct differential herd tick control practices across the district. Siakago and Gachoka Divisions had the least *T. parva* seroprevalence. Farmers in these two divisions had characteristically large herd sizes. On the other hand, Evurore and Mwea Divisions had the highest *T. parva* seroprevalence. In contrast, farmers in these two divisions had characteristically relatively smaller herd sizes.

'Division', as an area-level variable, had the most important and large effects associated with *T. parva* seroprevalence. As divisional boundaries are administrative in nature, a multivariable model without variable 'Division' was built to investigate significant effects which could have been masked by effects of 'Division'. Three additional effects became significant: age at which calf tick control was started, Normalized Differential Vegetation Index (NDVI) and agro-ecological zone (AEZ) indicating the importance of area-level effects.

Upon evaluation of sources of variation in *T. parva* seropositivity in the cattle population of Mbeere District, the absolute values of variance components for the hierarchical levels were as follows for random effects models (no fixed effects): herd (2.314), sub-location (0.393), division (2.151) and variance due to error (0.532). Presence of the significant fixed effects changed the herd-level variance component value only slightly to 2.2185 while the values for sub-location (0.71) and division (1.6878) changed substantially. The variance due to error for the mixed model was 0.519.

The study findings demonstrated that traditional mixed crop-livestock farming system was an important enterprise in Mbeere District. Although prevalence alone is not an adequate measure to make concrete conclusions on *T. parva* infection endemicity status, the low *T. parva* seroprevalence in Mbeere District indicated that ECF most likely occurred in the district in an endemic instability state. Thus, this requires more proof in a longitudinal study. The differential herd tick control strategies across the district probably arose out of differences in perceptions of ECF occurrence and importance. Substantial variation of *T. parva* seroprevalence in Mbeere District rested at herd and division levels though the variance due to herd was larger than that due to geographical areas in multilevel models. The large variation at the herd level appeared to be related to the differential tick control management practices across the district. It also indicated that outcomes within a herd had a common cause and/or strong dependency in terms of parasite exposure. The large geographical variation could be related to possible differential ecological and climatic variability in vector suitability habitats in the district. These observations suggested that *T. parva* seroprevalence in Mbeere District was mainly influenced by herd and environmental effects. These results implied that both among-farm and -division *T. parva* transmission factors were important in designing ECF control strategies in the district. Thus, practical ECF intervention strategies should be directed at all farms within the two high risk divisions of Mwea and Evurore for the control efforts to have the greatest impact.

CHAPTER 1

INTRODUCTION

1.1. Background

In sub-Saharan Africa (SSA), the agricultural sector accounts, on average, for close to 20% of total gross domestic product (GDP) and about 60% of the region's total labour force (FAO, 2003; World Bank, 2003). Many countries in the region, however, depend on agriculture to a greater extent than these regional averages (FAO, 2003; World Bank, 2003). Mixed farming systems, in which crops and livestock are integrated on the same farm, are widespread in the rain-fed SSA (FAO, 2003). Mixed farming systems are important in their contribution to the total output of animal products and in enhancing livelihoods of the poor through provision of food, income generation, draught power, and employment (Lenné and Thomas, 2005). Livestock account for 53% of the agricultural capital stock in SSA and contribute significantly (30%) to agricultural gross domestic product (GDP) (NEPAD, 2005).

Like the rest of SSA, the growth of Kenya's economy relies heavily on the agricultural sector despite the fact that only 25% of the landmass is suitable for agriculture (World Bank, 2003). Activities related to agriculture account for 50% of the direct or indirect contribution to the GDP of Kenya. These activities are a major economic resource for approximately 80% of Kenya's population (FAO, 2003). In Kenya, livestock plays a crucial role both at the national and household levels and contributes up to 10% (approximately Ksh. 79 billion) of the GDP (GoK, 2006). In

addition, 30% of the farm gate value of agricultural commodities originates from livestock (GoK, 2006). The livestock sub- sector is the main source of livelihood for the majority of the rural population especially in the arid and semi-arid lands (ASALs) and employs about 50% of the Kenya's agricultural labour force (GoK, 2006). About 75% of Kenya's land area is ASALs and holds over 50% and 58% of the country's large and small ruminants, respectively (GoK, 2006). Mbeere District, which was the focus of this study, is part of Kenya's ASALs.

The main livestock production systems in Kenya include commercial large scale, smallholder dairy, traditional mixed crop-livestock, and pastoralism among others (Jahnke, 1982). Mixed crop-livestock systems occur in several forms in Kenya. However, all forms of crop-livestock systems exist as closed systems in which waste products of one component serve as a resource for the other; manure from livestock is used to enhance crop production, whilst crop residues and by-products are used to feed the animals (Thornton *et al.,* 2002). In semi-arid lands, crop production depends on draught animals for tillage of cropping area, animal manure is used for enriching soil fertility while livestock depend on crop residues for dry season feeding (Thornton *et al.,* 2002).

All livestock production systems are, however, at risk from tick-borne diseases (TBDs). Four TBDs are of importance to the livestock industry: East Coast fever (ECF); Heartwater (HW); anaplasmosis; and babesiosis. The impact associated with these TBDs includes both direct losses from animal mortality and reduced livestock

production and the costs associated with control and treatment (Minjauw and McLeod, 2003). East Coast fever is the most economically important TBD of cattle in much of eastern and central Africa including Kenya (Mukhebi, 1992; Norval *et al.,* 1992; Lawrence *et al.,* 2004; Ndegwa 2005). Tick-borne diseases and inadequate fodder, in that order, have been reported as the main constraints to the livestock industry in Mbeere District (Kangara *et al.,* 1996; Onduru *et al.,* 2002).

Field studies investigating risk factors for TBDs and constraints to livestock production have been conducted in Kenya (Moll *et al.,* 1984; Moll *et al.,* 1986; Deem *et al.,* 1993; Maloo *et al.,* 1994; Gitau *et al.,* 1997; Peeler and Omore, 1997; O' Callaghan, 1998; Gitau *et al*., 1999; Maloo *et al.,* 2001a, b; Muraguri *et al.,* 2005; Ndung'u *et al.,* 2005; Okuthe and Buyu, 2006). The important risk factors that have been identified in these studies include animal age and breed, agro-ecological zone (AEZ), grazing management, livestock production system, tick control strategies and frequency of tick control. In particular, the occurrence and importance of ECF is a reflection of complex interactions involving the causative organism, the tick vector, the vertebrate host and the environment (Norval *et al.,* 1992). These interactions are driven and modified by a wide variety of factors ranging from climate, soil and vegetation, human activities including crop-livestock production systems and measures taken to control ticks and TBDs (Norval *et al.,* 1992). This complexity is clearly expected to result in variability and/or correlation in disease response at the different levels of cattle population organization (for example, animal-, herd- or at the area-level). The main limitation in the analyses of the studies cited above is that

the level at which the identified risk factor is associated with disease was not clearly defined. Furthermore, influences at the various levels may often be confounded. To adjust for such confounding, formal methods to partition variation among area-, herd- and individual-animal levels need to be conducted. Such information is required to plan disease control programmes, by identifying the highest risk populations and the most important risk factors in those populations.

The information that TBDs are a major constraint to livestock industry in Mbeere District and that no well-structured observational TBDs' study has been conducted in the district justified a rapid assessment of the present risk of *T. parva* infection in a cross-sectional survey. Previously, no well-structured observational TBDs' study has been conducted in a mixed crop-livestock system such as that found in marginal semi-arid rangeland in Mbeere District. The study was conducted with the objective of estimating the prevalence of serum antibodies to *T. parva,* the causative agent of ECF, in Mbeere District of Kenya. A further objective was to characterize the potential risks incurred by these factors and to help identify an indicator of the *T. parva* endemic status within the district. In order to come up with specific and targeted ECF intervention strategies suitable to the socio-economic and existing epidemiologic situations in the district, and also to take the clustered structure of the data into account, the study applied multilevel modeling techniques. The latter models were used both in studying the effects of risk factors and in estimating the contribution of the various levels of cattle population organization to the total variance of *T. parva* seropositivity in the district. Multilevel models have been used

to determine which levels of population organization contribute most to the variability of a number of infectious diseases of cattle in Kenya (Kadohira *et al.,* 1996). In this study, variation of *T. parva* seroprevalence was evaluated at four possible levels of population organization: geographic- (administrative division and sub-location), herd- and animal-levels. There have been no studies which have quantified the amount of variation existing at the various levels of population organization in TBDs observational studies. The current study was conducted within the four divisions (Siakago, Gachoka, Evurore and Mwea) of Mbeere District in March 2007.

1.2. Study objectives

1.2.1. Overall objective

The overall objective of this study was to identify and assess factors associated with *T. parva* infection in the cattle population of Mbeere District through a cross-sectional study and recommend targeted, suitable, and sustainable intervention strategies.

1.2.2. Specific objectives

1. To describe the characteristics of the traditional smallholder crop-livestock production system in Mbeere District, Kenya.
2. To estimate *T. parva* seroprevalence and identify and assess the effects of risk factors on *T. parva* seropositivity in the cattle population of Mbeere District, Kenya.
3. To determine the important sources of variation and subsequently quantify the variation in *T. parva* seropositivity in the cattle population of Mbeere District, Kenya.

CHAPTER 2

LITERATURE REVIEW

2.1. Livelihoods and livestock

A livelihood is ‘a means of living, maintenance, sustenance’ (Simpson and Weiner, 2000). This definition includes both the products of human undertaking (such as income and nutrition) and the means by which they are produced. Livestock contribute to the livelihoods of five main groups of people including owners, hired caretakers, vendors, consumers, and those who work in livestock related industries (Minjauw and McLeod, 2003). Livestock contribute to capital assets by providing natural capital (livestock are a form of natural capital and can be used to enrich the soil and to gain access to common forage resources), financial capital (through creation of income), human capital (through improved nutrition), physical capital (through provision of transport or energy), and social capital (ownership of livestock can increase access to social structures that strengthen the voice of an individual within the community) within all livestock production systems in different ways and to different degrees. A reduction in access to any of these capital assets may reduce the sustainability of a livelihood (Minjauw and McLeod, 2003).

Out of the groups of people that benefit from livestock, livestock owners are always and most immediately affected by livestock diseases. Livestock owners are also concerned with the risk of disease and the possible magnitude of its effect (Winrock International, 1992). The significant proportion of the rural poor (70%) in SSA that

depend on livestock for part of their livelihoods (Winrock International, 1992; FAO, 2003) calls for a better understanding of the role of livestock in poverty reduction.

2.2. Farming system in Mbeere District

The farming system in Mbeere District has been extensively reviewed (Onduru *et al.,* 2002). Mbeere District falls under ASALs and is dominated by mixed farming subsistence small-scale producers. The dominant system in the district is the traditional crop-livestock system (Onduru *et al.,* 2002). Generally, in these systems, indigenous livestock are closely integrated with other farm enterprises and provide a wide range of inputs to livelihoods (Minjauw and McLeod, 2003).

The livestock species kept in the district are mainly indigenous breeds of cattle (*Bos indicus*) which include the Small East African zebu, the Sahiwal, the Boran or their crosses which are well adapted to local harsh environmental conditions like poor feed supply, local diseases, and harsh climate existing in the district. In addition, indigenous sheep, goats and poultry are also kept and all are managed under the free-range system. The cattle free-range grazing system ranges from communal, extensive grazing or tethered grazing on smallholdings with limited or no opportunity for commercial supplements. Cattle are then corralled at night. The district has few exotic dairy cattle breeds or their crosses. For the period 1991-2001, exotic cattle breeds accounted for only about 1.3% of total cattle population in Mbeere District

(Onduru *et al.,* 2002). Where present, the exotic breeds of cattle are kept under semi-zero to zero grazing systems in the district (Onduru *et al.,* 2002).

Livestock plays a production role in the district by providing human capital in form of nutrition (meat and milk) and financial capital (cash income) when the products surplus is sold (Onduru *et al.,* 2002). Livestock are a buffer investment when crops fail (diversification), investment for old age, and a form of savings (Onduru *et al.,* 2002). Livestock also plays a socio-cultural role in Mbeere District as the number of animals in a farm is an element of status symbol and they are also used for social obligations like payment of dowry during marriage (Onduru *et al.,* 2002). Cattle in Mbeere District also provide physical capital in form of draught power and natural capital in form of manure to enrich the soils (Onduru *et al.,* 2002).

Food crops grown in Mbeere District include maize, millet, sorghum, beans, cowpeas, green grams, cassava, and bananas. The main cash crops grown are cotton, tobacco, and to a lesser extent sunflower (Onduru *et al.,* 2002). Forage legumes and farm byproducts such as cereal stovers (maize, millet, and sorghum) are important livestock feed sources during dry periods when other fodder types are scarce (Onduru *et al.,* 2002). The main constraints to livestock production in Mbeere District are TBDs and inadequate fodder, in that order, among others (Kangara *et al.,* 1996; Onduru *et al.,* 2002). However, despite the harsh environment, there are opportunities to improve cattle production such as strategic livestock disease management, introduction of drought tolerant fodder, and promotion of leguminous

fodder (Onduru *et al.,* 2002). Leguminous trees in mixed farming systems are capable of enhancing both crop production (through soil fertility maintenance) and livestock production (through increased availability of high quality feeds). The efficiency of improved production, however, depends on the livestock species used and the extent of integration and management within the cropping system (Thornton *et al.,* 2002).

2.3. Tick-borne diseases and their economic importance

Tick-borne diseases of cattle are the most prevalent and exert their greatest impact in the world's tropical and sub-tropical regions (Minjauw and McLeod, 2003). They include theileriosis, heartwater (HW), babesiosis and anaplasmosis. However, for anaplasmosis, mechanical transmission through contaminated hypodermic needles and biting flies also plays an important role. East Coast fever, a form of theileriosis, was earlier reported to kill 1.1 million cattle and to cause an economic loss of US $168 million annually (Mukhebi, 1992; Norval *et al.,* 1992). Recent estimates put the figure at $300 million annually (Ndegwa, 2005). From the latter figure, losses from the lowered milk production account for 47% of total losses, followed by losses incurred by the costs of tick control and treatment (28%), losses to traction (13%), and losses in meat (12%) (Ndegwa, 2005). Mortality is higher (up to 100%) in the more susceptible exotic cattle breeds than in the indigenous zebu breeds (where the

average mortality is estimated at 10% (Lawrence *et al.,* 1988) or higher particularly in calves in pastoral systems (Di Giulio *et al.,* 2003).

Tick-borne diseases, particularly ECF, are considered to be the most important constraints within all the livestock farming systems in Kenya (Moll *et al.,* 1984; Moll *et al.,* 1986; Mukhebi, 1992; Deem *et al*., 1993; Peeler and Omore, 1997; Gitau *et al*., 1999; Maloo *et al.,* 2001a, b*;* Okuthe and Buyu, 2006). Tick-borne diseases of cattle are considered to have important direct economic effects. In Kenya, estimated annual costs associated with TBDs in smallholder dairy system and traditional systems (both traditional crop-livestock and pastoral systems) were US$ 54 and US$ 34 respectively (Minjauw and McLeod, 2003). Furthermore, there is opportunity cost associated with the inability to introduce the more productive breeds or to intensify production systems in areas with high-risk of TBDs (Minjauw and McLeod, 2003).

The mortality rate due to ECF varies from 0-50% in endemically stable conditions (conditions with stable relationships between host, agent, vector and the environment where all coexist such that minimal morbidity and mortality are present in the host population) (Moll *et al.,* 1986; Norval *et al.,* 1992). In endemically unstable conditions, high ECF morbidity and mortality (as high as 80-100%) are present in the host population (Moll *et al.,* 1986; Norval *et al.,* 1992).

In most farming systems, the most widely used methods for control of ticks and TBDs are the direct application of acaricides to host animals despite their well-known ecological disadvantages (Minjauw and McLeod, 2003). Control of TBDs in East Africa, however, has proved difficult largely because of lack of epidemiological information (Norval *et al.,* 1992). In addition, control strategies currently applied are not integrated in the production system dynamics as previous investigations were not production system-specific and did not target biological, managemental, environmental, and socio-economic parameters unique to a given production system (Perry and Young, 1995; Minjauw and McLeod, 2003).

A combination of strategic application of acaricides, improved exploitation of genetic resistance and the wider use of specific vaccines would be more sustainable than current approaches (Minjauw and McLeod, 2003). Research on production system-specific epidemiology of ticks and TBDs is therefore necessary as it could have a significant positive long-term impact on food security and poverty alleviation in farming systems that largely rely on productivity of their livestock.

2.4. Tick-borne diseases aetiological research

2.4.1. The concepts of causality, risk and web of causation

Classically, the definition of '*causality'* is one of pure determinism in which a steady, distinctive, and perfectly predictable relationship is assumed between two factors *X* and *Y*. *X* is a cause of *Y* if, in a completely stable system (assuming all

factors are initially fixed), any manipulation or change in *X* alone induces a subsequent change in *Y* (Blalock, 1964). This concept requires that in every instance, the effect invariably has to follow the cause (e.g., infection by infective *T. parva* sporozoites in susceptible cattle invariably causes ECF). However, because of the lack of certainty in epidemiological results, the term *'risk factor'* is mostly used instead of *'cause'*. A *'risk factor'* is a variable that is related to the probability of an animal developing a disease, prior to the point of initiation of the disease (Dohoo *et al.,* 2003). For a variable to be a risk factor for a particular disease, the variable must be observed to co-vary with the disease, the presence of the risk factor (or a relevant change in the risk factor) must precede the occurrence of disease and the observed association must not be entirely due to any source of error (Martin *et al.,* 1987). Examples of risk factors for ECF include grazing management and ecological suitability for vector ticks, breed and human interventions such as tick control. A good example is the observation that ECF prevalence and risk rates vary across unrestricted and restricted grazing systems (Gitau *et al.,* 1997; Gitau *et al.,* 1999).

In a cross-sectional study, epidemiological studies are conducted to identify risk factors through the comparison of prevalence between groups exposed and not exposed to a risk factor. Probabilities of disease occurrence are then compared using measures of strength of association or measures of effects of the factor in exposed group or in the population (Martin *et al.,* 1987). Comparisons using measures of strength of association involves calculation of ratios such as relative risk (RR) and

odds ratio (OR) which measure the magnitude of a statistically significant association between the risk factor and disease (Dohoo *et al.,* 2003). Although the ratios are used to identify risk factors, they do not provide information on absolute risk. On the other hand, the measures of effects of the factor in an exposed group or in the population allow quantifying the consequences from exposure to a risk factor, and are used to predict, quantify the effect of prevention, and to plan disease control programmes (Martin *et al.,* 1987; Dohoo *et al.,* 2003).

The concept of the web of causation is used to describe disease occurrence where the presence or absence of disease is not just a matter of pathogen being present or absent. In this concept, disease occurrence is determined by a complex web of interacting risk factors involving pathogen, host and environment. Factors influencing the probability of disease occurrence which are associated with the pathogen are infectivity, pathogenecity, virulence, immunogenecity and antigenic stability. Those associated with host include species, age, sex, breed, genotype, physiologic and pathologic status, and nutritional status. Environmental factors include agro-climatic factors, housing, and management (Martin *et al.,* 1987).

2.4.2. Assessing tick-borne diseases at multiple levels of population organization

In a large population, animals are likely to be separated into herds, villages, agro-ecological zones (AEZs), etc. Under such circumstances, disease responses are often correlated. This is mainly because disease determinants are generally not evenly

distributed throughout the population (Rothman, 1990). An example of correlation is where a very low proportion of herds in a population may be affected by a particular disease - but within those affected herds, the prevalence of the disease amongst animals may be quite high. This phenomenon has been described in a TBDs' observational study by Deem et al. (1993) who reported that in areas of high tick suitability (endemic stability), all animals in a herd were infected, whereas areas marginal for tick survival had endemic instability status. These findings suggest that determinants of occurrence of TBDs operate at the various levels of population organization, i.e. ecological-level, village-level, herd-level or at animal-level. Under such circumstances, information on both environmental suitability and interaction between environment-specific factors and different population-level factors are required for planning and implementation of disease control programmes. Thus, estimation of correlation in TBDs research is of particular biological relevance.

The expected correlation at the various levels of organization necessitates special analytical approaches. The problem of correlated data is common in veterinary epidemiology and the scope of the problem – and some of the analytic options for dealing with the problem – have been summarized (McDermott *et al.,* 1994). One such analytical approach is multilevel analyses technique. Importantly, multilevel analysis allows division of total variability of the outcome of interest into variance components corresponding to the various population levels. Subsequently, the contribution of each level to the total variance of the outcome of interest can then be

estimated. In this manner, levels which account for an important amount of variability (high-risk levels) can be identified and targeted with the expectation that interventions at that level will have the greatest impact on outcome (Dohoo *et al.,* 2003).

2.5. East Coast fever

2.5.1. Definition and aetiology

East Coast fever (ECF), a form of bovine theileriosis, is an important TBD of cattle caused by the protozoan parasite, *Theileria parva,* and transmitted transtadially (transmission from stage to stage) by the three-host tick, *Rhipicephalus appendiculatus* (Figure 2.1).

a) **b)** **c)** **d)**

Fig 2.1: East Coast fever vector tick, *R. appendiculatus*: a) adult male, b) adult female, c) engorged female, d) female laying eggs. Source: DFID, 2001.

2.5.2. Disease distribution

East Coast fever is restricted to those areas where cattle and the main vector tick, *R. appendiculatus,* co-exist. In eastern and central Africa, this includes much of Kenya (Figure 2.2), Uganda, Rwanda, Burundi, the eastern part of Democratic Republic of Congo (DRC), areas of southern Sudan bordering Uganda, and much of Tanzania. In southern Africa, the range is more limited, and it is confined to the northern and

central regions of Malawi, the northern, eastern and central regions of Zambia, and the Tete Province of Mozambique, all lying to the north of Zambezi River (Lawrence *et al.,* 2004) (Figure 2.3).

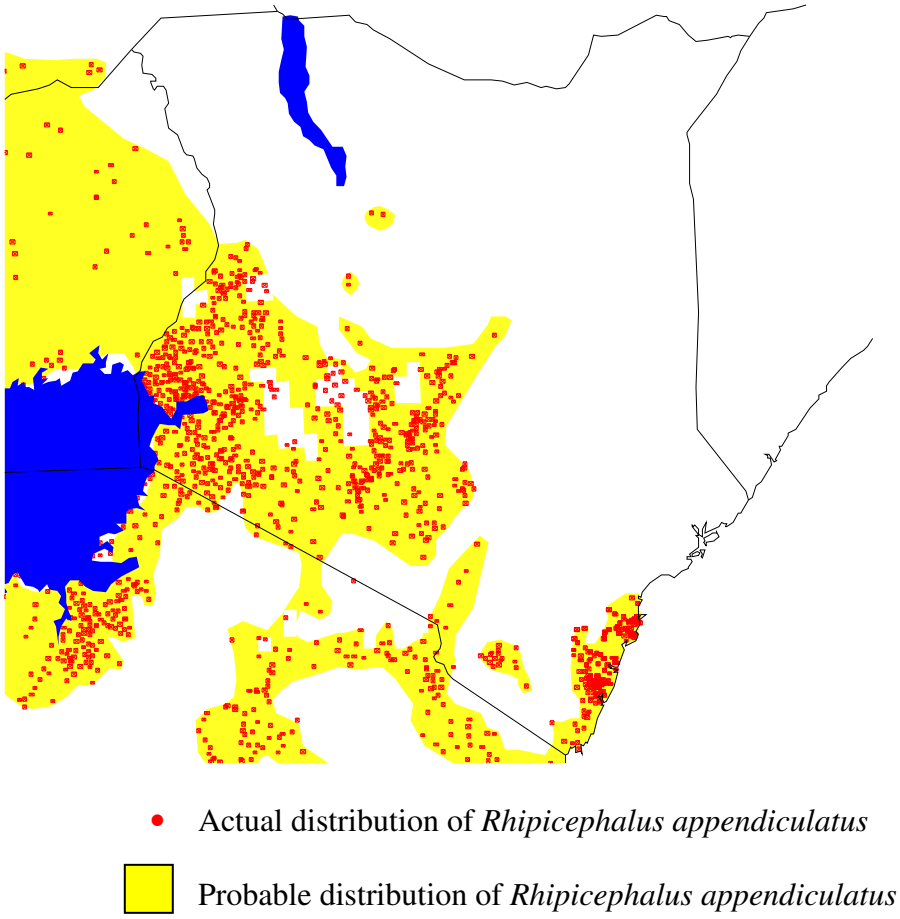


Fig 2.2: Map of Kenya illustrating the distribution of ECF vector tick (*R. appendiculatus*). Source: Lessard *et al.,* 1990.

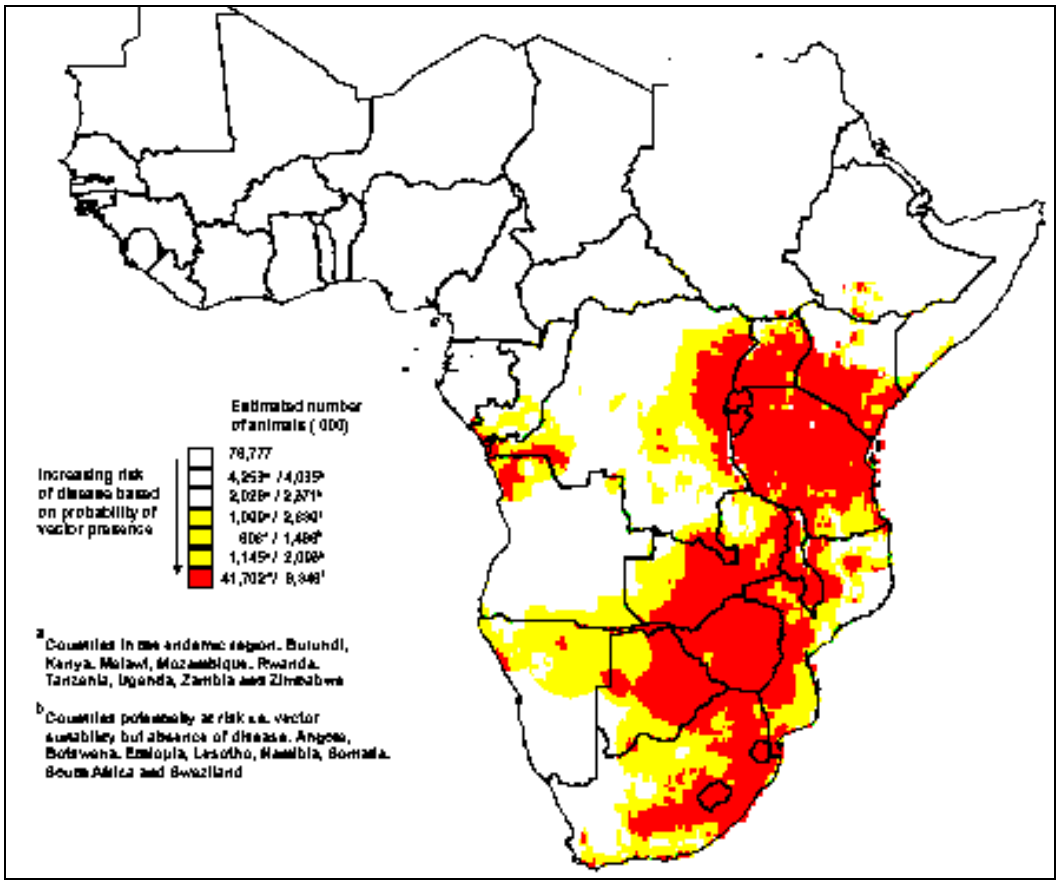
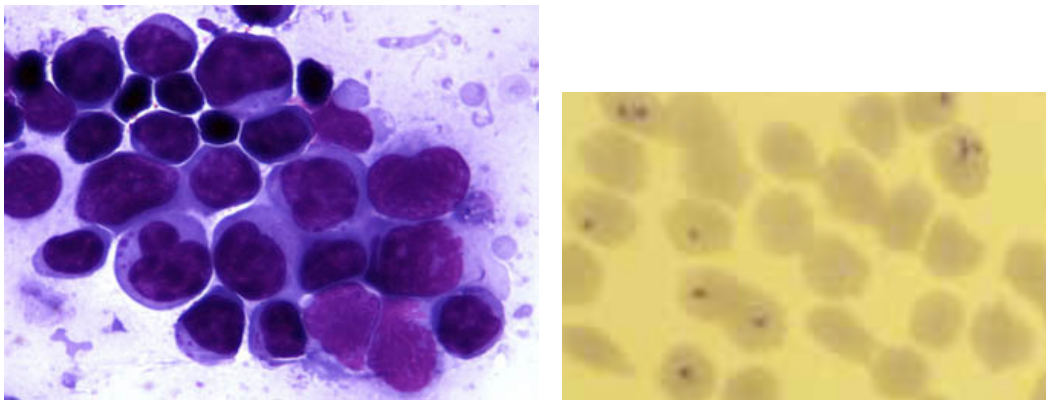


Fig 2.3: Map of Africa illustrating the potential distribution of ECF. Source: DFID, 2001

2.5.3. Lifecycle

Infected *R. appendiculatus* tick introduces the infective sporozoite stage to the host during feeding. The sporozoites are normally produced in large numbers in infected acinar cells of the tick salivary glands. The sporozoites then rapidly enter target host lymphocytes and develop into schizonts (Figure 2.4). At this stage, the infected lymphocyte becomes transformed into a lymphoblast. The lymphoblast divides synchronously with the schizont, giving rise to two infected daughter cells. Clonal expansion of infected lymphocytes ensue with an approximate tenfold increase of

schizonts every 3 days (Norval *et al.,* 1992). From day 14 after infection of cattle, individual schizonts undergo merogony to produce merozoites. Merozoites then invade the erythrocytes to become piroplasms (Figure 2.4). Piroplasm-infected erythrocytes are then ingested by ticks of the larval or nymphal stages and undergo a sexual cycle in the gut of the tick to produce zygotes, which in turn develop into motile kinete stages that infect the salivary glands of the next instar, the nymph or adult (Norval *et al.,* 1992) (Figure 2.5).



a) **b)**

Fig 2.4: a) Wright's-stained impression smear from an enlarged lymph node of a cow with ECF. Smear is composed of numerous large lymphoblasts with irregular nuclear outlines and prominent nucleoli (x100); b) *T. parva* piroplasms in a blood smear (x100). Source: DFID, 2001.

2.5.4. Risk factors for ECF

The epidemiological risk factors of ECF mainly depend upon complex interactions between the tick vector, agro–climatic factors, livestock production system, the causative organism, and vertebrate host.

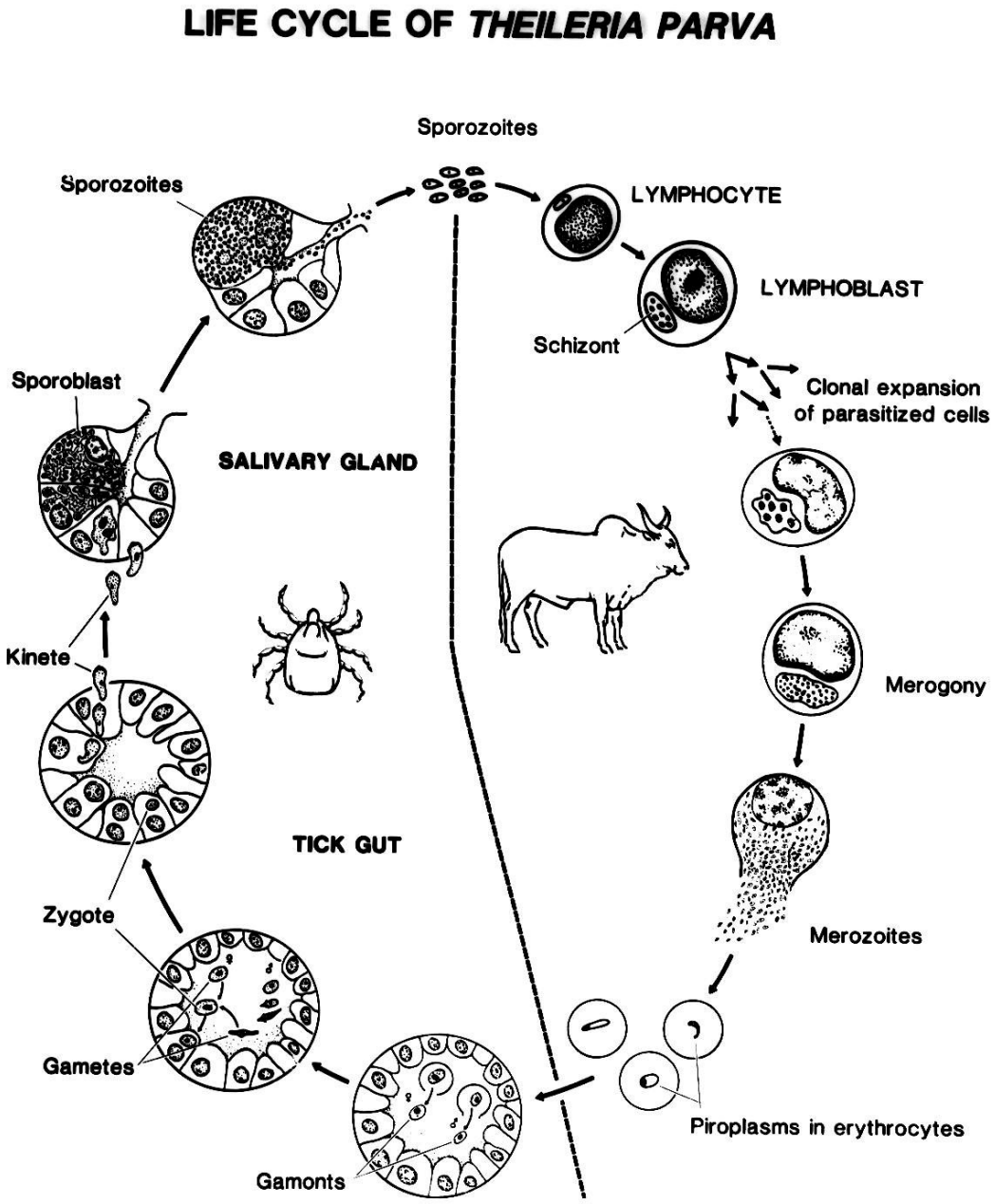


Fig 2.5: Life cycle of *Theileria parva* in cattle and in the vector tick (*R. appendiculatus*). Source: Norval *et al.,* 1992.

2.5.4.1. Causative agent risk factors for ECF

Ticks have the potential to transmit *T. parva* if, during the preceding stage of development, they have fed on an animal with circulating piroplasms. Infective cattle may be clinically ill, recently recovered, or persistent carriers (Lawrence *et al.,* 2004). A carrier state of *T. parva*, defined as the persistence of a tick-transmissible infection, is common among naturally recovered host animals, both in cattle and the major wildlife host, the African buffalo (*Syncerus caffer*) (Norval *et al.,* 1992; Lawrence *et al.,* 2004). Interestingly, it is possible for an infected animal to clear a *T. parva* infection while remaining immune, a condition described as sterile immunity, and found in 'Muguga' stocks (in Kenya) and 'Schoonspruit' stocks (in South Africa) (Lawrence *et al.,* 2004). However, investigations in Kenya have demonstrated that most *T. parva* stocks appear to induce immunity with either persistent or intermittent carrier state which is widely distributed in ECF endemic areas (Bishop *et al.,* 1992; Kariuki *et al*., 1995). This has been confirmed by the more sensitive polymerase chain reaction (PCR) assay based on species-specific sequences derived from the *T. parva* p104 rhoptry antigen gene (Skilton *et al.,* 2002). The infective dose the ticks receive, however, will be higher from clinically affected animals than from carriers (Lawrence *et al.,* 2004).

Although the optimum parasitaemia level that may be required for successful acquisition of *T. parva* by ticks upon feeding on reservoir hosts is not known, the level of parasitaemia in the reservoir hosts at the time of tick feeding is critical for

efficient acquisition of the pathogens by the ticks for certain TBDs (Eriks *et al*., 1993; Ueti *et al.,* 2005). It is known that the relationship between the parasite and tick vectors is complex, with evidence for a greater susceptibility of certain tick stocks for certain parasite stocks (Ochanda 1994). Within *R. appendiculatus* stocks there is a striking negative binomial distribution of infection with a small proportion of ticks containing a high proportion of the infection (Ochanda 1994). There are, however, conflicting reports on *T. parva* infection rates in vector ticks after feeding on animals with different levels of parasitaemia. Earlier observations indicated that *T. parva* infection rate in adult vector ticks feeding on low parasitaemic *T. parva*-carrier cattle was lower than in ticks feeding on calves with acute, high parasitaemic infections (Marcotty *et al.,* 2002; Ogden *et al.,* 2003). A xenodiagnosis experiment based on PCR assay performed on cattle that had been experimentally infected with two stocks differing in the carrier state induction ability resulted in unsuccessful parasite isolation from ticks at various time points (Skilton *et al.,* 2002). The latter finding led to the conclusion that an animal can still be PCR positive and, therefore, presumably infected, but apparently not able to transmit infection to ticks. This may be because parasitaemia is too low for effective transmission to ticks, or that the parasite may be present in the blood as the schizont and not as the infective piroplasm stage (Skilton *et al.,* 2002). Recently, however, it was experimentally found that vector ticks had the ability to acquire and transmit *T. parva* infection from carrier animals with low levels of parasitaemia (Konnai *et al.,* 2006). These findings are difficult to extrapolate to natural conditions but, nevertheless, suggest that the

efficiency of acquisition and transmission of *T. parva* infection by ticks may be dependant on the level of parasitaemia and parasite strain in the infected animals.

It is known that many strains of *T. parva* exist in the affected areas. Furthermore, heterogeneity between and within strains has been reported both between- (Irvin, 1987) and within-geographical areas (Matete *et al.,* 2004) and confirmed in molecular studies (Oura *et al*., 2005; Odongo *et al.,* 2006) suggesting that ECF could result from infection with a mixture of different parasite strains rather than a single strain.

2.5.4.2. Tick vector and ECF transmission dynamics

The dynamics of *R. appendiculatus* as a risk factor is influenced by numerous external factors, key among them being acquisition and transmission of the parasite (Ogden *et al.,* 2003) and agro-climatic factors (Norval *et al.,* 1992). The *R. appendiculatus* vector is a three-host tick, and transmission occurs from stage to stage. *Rhipicephalus appendiculatus* ticks can remain infected on the pasture for up to 2 years depending on the climatic conditions (the parasite dies out faster in hot climates) and the stage of infection (adult ticks survive longer than nymphs and the parasite dies out faster in nymphs compared with adult ticks) (Norval *et al.,* 1992).

Normally, for transmission to occur, the infected *R. appendiculatus* tick has to attach for several days to enable sporozoites to mature and be emitted in the saliva of the

feeding tick (Norval *et al.,* 1992). However, under high ambient temperatures, *R. appendiculatus* ticks on the ground may develop infective *Theileria* sporozoites, which can be transmitted to cattle within a few hours after attachment (Norval *et al.,* 1992). The tick clears itself of an infection during feeding though it may acquire a new infection if it later feeds on an animal in an active disease or a carrier state. Transmission of ECF may be expected to occur freely in areas where ecological factors permit an average total population of not less than 10 to 15 adult ticks per animal (Tatchell and Easton, 1986). However, information on the numbers of ticks that feed successfully (i.e. engorged ticks) may be more reliable than total tick counts.

The competence of different stocks of *R. appendiculatus* to transmit different stocks of *T. parva* has been studied (Ochanda *et al.,* 1998). In this study, there were significant differences in the patterns of infection of two *T. parva* stocks (Muguga and *T. parva* Boleni) in different stocks of ticks, and these differences were shown to be reproducible (Ochanda *et al.,* 1998). The Muguga tick stock from Kenya and the tick stock from Eastern Province in Zambia were reported to have the highest infections of *T. parva* Muguga and *T. parva* Boleni respectively (Ochanda *et al.,* 1998). Further, the Zambia Southern Province tick stock and the Zimbabwe Mashonaland West tick stock had the lowest infections of *T. parva* Muguga and *T. parva* Boleni respectively. These differences have direct influences on the epidemiology of the disease in the field.

2.5.4.3. Vertebrate host factors for ECF

Theileria parva is probably originally a parasite of African buffalo which has become adapted to cattle. Although it affects waterbuck (*Kobus defassa*) under natural conditions and the Asiatic buffalo (*Bubalus bubalis*) under experimental conditions, the parasite is not infective to other ungulates, or to any species of laboratory animal. However, it is possible for cattle to acquire the disease from buffalo (*Syncerus caffer*) though the resultant infection is rarely transmitted to other cattle (Lawrence *et al.,* 2004).

There is a marked variation in the susceptibility of cattle to infection. *Bos taurus* breeds of cattle are generally more susceptible to ECF than *Bos indicus* cattle (Norval *et al.,* 1992; Ndung'u *et al.,* 2005). Similar findings have been observed experimentally after induction of the infection using infected ticks as well as sporozoite stabilates (Paling *et al*., 1991). This variation could probably be due to the prolonged period of evolutionary time that indigenous cattle types have been exposed to ticks and TBDs (Minjauw and McLeod, 2003). This is supported by the observation that introduced cattle, whether of taurine, zebu, or sanga breed, are much more susceptible than cattle bred and reared in endemic areas (Lawrence *et al.,* 2004).

Increasing age has been associated with increased *T. parva* sero-prevalence (Moll *et al.,* 1986; O' Callaghan, 1998; Gitau *et al.,* 1999; Maloo *et al.,* 2001b; Swai *et al.,*

2005a; Rubaire-Akiiki *et al.,* 2006). This result may be expected since age is a surrogate measure of amount of exposure-time, and antibodies for *T. parva* persist in circulation for as long as six months (Katende *et al.,* 1998). This factor is indirectly correlated with environment, particularly AEZ, in that in endemically stable areas, there is high and continuous challenge by ticks and TBDs. This is in turn associated with persistent challenge of *Bos indicus* calves early in age (Moll *et al.,* 1986; Norval *et al.,* 1992; Gitau *et al.,* 1999). In such conditions, the majority of calves are expected to have seroconverted by six months of age and this continues throughout the animal's life. However, in some areas, higher prevalence of *T. parva* in calves than adults have been reported through use of the more sensitive PCR assay (Oura *et al.,* 2005; Bazarusanga *et al.,* 2007; Salih *et al.,* 2007).

Previous studies have not found any association between sex of the calf and *T. parva* seroprevalence (Gitau *et al.,* 1997; Muraguri *et al.,* 2005; Swai *et al.,* 2005a; Rubaire-Akiiki *et al.,* 2006). However, this factor is related to the importance that either sex is regarded in a given livestock production system. Thus, in the smallholder dairy system, a lot of value is attached to female calves because they are a future source of both financial and human capital (Gitau *et al.,* 1994). In traditional crop-livestock systems, cattle of both sexes mainly contribute to financial, human as well as physical capital (Minjauw and McLeod, 2003). There is no literature on the association of this factor with *T. parva* seroprevalence in traditional crop-livestock systems.

2.5.4.4. Herd-level management risk factors in relation to ECF control

There are few drugs for the treatment of ECF. These chemotherapeutic drugs, parvaquone (Clexon®, Wellcome Pharmaceutical Ltd, UK) and buparvaquone (Butalex®, Schering-plough Animal Health, UK), have the disadvantage of being relatively expensive, and this reduces their application in the field (Lawrence *et al.,* 2004). Both drugs do not achieve a complete parasitological cure and recovered animals may remain intermittent carriers (Lawrence *et al.,* 2004). The clinical cure rate for these drugs range between ranges 90 and 100% at dose rate of 2.5 to 5mg/kg body weight (buparvaquone) (Dolan *et al.,* 1992; Ngumi *et al.,* 1992; Thaiya *et al.,* 1993; Wanjohi *et al.,* 1995; Wilkie *et al.,* 1998; Mbwambo *et al.,* 2002; Mbwambo *et al.,* 2006; Muraguri *et al.,* 2006). East Coast fever immunization with live sporozoites and simultaneous treatment with 10 to 20% oxytetracycline (Radley *et al.,* 1975; Radley, 1981) also induces a carrier state with most *T. parva* stocks (Kariuki *et al*., 1995; Lawrence *et al.,* 2004). This concept is important in so far as establishment of new strains in a particular area is concerned (Skilton *et al.,* 2002).

East Coast fever can only be introduced by ticks which have dropped from infected cattle during the preceding stage of the life cycle. Therefore, the spread of infection is mainly through cattle movement (Billiouw *et al.,* 1999). Cattle maintained under strict confinement (zero-grazing) can be protected from ECF (Gitau *et al.,* 1999; Lawrence *et al.,* 2004). Animals raised under an open grazing system are frequently exposed to infected ticks, thus developing circulating antibodies. This has been

confirmed in Kenya where different livestock production systems have been reported to affect exposure of cattle to ticks resulting in considerable difference in tick infestation levels and corresponding infection prevalence between cattle reared under open grazing and zero-grazing management systems (Gitau *et al.,* 1997; Gitau *et al.,* 1999; Gitau *et al.,* 2000; Maloo *et al.,* 2001a, b). However, in certain circumstances, open grazed cattle could be reservoirs for both ticks and TBDs to zero-grazed cattle either in the same farm or in the neighbourhood cattle particularly if their grazing areas are also the main source of "cut and carry" forage for the zero-grazed cattle (Maloo *et al.,* 2001 b; Swai *et al.,* 2005a). Infected ticks are transported in the cut fodder to the zero-grazed cattle thus introducing the infection.

Theoretically, cattle can be maintained in an infected environment provided that they are kept completely free of infected *R. appendiculatus* through application of acaricides. Acaricide application is usually on a weekly basis, but this rate has to be increased when the challenge is high (Minjauw and McLeod, 2003). However, acaricides have the disadvantage of being relatively expensive and this reduces their application in the field. Moreover, high level of acaricide exposure leads to unacceptable residues in milk and meat, the resistance of vectors, and where successful, the creation of endemic instability conditions with a large proportion of cattle population becoming susceptible (Minjauw and McLeod, 2003). Previous studies have not found any association between the method and intensity of acaricide treatment and seroprevalence to *T. parva* (Gitau *et al.,* 1999; Swai *et al.,* 2005a;

Okuthe and Buyu, 2006; Swai *et al.,* 2007). This may be due to poor practices such as use of wrong dilutions or inadequate animal coverage. A good example is the discrepancy observed in the frequency of acaricide application stated by farmers between cross-sectional and longitudinal studies in the same area (Gitau *et al.,* 1997; Gitau *et al.,* 1999). In order to optimize the use of acaricides, farmers need to understand the biology and ecology of the tick species on their farms since an effective control strategy should use the available compounds at the most appropriate application frequency (Minjauw and McLeod, 2003).

2.5.4.5. Area-level risk factors for ECF

The distribution of ECF is strictly associated with the distribution of *R. appendiculatus* ticks and related species such as *R. zambiensis*. However, this relationship is influenced by external factors such as climate, vegetation, and host distribution (Norval *et al.,* 1992). *Rhipicephalus appendiculatus* is found from sea level to over 8,000 feet in areas where there is annual rainfall of over 500 mm. The influence of climate on the distribution of *R. appendiculatus* ticks is well demonstrated in some areas in eastern and southern Africa. Up to three generations of *R. appendiculatus* ticks can occur per year in favourable areas of East Africa particularly around the Lake Victoria basin (Norval *et al.,* 1992). Indeed, recent studies reported an all year-round prevalence of *T. parva* in almost all regions of Rwanda (Bazarusanga *et al.,* 2007; Bazarusanga *et al.,* 2008). In southern Africa there is one rainy season each year. As a result, the *R. appendiculatus* tick is strictly

seasonal, with one generation a year and subsequently, adult tick infestation on hosts becomes limited to a period of approximately three months of the year (Short and Norval, 1981).

Free-living *R. appendiculatus* have to seek sheltered microhabitats in which either to moult or to oviposit and undergo a period of development. The free-living stage survives best where there is mixture of grass and tree cover (savannah woodland) and rarely in open grassland. Conditions that are not suitable for the free-living stages include deep forests and areas that have undergone overgrazing and environmental degradation (Norval *et al.,* 1992; Lawrence *et al.,* 2004).

The main factors affecting the development rates of both engorged *R. appendiculatus* ticks and the unfed stages are mainly the combined effects of temperature and humidity (Norval *et al.,* 1992). *Rhipicephalus appendiculatus* suitability, based on temperature and vegetation can be used to describe ticks and TBDs distribution on an environmental or geographic basis. The distribution of climatic suitability has been plotted on the basis of eco-climatic indices (Figure 2.6). Interestingly, areas of eco-climatic suitability exist where the presence of *R. appendiculatus* has yet to be recorded (Lawrence *et al.,* 2004), probably due to the interrelationships of the factors

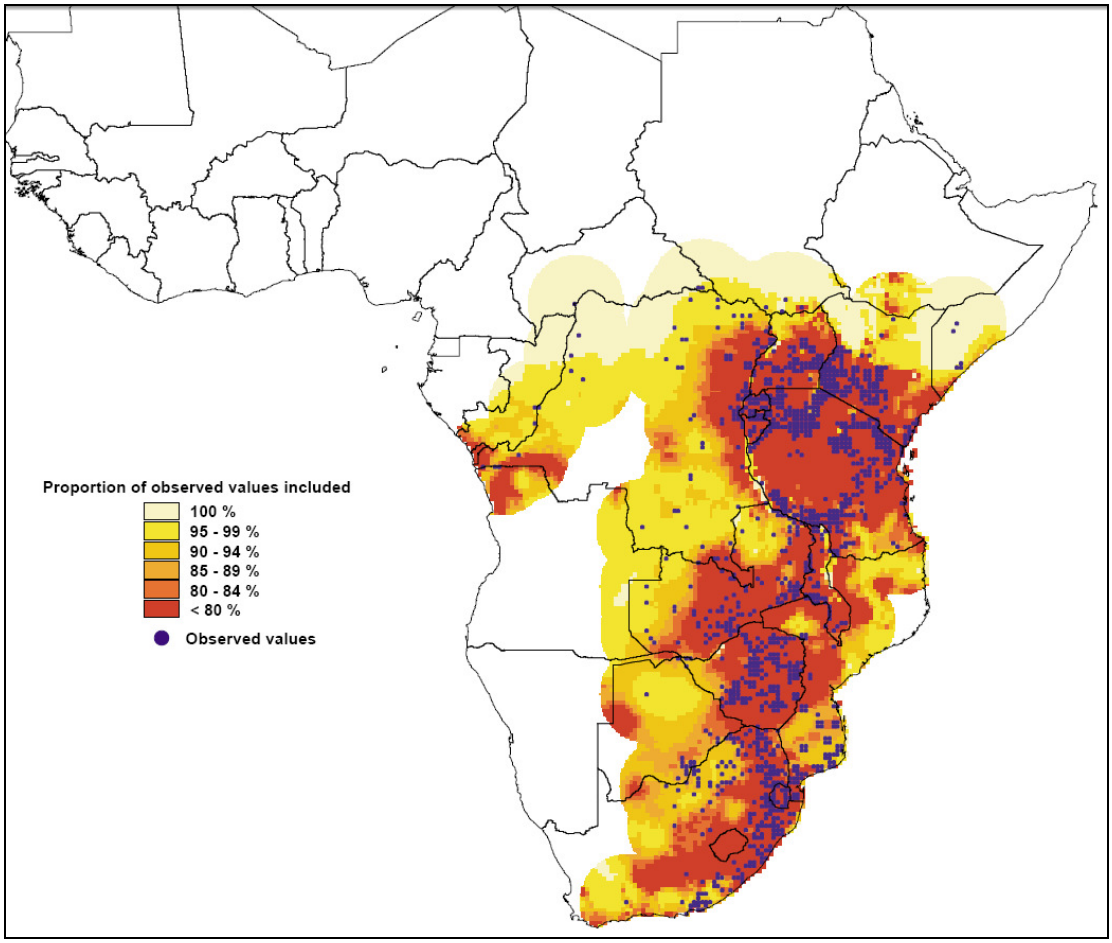


Fig 2.6: Distribution of East Coast fever based on the probability of the presence of the vector, the brown ear tick. Source: DFID, 2001.

described above. In addition, on the margins of *R. appendiculatus* distribution, the distribution may expand or contract with seasonal changes in climatic suitability (Lawrence *et al.,* 2004).

In Kenya, a strong correlation between grazing system, cattle breed and agro-ecological zone (AEZ) on one hand and prevalence of *T. parva* antibodies on the other has been found (Deem *et al.,* 1993; Gitau *et al.,* 1997; Gitau *et al.,* 1999;

Maloo *et al.,* 2001a, b). Generally, lower elevation AEZs are characterized by high seroprevalence of *T. parva* antibodies with resultant development of solid immunity (endemic stability). In the lower elevation AEZs, three factors predominate; *Bos indicus* cattle breeds and their crosses and open grazing system with little or no tick control. Higher elevation AEZs are commonly endemically unstable and characterized by low antibody prevalence and high disease incidence which is normally distributed in all age groups (Norval *et al.,* 1992; Perry *et al.,* 1992; Gitau *et al.,* 1997; Gitau *et al.,* 1999; Gitau *et al.,* 2000). In the higher elevation AEZs, *Bos taurus* cattle predominate and zero-grazing management is practised (Gitau *et al.,* 1997; Gitau *et al.,* 1999; Gitau *et al.,* 2000).

Tick abundances are known to vary with time (season to season and year to year) and space (between habitats and ecological zones) due to interactions of numerous factors, such as host diversity and climate (Norval and Lightfoot, 1982). Weather seasonality as a risk factor has been reported to affect *T. parva* prevalence in cattle (Norval *et al.,* 1992). Large numbers of *R. appendiculatus* have been observed during the rainy to hot dry seasons (Mulei *et al.,* 1989; Ndamukong, 1993; Swai *et al.,* 2006) and during rainy season (Billiouw *et al.,* 2005; Salih *et al.,* 2007). The amount of rainfall is the principal stimulus to *R. appendiculatus* activity (Yeoman 1966). Indeed, a recent study reported no significant differences in *T. parva* among seasons in Rwanda (Bazarusanga *et al.,* 2007).

2.5.5. Epidemiological states of East Coast fever

From the literature reviewed above on risk factors for *T. parva* seroprevalence, it can be clearly concluded that the occurrence and importance of ECF is a reflection of complex interactions involving the causative organism, the tick vector, the vertebrate host, and the environment. These interactions have led to the development of the concept of epidemiological states dependent on occurrence and extent of distribution of the disease. The epidemiological states include endemic stability, endemic instability and epidemic states (Norval *et al.,* 1992). The concept of epidemiologic states has been applied in developing appropriate and cost-effective TBDs control strategies. Although the epidemiology of ECF is well documented (Norval *et al.,* 1992), only a few epidemiological studies on the other TBDs have been undertaken. Consequently, principles developed during the study of the epidemiology of ECF are also speculated to apply for other TBDs.

2.5.5.1. Endemic stability

Endemic stability describes the situation where a stable ecological relationship between the host, vector and environment is established in which all co-exist with minimal effects in terms of clinical disease and mortality (Norval *et al.,* 1992; Perry *et al.,* 1992). In this state, infection of *Bos indicus* calves takes place by six months of age and hence development of immunity against the disease (Moll, 1986; Norval *et al.,* 1992; Perry and Young, 1995; Swai *et al.,* 2006). In addition, there is regular transmission of *T. parva* in all age groups of the cattle population and high levels of

population immunity are achieved. This situation is associated with suitable AEZs for the vector and where major seasonal fluctuations in vector abundance does not occur (Lawrence *et al.,* 2004). Infection prevalence in ticks may be around 1 to 2% whereas the infection intensity in the infected ticks is also low (Lawrence *et al.,* 2004).

2.5.5.2. Endemic instability

In endemically unstable conditions, the antibody prevalence is low and the disease incidence is normally high and distributed in all age groups (Norval *et al.,* 1992; Lawrence *et al.,* 2004). This is found in two forms, low incidence and high incidence instability. Low incidence instability is found in areas of very low infection challenge, either in areas marginally suitable for the vector or where acaricides are applied intensively. High incidence instability is characterized by an infection challenge that is insufficient to induce population immunity. This can be as a result of ineffective tick control, or intermediate levels of infection challenge (Lawrence *et al.,* 2004).

2.5.5.3. Epidemic East Coast fever

Epidemic ECF occurs when the disease is introduced to areas previously free of the disease, and often occurs on a seasonal basis at the margins of *R. appendiculatus* distribution (Lawrence *et al.,* 2004) and when intensive tick control fails in an endemic area (Perry and Young, 1995).

2.5.6. Clinical syndrome

The main clinical signs of ECF include fever, lymphadenopathy, pulmonary and subcutaneous oedema, petechial hemorrhages, characteristic cough and corneal opacity. In acute cases, there is sudden loss of weight while complete blindness may occur in chronic cases with some parasite strains. In terminal cases, recumbency, cachexia, hypothermia and nervous signs may be observed (Irvin and Mwamachi, 1983; Moll *et al.,* 1986; Norval *et al*., 1992; Lawrence *et. al.,* 2004).

2.5.7. Diagnosis of ECF

2.5.7.1. Clinical, parasitological and molecular diagnosis

Clinically, a febrile disease with high fever and signs of enlarged lymph nodes associated with infestation by the tick vector is suggestive of ECF (Norval *et al.,* 1992). Diagnosis is usually achieved by finding *Theileria* parasites in lymph node needle biopsy smears (schizonts) and Giemsa-stained blood smears (piroplasms) (Figure 2.4). Identification of schizonts in lymphoid cells is considered diagnostically definitive of theileriosis (Lawrence *et al.,* 2004). Molecular genetic technologies can be applied to material from cattle and ticks, including the use of probes and PCR. The blood-spot PCR assay based on a conserved region of the *T. parva* 104 kDa rhoptry antigen gene is potentially very sensitive and specific (Skilton *et al.,* 2002). However, molecular tools are used in research and are not routinely used to diagnose ECF.

2.5.7.2. Sero-diagnosis

Antibodies to *Theileria* antigens become detectable approximately 15-20 days after infection (Katende *et al.,* 1998) and their levels continue to rise for a further 20–30 days (Katende *et al.,* 1998). In the absence of re-challenge, antibody levels stabilize and decline at a variable rate, usually to 1:40 or less by six months after infection, as measured by indirect fluorescent antibody test although schizont antibodies decline more slowly than piroplasm antibodies (Lawrence *et al.,* 2004).

2.5.7.2.1. Indirect fluorescent antibody test

The indirect fluorescent antibody test (IFAT) employs cell culture schizont antigen (Norval *et al.,* 1992) but can also use piroplasm antigen. The IFAT is considered sensitive and reasonably specific (Norval *et al.,* 1992). However, the schizont and piroplasm IFAT for *T. parva* cross-reacts with *T. annulata* and *T. taurotragi.* The *T. parva* schizont IFAT does not cross-react with *T. mutans* (Lawrence *et. al.,* 2004). Whereas cross-reactivity with *T. taurotragi* is a major disadvantage of the test, that with *T. annulata* is not of major significance as *T. parva* and *T. annulata* occur in distinct geographical areas and are vectored by different ticks.

2.5.7.2.2. Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunoassays (ELISA) were previously developed using whole parasite lysates or specific antigens isolated by monoclonal antibodies (Katende *et al.,* 1990). Currently, the *T. parva* indirect ELISA is based on a recombinant form of

polymorphic immunodominant molecule (PIM) which is found on the surface of both sporozoites and schizonts. This assay is specific to *T. parva* (Toye *et al.*, 1996) and has demonstrated a sensitivity of over 99% and a specificity of between 94 and 98% in experimental and field sera (Katende *et al.,* 1998). It does not cross-react with other *Theileria* species such as *T. taurotragi, T. mutans, T. annulata,* or *T. buffeli* (Katende *et al.,* 1998). This assay detects antibodies for as long as 215 days from 15-20 days after a single infection with *T. parva* in experimental animals (Katende *et al.,* 1998). It is believed that the size and sequence polymorphism exhibited by PIM (Toye *et al.,* 1991) does not affect the detection of antibodies in field sera from cattle infected with different polymorphic stocks of *T. parva* (Katende *et al.,* 1998) largely due to the presence of conserved epitopes across many *T. parva* stocks (Toye *et al.*, 1996). However, serological tests for *T. parva* suffer from the inability to distinguish between animals that have cleared the parasite and those that remain carriers of infection (Skilton *et al.,* 2002).

In ELISA, percent positivity (PP) values are preferred to optical density readings as the PP values can be adjusted for variations associated with inconsistent background activity and provides standardization between assays (Wright *et al.,* 1993; Katende *et al.,* 1998).

2.5.8. Control

2.5.8.1. Treatment

The first chemotherapeutic drug for ECF management to be shown to have antitheilerial activity was naphthaquinone menoctone (McHardy *et al.,* 1976). Further development of this compound through laboratory evaluation and field studies (McHardy *et al.,* 1976; Chema *et al.,* 1986) culminated in the launching of the first and widely used drug for ECF, parvaquone (Clexon®, Wellcome Pharmaceutical Ltd, UK). Another naphthaquinone, buparvaquone, (Butalex®, Schering-plough Animal Health, UK) has been developed (McHardy *et al.,* 1985) and extensively evaluated (Dolan *et al.,* 1992; Ngumi *et al.,* 1992; Thaiya *et al.,* 1993; Wanjohi *et al.,* 1995; Wilkie *et al.,* 1998; Mbwambo *et al.,* 2002; Mbwambo *et al.,* 2006; Muraguri *et al.,* 2006) and is currently available for treatment of ECF. Halofuginone (Terit®, Hoechst Pharmaceutical, Germany) is also used for the management of ECF (Dolan *et al.,* 1986). A second type of parvaquone, (Parvexon®, Bimeda Export Ltd, Ireland), was developed and initial field evaluations indicated that its efficacy is comparable to that of Butalex® (Muraguri *et al.,* 1999). The efficacy and reliability of these compounds are however dependent on early diagnosis and administration of full therapeutic doses (McHardy, 1989). Unfortunately, the prohibitively high costs of these drugs have resulted in their limited use by smallholder farmers (Lawrence *et al.,* 2004).

2.5.8.2. Tick control

The primary method of controlling ECF and other TBDs has been the application of cattle with chemical acaricides. These acaricides previously included chlorinated hydrocarbons (CHCs), Organo-phosphoric acid esters (OPAEs), carbamates, macrocyclic lactones and benzoylphenylureas and the more recent cyclic amidines, (Stendel and Hamel, 1990; Georghiou and Lagunes-Tejeda, 1991; Seifert, 1996; Minjauw and McLeod, 2003). The methods used for the application of acaricides include plunge dipping, spray race, hand spraying, and hand dressing and use of pour-ons (FAO, 1984; Minjauw and McLeod, 2003). The principal objective of tick control is to kill the infesting ticks in order to break the life cycle and to ensure total coverage of all predilection sites of the various tick species.

2.5.8.3. Immunization

The most successful ECF immunization technique involves an "infection and treatment method" (ITM) (Radley *et al.,* 1975; Radley, 1981) initially using 20% or 30% oxytetracycline and more recently, parvaquone or buparvaquone (Lawrence *et al.,* 2004). Animals are inoculated with a potentially lethal dose of infective sporozoite stabilate prepared from experimentally infected ticks, and treated simultaneously with tetracyclines. This procedure induces a controlled disease reaction that results in a mild infection and a solid protective immunity to homologous parasite challenge (Lawrence *et al.,* 2004). Uilenberg (1999) has extensively reviewed the important aspects of immunization against ECF.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Study site

3.1.1. Location and description of Mbeere District

Mbeere District is one of the 12 districts constituting Eastern Province of Kenya and lies between Latitudes 0^{o} 20' and 0^{o}50' South and Longitude 370 16' and 370 56' East (Figure 3.1). The district was carved out of Embu District in 1996 (Central Bureau of Statistics, CBS, 2001).

Fig 3.1: Map of Kenya illustrating the geographical position of Mbeere District

Mbeere District borders with Embu District to the Northwest, Tharaka/Nithi District to the North, Mwingi District to the East, Machakos District to the South and Southeast and Kirinyaga District to the West (Ministry of Environmental Conservation, MEC, 1999). The district has a total area of about 2,093 km^2 and is sparsely populated with a population density of 82 persons per km^2 (Table 3.1) (CBS, 2001).

3.1.2. Topography of Mbeere District

Mbeere District slopes in a North-West to South-East direction from 1,200 m above sea level down to 500 m on the Tana River basin. The slope is however broken by a few hills. Mwea plains cover the southern part of the district and then gradually rise northwards culminating in hills and valleys to the northern and eastern parts of the district. Five permanent rivers serve the district and they include Tana, Ena, Rupingazi, Thuci and Thiba, all of which flow in a general south-easterly direction (GoK, 2001).

Table 3.1: Area and household characteristics of Mbeere District by division

Division	Area (km^2)	Area (% of total)	Number of households	Density (persons/km^2)
Siakago	365.3	17.5	7,852	93
Gachoka	800.3	38.3	7,677	74
Evurore	410	19.6	12,905	90
Mwea	514.9	24.6	8,602	79
Total	2092.5	100	37,036	82

Source: Central Bureau of Statistics (CBS), 2001.

3.1.3. Climate of Mbeere District

Temperature ranges from 20^{o}C to 32^{o}C depending on altitude. August is usually the coldest month with an average minimum temperature of 15^{o}C, while the warmest month is March with an average maximum temperature of 30^{o}C. The district has a bimodal rainfall pattern with long rains falling between March and June and short rains from October to December. Rainfall in the district ranges from 550-1100 mm per year depending on altitude. Most parts of the district receive less than 550 mm per year (Jaetzold and Schimdt, 1983). Rainfall is unpredictable and erratic in most parts of the district. The district is covered by three main AEZs (Jaetzold & Schimdt, 1983): the marginal cotton zone (Lower midlands 3), the Lower midland livestock-millet zone (Lower midlands 4) and Lowland livestock millet zone (Lowlands 5) (Jaetzold and Schimdt, 1983).

3.2. Study design

3.2.1. Selection of sub-locations and farms

The study was carried out in March 2007. The farms were selected by multistage random sampling method. To increase the geographical spread of the study, all the four administrative divisions (Siakago, Gachoka, Evurore and Mwea) (CBS, 2001) in the district were included in the study. A sampling frame of the sub-locations (the smallest administrative unit) in each division was obtained from the District Commissioner's office in Siakago. The 39 sub-locations in the district were listed according to divisions and two sub-locations from each division were randomly

selected giving a total of 8 sub-locations. In collaboration with the Assistant-chief and village elders from each selected sub-location, a list of all households owning cattle was compiled and ten from each sub-location were randomly sampled using random number tables. To create a map of the selected households, a Garmin® Global Positioning System (GPS) (Garmin International, Inc., USA) hand receiver was used to obtain the GPS readings (Easting, Northing and Altitude) in Universal Transverse Mercator (UTM) units. Arcview version 3.3 (ESRI, Buckinghamshire, UK) was used to create a detailed map of the study area (Figure 3.2).

3.2.2. Sample size and selection of animals

The *T. parva* antibody prevalence in cattle in Mbeere District was not known *a priori* and so, 50% prevalence and a 5% tolerable error were assumed when determining the desired sample size of cattle to be sampled. The sample size (400) was determined according to the method described by Martin *et al.* (1987) as follows:

$$n = \frac{1.96^2 p(1-p)}{L^2}$$

Where n is the desired sample size, 1.96 is the z value for the desired confidence level (95%), p is an estimate of the probable prevalence, and L is the level of precision (tolerable error) (distance of the sample estimate in either direction from the true population proportion considered acceptable). A total of 440 animals were sampled and examined in this study.

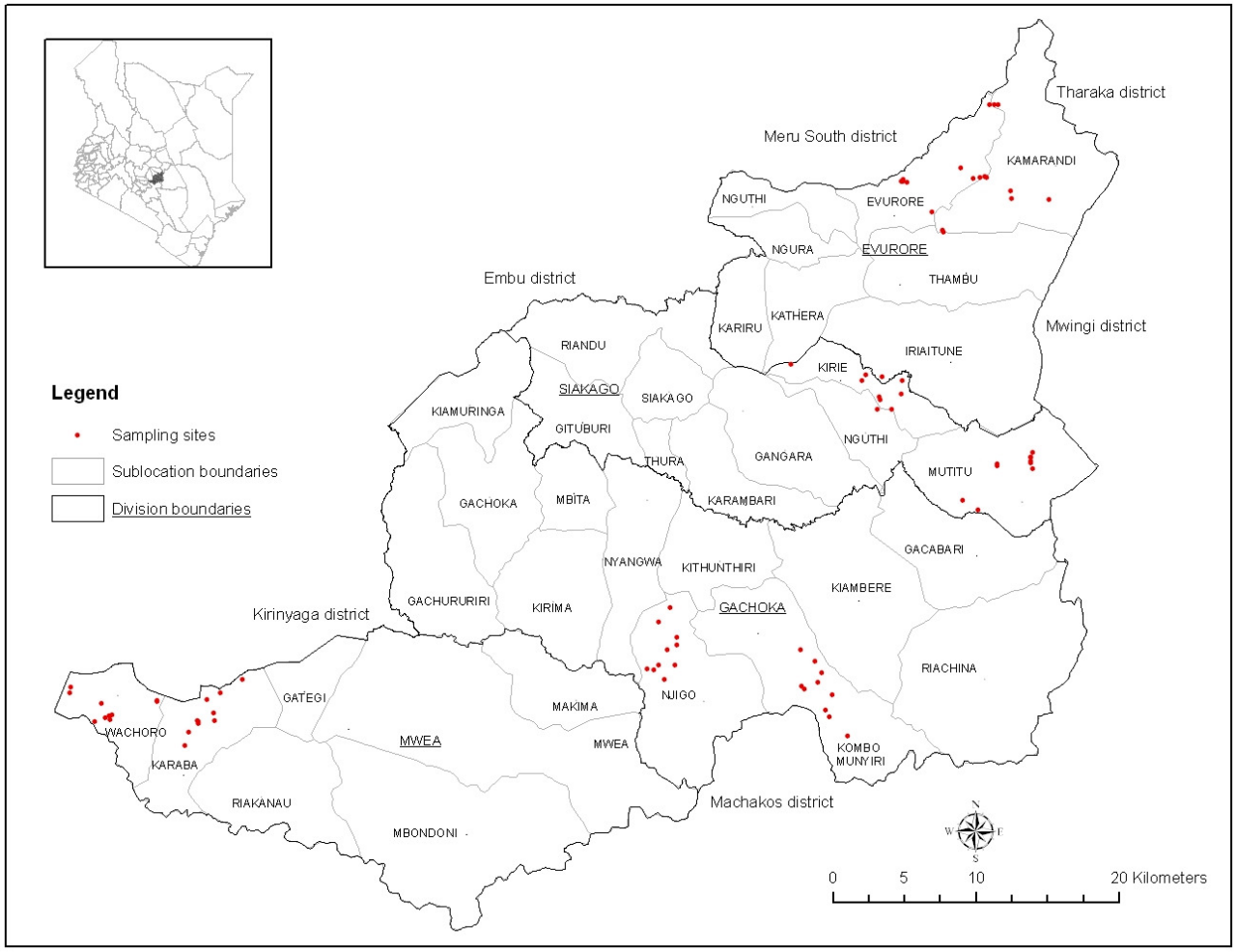


Fig 3.2: Map of Mbeere district showing the sampling sites (location of selected households/farms/herds), March, 2007.

Sampling of individual animals used proportional allocation approach. Thus, as the herd sizes were not known ahead of time, a constant 50% proportion of animals in each herd were selected using systematic random sampling to ensure that each animal had the same probability of selection. In herds that had 6 or less cattle, all animals in that herd were sampled. Details of the number of animals and number selected per farm (herd), number selected per sub-location, AEZs and division are shown in Appendix 1. Further, number of cattle selected during the study relative to the number reported from livestock census held in 2001 per division is shown in Appendix 2 (Ministry of Livestock and Fisheries Development, MoLD, 2003)

3.3. Data handling and management

3.3.1. Farming system data collection

After farm selection, an adult resident (18 years or older) from the selected household was asked for verbal consent to participate in a brief interview. Using a structured questionnaire, information was gathered on general farm data and information on animal and farm level management practices (Appendix 3).

General farm data included family biodata, household farming activities, land tenure and animal ownership and management in relation to gender. Livestock management information included the species and breed of domestic animals kept in the farm, reasons for keeping livestock, cow milk production and constraints to livestock production. Details of animal diseases as a constraint were sought including the common disease

syndromes encountered in the farm. Crops grown on the farm were listed and details taken on whether crop residues were offered to cattle.

3.3.2. Animal data collection

The selected animals were first restrained, their body conditions scored, and sex, breed and the respective ages recorded from the owner and/or respondent. Approximately 5 ml of blood were collected from each animal in a labeled 10-ml vacutainer tube (Becton-Dickinson, UK) by jugular venipuncture. The vacutainer tubes were kept slanted for about 2 hours at ambient temperature in the field and later transferred into a cool box for storage overnight. Serum samples were separated the following day and were stored at -20°C at Mbeere District Veterinary Office, Siakago, and later, also, at -20°C at the Kenya Agricultural Research Institute, Veterinary Research Centre (KARI-VRC) until analysis. Analysis was done at the International Livestock Research Institute (ILRI), Nairobi by use of indirect Enzyme Linked Immunoassay (ELISA) to determine individual animal's *T. parva* serostatus.

3.3.3. Statistical analyses

Data collected from farmers through questionnaires during the cross-sectional study were coded and stored in Microsoft Excel software. All statistical analyses were conducted in Genstat statistical software, 2nd Edition (GenStat Release 4.24DE, 2005).

Analyses of all farm data, variables on animal husbandry and management practices, perceptions of the importance of different diseases and disease management were undertaken using descriptive statistical procedures.

Most of risk factor information for *T. parva* seropositivity was obtained from questionnaire responses. Relationship between the risk factors and *T. parva* seropositivity was investigated by logistic regression models. To investigate the contribution of the levels of cattle population organization to the total variance of *T. parva* seropositivity, this study applied multilevel models.

CHAPTER 4

DESCRIPTION AND CHARACTERISTICS OF TRADITIONAL SMALLHOLDER CROP-LIVESTOCK PRODUCTION SYSTEM IN MBEERE DISTRICT, KENYA

4.1. Introduction

The importance of agriculture in the growth of Kenya's economy is well documented in various reports (GoK, 1994; GoK, 1997; FAO, 2003; World Bank, 2003). It is perceived that the needs of the high agricultural frontier in Kenya (which constitutes 25% of Kenya's landmass) have been met through research and extension whereas the ASALs have not benefited from agricultural innovations or appropriate technologies (Onduru *et al.,* 2002). This is in spite of the fact that Kenya's ASALs' resources are enormous and their ecosystems fragile, thereby requiring appropriate management strategies to ensure sustainable productivity (Kamau, 2004). Livestock production is the main form of ASALs use through pastoralism, agro-pastoralism and mixed crop-livestock farming systems (FAO, 2003).

Rangeland mixed crop-livestock production systems are characterized by small capital investment and less attention of farmers to key management issues such as nutrition and disease preventive measures resulting in low return on investments (Minjauw and McLeod, 2003). Yet, these systems have the potential for intensification and for contributing to overall agricultural productivity and sustainability (McDermott *et al.,* 1999).

Milk production has been identified as one livestock component that can be improved by such intensification in these systems (Thorpe *et al.,* 1993). Livestock production intensification would require improved management and increased resources per animal. Identification of the major challenges and viable prospects would be an important step in evaluating potential development alternatives for increased productivity in these systems.

As part of a larger study to identify risk factors associated with *T. parva* infection in cattle in Mbeere District, this study component was conceptualized with the objective of describing characteristics of the traditional smallholder crop-livestock production system in the semi-arid district of Mbeere, Kenya. The study further attempted to identify and qualify potential livestock production constraints and opportunities in the district, through a cross-sectional study.

4.2. Materials and methods

4.2.1. Study site, study design and data management

The details of the study site are as given in sections 3.1., 3.2. and 3.3.

4.3. Results

4.3.1. Sampling frame profiles

In the first stage of sampling, there were 39 sub-locations in the district out of which two were randomly selected from each division. Table 4.1 shows the total number of

households owning cattle in the selected sub-locations as given out by the area Assistant-chief and village elders. Ten households from each sub-location were then randomly sampled to give a total of 80 households. A 100% voluntary participatory rate was obtained as all selected households participated in the study.

Table 4.1: Sampling frame for households from the randomly selected sub-locations in Mbeere district, March 2007

Sub-location	Division	Number of households owning cattle
Kerie	Siakago	373
Mutitu	Siakago	449
Kombo Munyiri	Gachoka	914
Njigo	Gachoka	801
Ishiara	Evurore	941
Kamarandi	Evurore	1677
Karaba	Mwea	1456
Wachoro	Mwea	1743
Total		**8354**

4.3.2. Household biodata

The household survey results showed that the average family size was 7.3 persons (range 1-15) with most (54.4% (n=43/79)) of the household heads having primary education, 19% (n=15/79) with secondary education and 8.9% (n=7/79) with tertially education while 17.7% (n=14/79) had no formal education (one respondent failed to give a response to this question). Most (88.8% (n=71/80)) families were headed by men, although a few households (11.2% (n=9/80)) were headed by women who were either widowed or single mothers.

4.3.3 Smallholder unit farming profiles

All 80 farms (with 440 animals) that represented approximately 1.7% of all farms in the sampling frame (n=4783) were visited and a questionnaire administered via personal interviews.

4.3.3.1. General farming activities

Figure 4.1 *a*, *b* and *c* illustrates the characteristics of household farm activities and enterprises important for the subsistence and cash income for the household. Ninety-four percent (n=75/80) of the households depended on both crops and livestock as the main farm activity while the rest depended on livestock alone. Sixty three (n=50/80) and 78.5% (n= 63/80) of the households depended on both crops and livestock for household cash income and household subsistence, respectively. The majority of the farms (88.8% (n=71/80) were under private land tenure system while the rest were in a government corporation land (Tana and Athi Rivers Development Authority; TARDA).

4.3.3.2. Livestock production, management and constraints

The average number of cattle per household was 10.6 (range 2-30) with averages reported to be 6 (range 2-11), 9.3 (range 2-18), 13.2 (3-26) and 13.3 (3-30) in Evurore, Mwea, Siakago and Gachoka divisions, respectively. Figure 3.3 *d* illustrates the mean cattle herd sizes per division. Overall, 90% (n= 72/80) of the households kept only indigenous cattle breeds and their crosses, 5% (n= 4/80) kept exotic animals only, while the remaining 5% (n= 4/80) kept both indigenous and exotic breeds of cattle. The majority of cattle were reared under open grazing management systems.

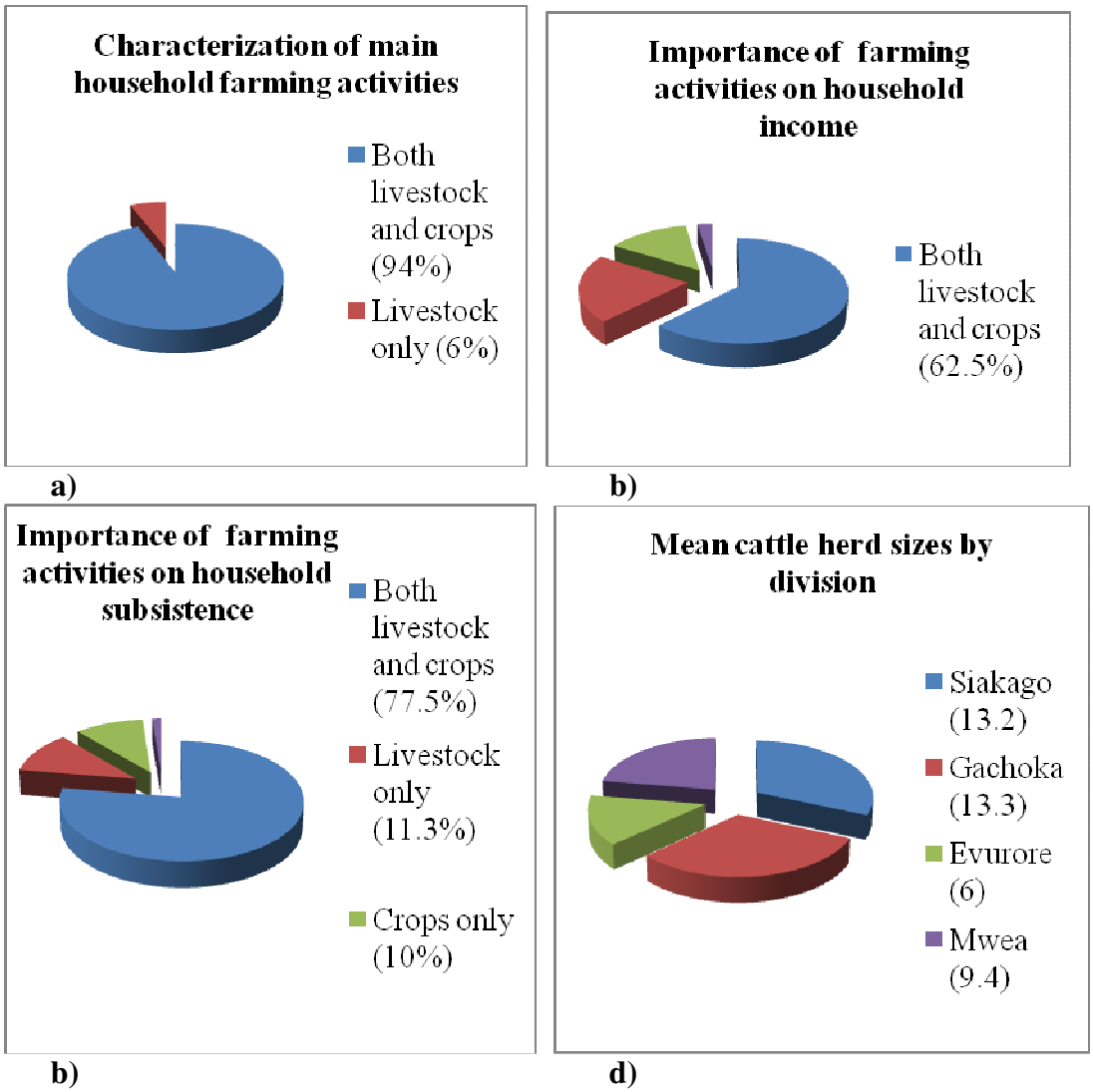
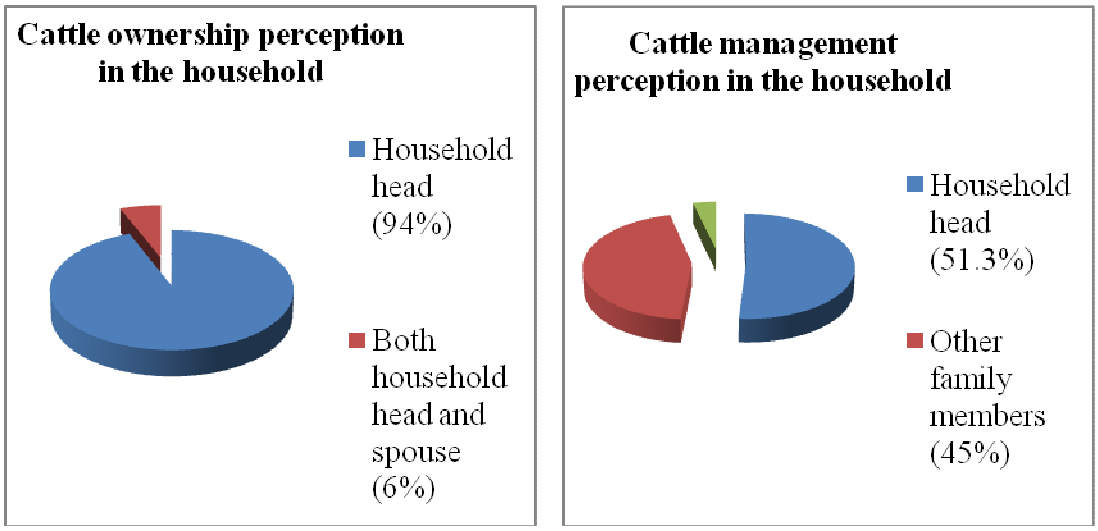


Fig 4.1: Pie-chart illustrations showing main farming activities and their importance in household subsistence and income, mean cattle herd sizes by division, and perceptions of cattle ownership and management as expressed by respondents in questionnaire survey, Mbeere District, March, 2007. a) descriptions (%) of household farming activities; b) descriptions (%) for main sources of household cash income; c) descriptions (%) for main sources of household subsistence; d) mean number of cattle herd sizes per division.

Specifically, grazing management systems included free grazing (60% (n = 48/80)), tethering (13.8% (n = 11)), alternate free grazing and tethering (10% (n= 8)), free grazing and stall feeding (6.3% (n= 5)), alternate tethering and stall feeding (3.8% (n= 3)) and stall feeding (6.3% (n= 5)) depending on the cattle breeds kept and the area. All exotic breeds were stall fed.

Figure 4.2 illustrates household cattle ownership and management perceptions as expressed by respondents. Although cattle were mainly owned by men, other household members of the family were involved in their day to day management (Figure 3.4). For farmers practicing free grazing, 54% (n=43/80) utilized family labour, 46% (n=37/80) utilized hired labour while the rest utilized both categories.



a) b)

Figure 4.2: Pie-chart illustrations showing: a) descriptions (%) for cattle ownership as perceived in the household; b) descriptions (%) for day to day cattle management as perceived in the household in Mbeere District, March 2007.

Reasons advanced for keeping cattle in order of importance were milk, homestead "security" banks, traction and manure. The average peak milk production for indigenous cattle breeds was 2.2 liters per day (ranged between 0.5 and 8 liters) with the maximum value being for zebu and Sahiwal cross breeds. The average peak milk production for exotic cattle breeds was 6 liters per day (ranged between 2 and 12 liters). Fifty six percent (n=45/80) of the households utilized milk both for home consumption and selling in the neighborhood while the rest (44% (n=45/80)) used milk for home consumption only.

Overall, 94% (n=75/80) of the farmers were reported to apply acaricides to cattle for control of ticks. The rest used traditional methods such as hand picking and application of kerosene. One farmer was reported not to apply any method for tick control. The majority of the farmers (90% (n=72/79)) applied the acaricides by hand spray and hand wash, 5% (n=4/79) used cattle dips and 3% (n=3/79) used pour-on formulation whereas the rest were reported not to apply acaricides at all.

The proportions of farmers keeping other livestock per division are shown in Table 4.2. Overall, indigenous sheep, goats and poultry and bee rearing were reported to be the main livestock production activities in the district though this differed across divisions (Table 4.2). Different animal species in the district supplied different products; e.g. goats and cattle provided milk. Donkeys and oxen provide draught power in the relatively drier Siakago and Gachoka divisions.

Goats and sheep were kept to cover certain home expenditures and for slaughter, particularly during festivities. Chickens provided eggs and meat and were also sold to cover home medium expenditures. Cattle were sold to meet major expenditures such as paying school fees. More importantly, manure from all species of animals was used to enrich soil fertility for enhanced crop production, thus, playing a very important role in nutrient recycling.

Table 4.2: Proportions of farms (%) rearing different livestock species across divisions in Mbeere District, March 2007.

Livestock category	Siakago	Gachoka	Evurore	Mwea	Overall
Sheep & goats	100	95	95	100	97.5
Donkeys	55	65	15	0	33.8
Poultry	82	90	95	100	92.2
Bee keeping	60	75	75	3	56.3

Overall, feed and water availability, and livestock diseases were ranked as the most important farm constraints in all divisions (Table 4.3). Feed availability was ranked first in all divisions. However, livestock disease was ranked second in both Evurore and Mwea Divisions whereas water availability was ranked second in both Siakago and Gachoka Divisions. Other constraints reported included difficulty in accessing veterinary services and lack of markets for livestock and livestock products.

Overall, anaplasmosis, East coast fever and foot lameness were ranked as the most common disease constraints in that order (Table 4.4). Anaplasmosis was ranked first in all divisions except Mwea where ECF was ranked first. None of the farmers from Siakago Division ranked ECF either in the first or second position.

Other diseases reported included contagious bovine pleuropneumonia (CBPP), foot and mouth disease (FMD) and blackquarter.

Table 4.3: Major farm constraints classified by divisions as expressed by respondents in the Mbeere District households survey, March 2007

	Siakago (n=20)		Gachoka (n=20)		Evurore (n=20)		Mwea (n=20)	
Constraint	% *	Rank	%*	Rank	%*	Rank	%*	Rank
Feed availability	55%	1	50%	1	80%	1	80%	1
Diseases	30%	2	23.5%	2	62.5%	2	45%	2
Water availability	40%	2	29.4%	2	25%	2	30%	2

*Percentages along columns correspond to proportions of respondents who ranked each constraint per division.

Table 4.4: Number of farms ranking common disease constraints by divisions in the Mbeere District households survey, March 2007

	Siakago (n=18††)		Gachoka (n=19†)		Evurore (n=19†)		Mwea (n=20)	
Constraint	No.*	Rank	No.*	Rank	No.*	Rank	No.*	Rank
Anaplasmosis	4	1	7	1	6	1	2	1
ECF	0	1	4	1	4	1	5	1
ECF	0	2	3	2	1	2	3	2
Foot lameness	1	2	1	2	7	2	1	2

*Number of farms along columns indicate number of farms which ranked each constraint per division;.ECF: East Coast fever. Other diseases ranked across all divisions included FMD, CBPP and Black quarter, Mange, wasting syndrome (trpanosomosis), helminthosis, Babesiosis and heatwater. † one respondent each failed to answer this question in Gachoka and Evurore Divisions; †† two respondents failed to answer this question in Siakago Division

4.3.3.3. Food crops

Ninety-four percent (n=75/80) of the households grew food crops for household subsistence and sale of surpluses. These included cereals, legumes, fruit and root crops such as maize, sorghum, millet, beans, cowpeas, green grams, pigeon peas, pumpkins, mangoes, pawpaw, sweet potatoes and cassava.

All farmers reported using by-products from cereals and legumes as important feed sources, particularly during the dry periods. Similarly, all farmers reported using manure from livestock to enhance crop production.

4.4. Discussion

The high proportions of farmers depending on crops and livestock in their households in Mbeere District for subsistence and cash income underscored the overall importance they attach to the mixed crop-livestock farming systems. This is probably because the system can reduce risk, spread labour and re-utilize resources (FAO, 2003). The cereal crops provided grain, straw and stover residues while legumes provided grain, organic matter, fodder and nitrogen. The livestock excreted dung and urine which contain several nutrients such as nitrogen, phosphorus and potassium, and the solid fraction contains organic matter that is important in maintaining soil structure and fertility (Velthof *et al.,* 2000). Similar to traditional farming systems in western Kenya (Waithaka *et al.,* 2004), farmers tended to look for high yield of the combination of the components rather than for the (high) yield of one component. According to Lenné and Thomas (2005), mixed crop-livestock systems are more important than any other system in terms of their contribution to the total output of farm products and contribution in enhancing the livelihoods of the poor through provision of various household capital assets.

The high proportions of farmers depending on livestock either when integrated with crops or when alone in this district agrees with observations by FAO (2003) that livestock production is an important economic sub-sector where mixed crop-livestock production system is the main agricultural activity in sub-Saharan Africa. The proportion of farmers keeping several species of livestock was high across the district. By keeping several livestock species, farmers could exploit a wider range of feed resources than if only one species is kept and also these animals supplied different products. The climatic conditions in this district are harsh and prone to droughts and outbreak of epidemic animal diseases such as FMD (Onduru *et al.,* 2002). Keeping more than one species of livestock may thus be a risk-minimizing strategy. An outbreak of disease may affect only one of the species. Some species or breeds are better able to survive droughts, particularly goats, and thus help support a family through such difficult periods. Advantage can also be taken of the different reproductive rates of different species to rebuild livestock holdings after a drought; for example, the greater fecundity of sheep and goats permit their numbers to multiply quicker than cattle or donkeys. The small ruminants can then be exchanged or sold to obtain the larger animals.

Milk is an important product from animals in mixed farming systems (Thorpe *et al.,* 1993). However, the generally poor feed quality in Mbeere District (Onduru *et. al.,* 2002) could have been the reason for the low peak milk production reported in both exotic and indigenous breeds in this study compared to national averages of about 6.5 liters to 7.3 liters in small scale zero grazing and large-scale open grazing systems, respectively (Karanja, 2003).

The milk-producing animals in the district are mainly traditional cattle and goats; however, each of these has a place in mixed crop-livestock systems. Both cattle and goats possess a digestive system that allows them to utilize coarse feeds like straws, grasses and tree leaves. Apart from the traditional attachment that people have to the traditional breeds, it is probably their small size and tolerance to local diseases and harsh environmental conditions that determine their suitability in the area. Goats are known to produce much less milk and meat per animal than cattle and they also eat less, so it can be argued that the production per kilogram of feed is quite similar for small and large animals (FAO, 2003).

Availability of feed and water, and livestock diseases were reported to be the main livestock production constraints in the district. This is similar to findings by Kangara *et al.* (1996) and Onduru *et al.* (2002). This is probably due to the harsh climatic and environmental conditions existing in the district (Onduru *et al.,* 2002). In Mbeere District, livestock depend mainly on grazing on wastelands, fallowed croplands or distant grazing areas, and are fed on crop residues when the feed are scarce. This may have led to the generally low livestock production reported in the district. To achieve higher production of milk, meat, or draught power over long periods, low-quality fodders should be supplemented with better feeds such as drought tolerant fodder, leguminous forages such as *Calliandria* and sweet potato vines. Increased production of on-farm fodder and adoption of feed conservation technologies based on local feed resources should be promoted as long-term measures.

Dual-purpose crops should be promoted, and at the same time, increase farmer awareness of the value of residues from these crops as fodder. The problem of water availability could be solved by harnessing water resources through community-based projects.

The other constraints cited could be solved by upgrading the local small East African zebu with other zebu breeds of higher genetic potential such as the Sahiwal and Boran. Marketing for livestock and livestock products particularly milk was ranked fourth by farmers as a constraint to livestock production. Poor marketing systems linked with poor transport systems and storage facilities hinder the successful marketing of quality products by small-scale farmers and this has been perceived to be a significant constraint to development of the livestock sector in the whole of SSA (FAO, 2003). It is unlikely that smallholder farmers will adopt productivity-enhancing technologies if effective and secure markets are not available. There is, therefore, an urgent need for more comprehensive studies of the marketing systems for livestock and livestock products in SSA to identify the most practical and feasible solutions and improvements (FAO, 2003).

Animal health is essential in mixed farming systems. The main diseases reported to constrain livestock production were vector diseases such as anaplasmosis, ECF and trypanosomosis. The occurrence of these diseases depends on local ecological and socio-cultural conditions (unrestricted grazing). Anaplasmosis was reported more in the drier divisions of Siakago and Evurore while ECF was reported in the wetter Mwea Division probably due to differences in local ecological suitability for their vectors.

Outbreaks of devastating diseases such as FMD and blackquarter were reported to hamper livestock production. Veterinary care in terms of prevention can help to improve livestock production. However, in traditional livestock systems such as that in Mbeere District, farmers try to cope with disease by spreading risks (rearing large herd sizes), by using animals tolerant to local diseases, and by running low-cost operations (Minjauw and McLeod, 2003). This scenario was prevalent in Mbeere District as *Bos indicus* cattle breeds reared possess natural resistance to these diseases and are also managed with a minimum expense. These breeds may therefore continue to be important in traditional forms of crop-livestock farming systems. A sustainable strategy on ticks and TBDS control programmes may aim at promoting integrated control methods that include genetic resistance, strategic tick control using communal dips to take advantage of economies of scale, immunization where applicable and increasing awareness about resistance of ticks to acaricides (FAO, 2003).

CHAPTER 5

SERO-EPIDEMIOLOGY OF *T. PARVA* INFECTION IN THE CATTLE POPULATION OF MBEERE DISTRICT, KENYA

5.1. Introduction

East coast fever (ECF) is a fatal tick-borne disease (TBD) of cattle caused by the parasitic protozoan, *Theileria parva,* and transmitted by the three-host tick, *Rhipicephalus appendiculatus.*

Epidemiologically, diseases are governed by multiple risk factors which normally operate at the different levels of population organization, i.e., animal-level, herd-level, and area-level. Interactions between these determinants could also influence the occurrence of diseases. This concept has rarely been studied and applied in TBDs epidemiological studies that have been conducted in Kenya (Moll *et al.,* 1984; Moll *et al.,* 1986; Deem *et al.,* 1993; Maloo *et al.,* 1994; Gitau *et al.,* 1997; Peeler and Omore, 1997; O' Callaghan, 1998; Gitau *et al*., 1999; Maloo *et al.,* 2001a, b; Muraguri *et al.,* 2005; Ndung'u *et al.,* 2005; Okuthe and Buyu, 2006). In addition, there seems to be virtually no epidemiological work carried out on the prevalence and distribution of *T. parva* infection in a typical mixed crop-livestock production system such as that in semi-arid rangelands of Mbeere District.

Considering the importance of understanding the epidemiology of ECF in all production systems, this study was conducted in the crop-livestock production system in Mbeere District with the objective of providing a baseline quantitative assessment of seroprevalence of *T. parva* infection in cattle. The purpose of the study was to characterize the potential risks incurred and estimate the contribution of unexplained variation at the various levels of organization to the total variance of *T. parva* antibody levels in the district. In this manner, high-risk levels of organization with large unexplained variation can be identified and targeted with the expectation that interventions at those levels might indicate a substantial room for improvement on the outcome of interest (Dohoo *et al.,* 2003).

5.2. Materials and methods

5.2.1. Study site and study design

The details of the study site and study design are as given in sections 3.1. and 3.2.

5.2.2. Data collection, handling and management

The details of the data collection, handling and management are as given in section 3.3. Details of these data are, however, provided below.

5.2.2.1. Serology: *T. parva* indirect enzyme-linked immunosorbent assay (ELISA)

Serum samples were assayed for antibodies to *T. parva* by enzyme-linked immunosorbent assay (ELISA) using the *T.* parva-specific recombinant polymorphic immunodominant molecule (PIM) specific to *T. parva* as the antigen (Katende *et al.,* 1998). Purified *T. parva* antigen was coated in 96-well microtiter plates (Nunc-Immuno Lockwell Modules, framed, No. 448496, Denmark): the antigen was diluted with 15 ml of coating buffer (Phosphate buffered saline [PBS], pH 7.4) in a 50-ml Falcon tube (Becton Dickinson, USA) and mixed well. Using a pipette, 150µl of the diluted antigen was dispensed into each of the plate wells. The plates were then incubated for 2 hours at 37^0C in an Insel incubator (Insel Hamble S03, 8DH, England®) shaking continuously to guarantee maximum coating. At the end of the incubation, the excess antigen was discarded by flicking out the contents into a sink and then gently blotting the plates onto hand paper towels. The plates were then left inverted on the towels for about 15 minutes to drain off completely. The plates were either used immediately or sealed with an adhesive plate sealer (Dynatech Microtiter System, Cat No. M30) and stored at -20^0C.

During the assay, the coated plates were first blocked by dispensing 300µl of blocking buffer (0.2% w/v casein in PBS-Tween 20) into each well and incubating for 20 minutes at 37^0C in a continuously shaking Insel® incubator. The excess buffer was then discarded and the plate washed 3 times by filling and sucking washing buffer (PBS-Tween 20) using an 8 channel washing bar (Nunc Immunowash 8, No. 470174) fitted to a washing buffer reservoir and a vacuum system. The plates were then drained off by slapping the plates onto hand paper towels.

Initially, the test sera and both positive and negative control sera were diluted by dispensing 5µl of sera into each of the respective wells of a 96-well flat-bottomed diluting plate (Nunc 96 Microwell plates, No. 260895) in duplicates. Then, 195µl of serum diluent (1% w/v skimmed milk in PBS-Tween 20) was added to each well to give a dilution of 1:40. The plates were then agitated in a shaker (Heidolph shaker, Bioblock Scientific) at ambient temperature for about 20 minutes. Then, 120µl of serum diluent was dispensed into each well of the coated plate and 30µl of the 1:40 dilution pre-diluted sera added to each well in duplicates to give a final dilution of 1:200. Each ELISA test plate included the predetermined positive and negative control sera. The plates were then incubated at 37^0C for 30 minutes in a continuously shaking Insel® incubator. The excess sera were then discarded and the plate washed 5 times as described above. The plates were then drained off by slapping the plates onto hand paper towels. The wells were then soaked by filling with 250µl of PBS-Tween 20 buffer and the plate incubated at 37^0C for 10 minutes in a continuously shaking Insel® incubator. The wash buffer was then discarded.

To each of the wells, 150µl of secondary antibody (horseradish peroxidase conjugated mouse anti-bovine IgG monoclonal antibody) (Svanova®) at a dilution of 1:35000 was dispensed and the plates were incubated at 37^0C for 30 minutes in a continuously shaking Insel® incubator. The excess conjugate was then discarded and the plate washed 3 times as previously described. The wells were then soaked by filling with 250µl of PBS-Tween 20 buffer and the plate incubated at 37^0C for 10 minutes in a continuously shaking Insel incubator and then discarded.

The substrate was prepared by adding 100µl of hydrogen peroxide and 125µl of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to 25ml of substrate buffer and mixed well. Then, 150µl of the final substrate solution was dispensed to each well and the plates incubated at 37^0C for 30 minutes in a continuously shaking Insel incubator to allow the conversion of the substrate by the enzyme conjugate. Subsequently, a green colour development from the chromogen indicated a positive result. The absorbance was measured using an Immunoskan ELISA reader (BDSL Immunoskan MS) at 405nm wavelength, and the resultant optical density values were expressed as percent positivity (PP): PP = (optical density of test serum/optical density of strong positive) X 100 (Wright *et al.,* 1993). A sample was considered positive if the PP value was 20 or above.

5.2.2.2. Extraction of Normalized Difference Vegetation Index (NDVI)

Normalized Difference Vegetation Index (NDVI) is a simple numerical index or indicator that provides a standardized method of comparing vegetation greenness between satellite images (Chen and Brutsaert, 1998). In effect, it measures the amount of green vegetation in an area. In this study, Landsat satellite imagery was used to obtain NDVI values for the sampled sub-locations. The processing for derivation of the NDVI values was by combination of bands 3 and 4 of the Landsat^{TM} 7 bands image of the study area where their normalised ratios were used (Square root (band4-band3)/band4+band3) +0.5). This was done through the procedures embedded in the ERDAS IMAGINE 8.5 software where the available satellite imagery of the year 2001 was used to obtain proxy conditions of the vegetation at a spatial resolution of 30x30m pixel size.

The processed NDVI image was then subjected to extraction of location specific NDVI values by use of ArcGIS spatial analysis toolset. Thus, sub-locational values were derived from zonal means from the original data which covered all the pixel values from the image within a sub-location.

Normally, index values can range from -1.0 to 1.0, but vegetation values typically range between 0.1 and 0.7. As the calculated NDVI values from bands 3 and 4 were likely to yield negative values, the values were normalised with an addition of 0.5 as noted in the formula above to have the data start from zero to 1. Higher index values are associated with high levels of healthy vegetation cover whereas low figures show little to no vegetation cover.

5.2.2.3. Statistical handling

5.2.2.3.1. Variables of interest

5.2.2.3.1.1. Outcome variable

Individual-animal serostatus (positive or negative) to *T. parva* antibodies was the outcome of interest.

5.2.2.3.1.2. Fixed-effect variables

Fixed-effect variables were collected at three levels: animal, herd and administrative /agroecological zone (AEZ). Herd and the administrative variables (sub-location and division) were also included as random effects in different models.

5.2.2.3.1.2.1. Animal-level variables

Animal-level variables included age, sex, breed and body condition. Age was categorized into three classes according to owner information: adult (> 2years), yearling (≥ 13 to 24 months) and calf (4 to 12months). Sex was female versus male while breed was categorized into two classes: exotic and indigenous (both indigenous animals and their crosses). Body score was assigned subjectively as poor, average or good based on a combination of visual and palpation of key bone structures for fat cover. This was done mostly for yearlings and adults.

5.2.2.3.1.2.2. Herd-level variables

Herd-level variables included grazing management, herd and calf tick control management practices, and herd size. Others included crude calf mortality during the preceding year, common veterinary drugs (acaricides versus any other drug (antihelmintics, multivitamin and antibiotics) used in the farm, whether the farm had experienced an ECF case within the last three years, distance to the nearest veterinary clinic consulted, and whether any cattle had joined the herd during the preceding year. Lastly, information on whether the vector tick (*R. appendiculatus)* was found in the farm or not was collected through examination of ticks on all animals in a sampled farm.

5.2.2.3.1.2.3. Area-level variables

Area-level variables included NDVI, division and agro-ecological zone (AEZ). From the three AEZs in the district, cattle were found to have been retrospectively sampled from Lower Midlands 4 (LM4) and Lowlands 5 (L5). In Mbeere District, LM4 is characterized by a short and a short to very short cropping season which is comparable to the more potential LM3. Lower Midlands 4 (LM4) zone's grassland and forage is dominated by mixed medium grass savannah and fast growing fodder legumes. On the other hand, L5 is characterized by a very short to short cropping season with short grass savannah predominating on overgrazed and eroded places (Jaetzold & Schimdt, 1983).

5.2.2.3.1.3. Random-effects variables

Herd, sub-location and division were variably included as random-effects variables in different models.

5.2.2.3.2. Descriptive statistics

5.2.2.3.2.1. Summary statistics and distribution of risk factors

Exploratory data analysis was undertaken by carrying out relevant summary descriptive statistics for all variables. Distribution of risk factors at each level was calculated as proportions corresponding to categories for each variable.

5.2.2.3.2.2. Relationships between independent predictors

Relevant relationships amongst predictors were done using regression analysis or chi-square statistics.

5.2.2.3.2.3. *T. parva* seroprevalence and variation estimation

The overall- and between-risk factor levels *T. parva* seroprevalence and the corresponding 95% confidence intervals (95%, CI) were calculated using the method described by Collett (2002).

The *T. parva* seroprevalence and variance were estimated both under the binomial model and in the presence of extra-binomial variation. Under the binomial model, the prevalence was estimated as $p = y/n$, where y denoted the total number of animals

positive for *T. parva* antibodies out of the total sample size, *n.* Under the assumption of simple random sampling, the naive expected variance using the binomial model was calculated as Var $(p) = p\ (1 - p)/n$. The 95% confidence interval for the prevalence p according to the Normal approximation was calculated as:

CI $(p) = p \pm 1.96\ [\text{Var}\ (p)]^{0.5}$.

Extra-binomial variation arises from the usual variation between individual animals plus the variation that is due to differences between herds. Thus, the variance estimate calculated under the binomial model was not considered adequate due to the herd effect that resulted in variance inflation as the number of independent observations (80 herds) was less than the denominator n (440). This effect was analytically accounted for using the variance-inflation factor (VIF), $c = 1+\rho\ (\tilde{n}-1)$ (Dohoo *et al.,* 2003) where ρ and $\tilde{n}$ denote the intraherd correlation coefficient (ICC) and the average herd sample size, respectively. Intraherd correlation coefficient is a measure of the degree of similarity among the outcomes of herdmates. Denoting the number of cases y_i and sample size $n_{i,}$ for the i^{th} herd, ρ was estimated from the data as: $\rho = (\text{MSB} - \text{MSW})/(\text{MSB}+\text{MSW}(\tilde{n} -1))$ where MSB and MSW denote the mean square between herds and the mean square within herds respectively (Dohoo *et al.,* 2003). The MSB and MSW were derived from one-factor Analysis of Variance (ANOVA) with the herd identification as the independent variable and the individual animal *T. parva* antibody serostatus as the response variable.

The inflated (design-based following the ICC-approach) variance estimate was calculated as *c* X variance calculated using the binomial model.

Chi-square tests were used to relate the *T. parva* seroprevalence (dependent variable) with the risk factors (independent variables) at different levels using the maximum likelihood method executed in Genstat Discovery Edition 2 software (Genstat RELEASE 4.24DE, 2005).

5.2.2.3.2.4. Statistical models

Investigation of risk factors was carried out by fitting data into logistic regression models using the maximum likelihood estimation process. The strength of association between the risk factor and *T. parva* seropositivity was estimated by odds ratios (OR) which were directly derived from estimates of logistic regression. Odds ratios from logistic regression are interpreted as a multiplicative factor of risk of disease when the risk factor is present. The OR is a relative measure of risk that describes how much more likely it is that an animal which is exposed to the factor under study will develop the outcome as compared to an animal which is not exposed. In this study, the OR were interpreted as the odds of being *T. parva* seropositive among those exposed to the suspected risk factor as being OR *times* the odds of disease among those with no such risk factor. If OR is close to 1, the risk factor is unlikely to be associated with the risk of disease. For an OR greater or smaller than 1, the likelihood that the risk factor is associated with risk of disease

increases, and the stronger the association. Further, if the 95% CI of the ORs includes the value 1, this implies that the OR obtained in the study is statistically consistent with a true OR of 1, "not statistically significantly different." Univariable models were first run to investigate the relationship between *T. parva* seroprevalence and individual risk factors to compute crude ORs and their corresponding 95% CI. The logistic model for the probability p_i of the i^{th} animal being positive for *T. parva* antibodies with only one predictor was computed as : logit $(p_i) = \beta_o+\beta_1 X_i+e_i$. The significance level was set at $p\leq 0.1$. For continuous predictors, the assumption that the log-odds of disease increases with the predictor (Dohoo *et al.,* 2003) was thoroughly investigated.

A multivariable logistic regression model was then built using significant variables in the univariable analysis by extending the univariable model to include other predictors as follows: logit $(p_i) = \beta_o+ \beta_1 X_i + \ldots+ \beta_k X_{ki} + e_i$. In this analysis, all the significant risk factors were initially included in the model. Model building used backwards elimination method to decide on the factors to exclude from the model using the likelihood ratio test ($p<0.05$). The multivariable model was built first without accounting for internal correlation at herd level. At this level, the resultant 'adjusted' ORs measures the effect of a given factor assessed conditional on other factors included in the model. Crude ORs from the univariate analysis were compared with those from multivariable analysis. If those ORs differ considerably, the variable might be a confounder or an interaction term (Dohoo *et al.,* 2003).

Lastly, correlation at herd-level was accounted for using generalized linear mixed model (GLMM). The GLMM was a generalization of the above multivariable logistic model to accommodate overdispersion expected in the outcome using the Schall method (1991).

Schall's mixed generalized linear model is an adapted version of Fellner's algorithm (Fellner, 1986) derived from the best linear unbiased predictor (BLUP) method of Henderson (1963). In this method, fixed- and random-effect parameters are estimated in two steps. The first step is a modified Fisher's scoring method for both fixed and random parameters. The second step is an approximate restricted maximum-likelihood (REML) method for estimating random effects from initial estimates at single or multiple levels of population organization. In an illustration of a single level clustering at herd level, assuming *T. parva* seroprevalence was a binary outcome variable Y_{ij}, where i indexes herds and j indexes animals within herds, within the i^{th} herd, the Y_{ij} were independent with probability p_i of the j^{th} animal being positive for *T. parva* antibodies being: logit (p_i) = $\beta_o + \beta_1 X_i + \ldots + \beta_k X_{ki} + u_i$, where u_i is a herd-specific effect (which contains animal j) to account for seroprevalence differences amongst herds. As herds were randomly sampled from a population of herds from the district, the herd specific effect was a random variable assumed to be normally distributed with a mean of zero and variance σ^2_{herd}. The adjusted ORs obtained before and after accounting for correlation were then compared.

In all the models, level 1 (animal level) variance was not constrained (i.e. extra binomial variation was permitted).

In the evaluation of the contribution of each level of organization to the total variance, two types of mixed generalized linear models were built. The first model was built sequentially by adding higher level random effects without any fixed effects. The second model was built sequentially as the first one but in the presence of significant fixed effects. In each case, the changes in the absolute values of variance estimates that accompanied the sequential addition of higher level random effects were evaluated. These models utilized the mixed generalized linear model procedure developed by Schall (1991) and executed in Genstat Discovery Edition 2 (Genstat RELEASE 4.24DE, 2005).

5.3. Results

5.3.1. Descriptive statistics and distribution of risk factors

5.3.1.1. Distribution of animal-level risk factors

Table 5.1 lists the characteristics of animal-level variables recorded for the 440 cattle sampled in the study area. The pattern of age distribution in the sample was similar in all divisions where the adults, yearlings and calves constituted approximately 60%, 17% and 20%, respectively, except in Mwea where calves comprised 13% of the sampled animals. Ninety-two percent of the sampled animals consisted of the indigenous breed. All divisions had more than 60% females except Mwea where the proportion of females was 55% (Table 5.1).

Table 5.1: Descriptions (%) of animal-level variables for cattle sampled in the four divisions of Mbeere District, Kenya, March 2007

Variable	Siakago (n=102)	Gachoka (n=133)	Evurore (n=103)	Mwea (n= 102)	Overall (n=440)
Age					
Calves	23	21	21	12	20
Yearlings	18	17	16	20	17
Adults	59	62	63	68	63
Sex					
Female	67	63	69	55	63
Male	33	37	31	45	37
Breed					
Exotic	0	14	15	1	8
Indigenous	100	86	85	99	92
Body score					
Good	25	37	43	40	36
Average	70	61	55	58	61
Poor	5	2	2	2	3

5.3.1.2. Distribution of herd-level risk factors

In all divisions, free grazing management comprised of at least 80% of the herds sampled. All herds from Siakago Division practiced free grazing (Table 5.2). Herd size was converted into a categorical variable with three levels. Based on this category, 28%, 31% and 41% of the sampled herds had sizes of 1 to 5, 6 to 10 and over 10 cattle, respectively, with Evurore Division having significantly more smaller sized herds compared to all other divisions ($p<0.005$) (Table 5.2). The proportion of herds where acaricides had either been used or not used 2 weeks prior to the farm visit was approximately 50% (Table 5.2). Farmers from Mwea Division were more likely to start calves tick control at an earlier age and also apply acaricides more frequency on calves than farmers from other divisions ($p<0.05$) (Table 5.2). Overall, the majority of the farms (71%) had not experienced calf mortality in the preceding year (Table 5.2).

Fifty-eight percent of the farms acknowledged having observed ECF in their cattle according to the clinical syndrome described by the author during the interview (Appendix 3). Overall, *R. appendiculatus* tick was found in cattle from only 23% of the farms though this differed significantly ($p<0.05$) across divisions (Table 5.2).

5.3.1.3. Distribution of area-level risk factors

Approximately equal proportions of animals were sampled from Siakago, Evurore and Mwea Divisions (Table 5.3). Animals sampled came from two agro-ecological zones. Majority of the animals (63.2%) sampled came from LM4 (Table 5.3). Table 5.4 lists sub-locational NDVI values.

5.3.2. Relationships between independent variables

Analysis between independent variables focused mainly on relationships between herd-level variables on one hand and area-level variables on the other (Table 5.5). The herd levels included grazing system practiced, herd size, recent use of acaricides, initial age of calf tick control, frequency of calf tick control, calf mortality in the preceding year of the study, common drugs used in the farm and whether cattle in farm had experienced ECF syndrome in the preceding years.

Table 5.2: Descriptions (%) of herd-level variables for cattle sampled in the four divisions of Mbeere District, Kenya, March 2007

Variable	Siakago	Gachoka	Evurore	Mwea	Overall
Grazing type					
Open	100	80	80	85	86
Mixed open + stall	0	20	20	15	14
Herd size					
1 to 5	20	45	65	35	41
6 to 10	35	25	30	35	31
Over 10	45	30	5	30	28
Acaracide use last 2 wks					
Yes	45	55	25	65	48
No	55	45	75	35	53
Initial calf tick control age					
<3 months	25	20	15	45	26
3-6 months	55	75	60	55	61
Over 6 months	20	5	25	0	13
Freq. of calf tick control					
≤ 5	75	50	74	35	58
>5	25	50	26	65	42
Calf mortality in preceding year					
Yes	50	30	15	25	29
No	50	70	85	75	71
Common drug used					
Acaricide	25	25	25	40	29
Others[a]	75	75	75	60	71
ECF seen on farm in preceding years					
Yes	35	70	30	95	58
No	65	30	70	5	43
Vet distance					
0 to 5km	10	80	70	90	63
6 to 10km	10	15	15	5	11
over 10km	80	5	15	5	26
***R. appendiculatus* found on farm**					
Yes	15	40	10	45	27
No	85	60	90	55	73

Legend for Table 5.2: Open grazing: grazing animals on free range or tethering in communal or privately owned land; Mixed open + stall: Feeding animals both on free range and within an enclosure with fodder collected from the fields either spatially or temporally; Freq. of calf tick control: Number of times calves exposed to acaricides before 6 months of age; Vet distance: distance traveled by farmer to the nearest veterinary clinic; Others[a]: Other drugs including antihelmintics, antibiotics and vitamin supplements.

Table 5.3: Descriptions (%) of area-level variables for cattle sampled in Mbeere District, Kenya, March 2007

Variable	Level	Proportion (%) of animals sampled
Division	Siakago	23.2
	Gachoka	30.2
	Evurore	23.4
	Mwea	23.2
AEZ	LM4	63.2
	L5	36.8

AEZ: Agro-ecological zone; LM4: Lower midlands 4; L5: Lowlands 5

Table 5.4: Mbeere District sub-locational NDVI values, 2001

Division	Sub-location	NDVI value
Siakago	Kerie	0.6307
	Mutitu	0.6034
Gachoka	Kombo Munyiri	0.6347
	Njigo	0.6021
Evurore	Ishiara	0.6692
	Kamarandi	0.5945
Mwea	Karaba	0.6687
	Wachoro	0.6137

NDVI: Normalized Difference Vegetation Index

Other herd-level variables included the presence of *R. appendiculatus* vector tick on farm and distance to the nearest veterinary clinic. Area-level variables included division, AEZ and NDVI. All herd-level variables were significantly associated with division ($p < 0.001$) while majority of the herd-level variables were also associated with NDVI ($p < 0.05$) (except recent use of acaricides, frequency of calf tick control and calf mortality in the preceding year of the study) and AEZ ($p < 0.05$) (except recent use of acaricides and frequency of calf tick control) (Table 5.5). The three area variables (division, NDVI and AEZ) were significantly related to each other ($p<0.001$) (Table 5.5).

5.3.3. *T. parva* exposure prevalence

5.3.3.1. Estimating *T. parva* seroprevalence and variance under the binomial model

The overall *T. parva* prevalence was estimated to be 19.3% (n=85/440). Under the assumption of simple random sampling, the naive expected variance using the binomial model was calculated to be 3.5×10^{-4}. The 95% CI for the prevalence *p* according to the Normal approximation was therefore [15.6%, 23%] for the naive variance estimation.

Table 5.5: The *p*-values obtained from either logistic regression analysis or chi square statistics investigating relationships between independent variables

Variable	NDVI	AEZ	Division
Acaricide use last 2 weeks	0.272	0.376	<0.001
Initial calf tick control age	<0.001	<0.001	<0.001
Grazing type	0.019	<0.001	<0.001
Herd size	0.002	<0.001	<0.001
Freq. of calf tick control by 6 months of age	0.214	0.671	<0.001
Calf mortality in preceding year	0.163	0.042	<0.001
Common drug used	<0.001	<0.001	<0.001
ECF on farm in preceding years	<0.001	<0.001	<0.001
Vet distance	<0.001	0.004	<0.001
R. appendiculatus seen on farm	<0.001	0.002	<0.001
NDVI	-	<0.001	<0.001
AEZ	<0.001	-	<0.001
Division	<0.001	<0.001	-

NDVI: Normalized Differential Vegetation Index; AEZ: Agro-ecological zone.

5.3.3.2. Estimating *T. parva* seroprevalence and variance in the presence of extra-binomial variation

For this study data, the mean herd size was 5.5 (range 2–30), the mean square between herds (MSB) was 0.405, mean square within herds (MSW) was 0.102 and ρ was calculated to be 0.3 resulting in the estimate for the VIF (c) to be 2.35. The inflated (design-based following the intra-herd correlation coefficient approach) variance estimate (calculated as c X variance using the binomial model) was 8.2×10^{-4} giving a 95% CI for prevalence as [13.7%, 24.9%]. The differences in the variances calculated above was evidenced by the pattern of clustering observed mostly at herd level as herds had either low or no infection to high seroprevalence across divisions (Table 5.6). Table 5.6 classifies herd prevalence into either more or less than 70% prevalence which is perceived as an indicator of tick-borne diseases endemic stability. Only 10% of the herds had herd prevalences of over 70% with the majority of them coming from Mwea Division (Table 5.6)

Table 5.6: Number of herds classified into either >70% or <70% herd prevalence and *T. parva* herd prevalence range (%) by division in the cattle sampled from 80 farms in Mbeere District, Kenya, March 2007.

Division	No. of herds with herd prevalence		Divisional herd prevalence range (%)
	>70%	<70%	
Siakago (n=20)	0	20	0-33
Gachoka (n=20)	0	20	0-41
Evurore (n=20)	2	18	0-83
Mwea (n=20)	6	14	0-100

5.3.3.3. Comparisons of *T. parva* seroprevalence between levels of categorical variables

Chi square statistics for comparison of age, breed, sex, and body score categories and *T. parva* seroprevalence did not reveal significant differences ($p > 0.05$) (Table 5.7). For herd-level variables, there were no significant differences between *T. parva* seroprevalence for cattle in which an acaracide had been used or not used in the 2 weeks prior to the time of the farm visit ($p = 0.18$) and in those herds in which either cattle from outside had joined the herds or not ($p = 0.172$) (Table 5.8). There was only weak evidence against the null hypothesis of no difference in *T. parva* seroprevalence between herds which had experienced calf mortality in the preceding year and those which had not ($p = 0.1$) (Table 5.8). *Theileria parva* seroprevalence, however, differed significantly ($p<0.05$) between categories of all other herd-level factors (Table 5.8).

Theileria parva seroprevalence in animals from herds in which calf tick control was initiated at less than 3 months of age (24.8%) and at between 3 to 6 months of age (18.6%) were not significantly different from each other ($p = 0.24$). However, these seroprevalence values were significantly different from that of animals in which the control measures were initiated at over 6 months of age (5.5%) ($p < 0.05$) (Table 5.8). Similarly, *T. parva* seroprevalence in cattle from herds in which acaricides were the most commonly drugs used (28.8%) were significantly higher compared to cattle from herds in which 'other drugs' (antihelmintics, multivitamin supplements, antibiotics) were commonly used ($p=0.008$) (Table 5.8).

The shape of the relationship between the continuous variables (herd size and veterinary distance) and the outcome was evaluated by adding a quadratic term (the variable squared) to check whether the assumption of linearity was violated or not. The quadratic term for 'veterinary distance' was not significant (p=0.94) suggesting that a linear relationship was acceptable. Thus, this variable was modeled as a continuous variable (Table 5.8). The quadratic term for 'herd size', however, was highly significant (p<0.001) implying that the linear relationship assumption had been violated. This variable was thus divided into categories which were fitted in the model as categorical variables (Table 5.8). The seroprevalences and their 95% CI for herd size categories of 1-5 cattle, 6-10 cattle and over 10 cattle was 13.2% [7.2%, 19.2%], 25.3% [18.1%, 32.5%], and 18.5% [12.8%, 24.2%], respectively. Chi square statistics for comparison of herd-size categories for *T. parva* seroprevalence were not significantly different (χ^2 = 1.08, df =2, p=0.58).

Both division- and AEZ-specific *T. parva* seroprevalence differed significantly across all divisions (p < 0.0001) and the two AEZs (p = 0.005) (Table 5.9). *Theileria parva* seroprevalence in cattle from herds in which *R. appendiculatus* tick was found in the farm (31.8%) were significantly higher (p< 0.001) from that in cattle from herds in which the vector tick was not found in the farm (12.4%) at the time of the visit. The shape of the relationship between the NDVI values (a continuous variable) and the outcome was evaluated with the quadratic term being not significant (p=0.54) suggesting that the linear relationship assumption had not been violated. This variable (NDVI) was, thus, modeled as a continuous variable (Table 5.9).

5.3.4. Univariate analysis

Tables 5.7, 5.8 and 5.9 also show the crude ORs from univariable logistic regression models of individual risk factors postulated to be associated with *T. parva* antibody prevalence. As indicated above, the strength of association between the risk factor and *T. parva* seropositivity was estimated by ORs which were directly derived from estimates of logistic regression models.

Of the animal level factors, only breed was significantly associated with *T. parva* antibody prevalence as indigenous breeds of cattle were 2.7 times more likely ($p = 0.067$) (level of significance was set at 0.1% under univariate analysis) to have *T. parva* antibodies compared to exotic breeds (Table 5.7). All herd-level risk factors except grazing type were significantly associated with *T. parva* antibody prevalence ($p < 0.05$) (Table 5.8). All area-level variables were significantly associated with *T. parva* antibody prevalence ($p < 0.001$) (Table 5.9).

Table 5.7: Animal-level risk factor-stratified seroprevalence, 95% CI and univariate analysis for exposure to *T. parva* in cattle sampled from Mbeere District, Kenya, March 2007

Variable	Level	*n*	Prevalence % [95% CI]	Crude OR [95%CI]	p-value
Age					0.49
	Calves	88	16[a] [8.3, 23.7]	0.71[0.53, 0.95]	
	Yearlings	76	17[a] [8.6, 25.6]	0.78[0.4, 1.5]	
	Adults	276	21[a] [16.2, 25.8]	1.0	
Sex					0.14
	Female	279	17[a] [12.8, 21.6]	1.4 [0.9, 2.3]	
	Male	161	23[a] [16.5, 29.5]	1.0	
Breed					0.067
	Exotic	35	8.6[a] [0, 18]	1.0	
	Indigenous	405	20.2[a] [16.2, 24.2]	2.7 [0.8, 9]	
B/score[x]					0.244
	Good	150	24[a] [17.2, 30.8]	1.5 [1.1, 2.5]	
	Average	251	17[a] [12.4, 21.8]	1.0	
	Poor	12	16.6[a] [0, 37.6]	0.96[0.2, 4.6]	

[a,] Values with same superscript letters are not significantly (p >0.05) different along column of comparison for levels of each variable; CI: Confidence interval; OR: Odds ratio; B/score: Body score, [x] n<440 as some calves were too small for body scoring.

Table 5.8: Herd-level factors-stratified seroprevalence, 95% CI and univariate logistic regression analysis for exposure to *T. parva* in cattle sampled from Mbeere District, Kenya, March 2007.

Variable	Level	*n*	Prevalence % [95% CI]	Crude OR [95%CI]	p-value
Grazing type	Open	383	19.1[a] [15, 23]	1.1 [0.57, 2.2]	p= 0.725
	Open + stall	57	21.1[a] [10.5, 31.7]	1.0	
Acaracide use last 2 weeks	Yes	224	22.3[a] [16.9, 27.8]	1.5 [0.92, 2.4]	p=0.1
	No	216	16.2[a] [11.3, 21.1]	1.0	
Initial calf tick control age	<3 mnths	165	24.8[a] [18.2, 31.4]	1.0	p=0.002
	3-6 mnths	220	18.6[a] [13.5, 23.7]	0.7[0.43, 1.13]	
	> 6 mnths	55	5.5[b] [0, 12]	0.17 [0.05, 0.6]	
Freq of calf tick control[c,x]	≤ 5	225	12.4[a] [8.1, 16.7]	1.0	p<0.001
	>5	212	26.9[b] [21, 32.9]	2.6 [1.6, 4.3]	
Calf mortality in preceding yr [y]	Yes	147	14.3[a] [8.6, 20]	0.59 [0.34, 1]	p=0.045
	No	284	22.2[a] [13.2, 31.2]	1.0	
Common drug used in farm	Acaricide	132	28.8[a] [21.1, 36.5]	1.0	p<0.001
	Others[d]	308	15.3[b] [11.3, 9.3]	0.45 [0.27, 0.73]	
ECF found on farm	Yes	265	24.9[a] [19.7, 30.1]	2.7 [1.57, 4.73]	p<0.001
	No	175	10.9[b] [6.3, 15.5]	1.0	
Cattle joined herd	Yes	102	13.7[a] [7, 20]	0.6 [0.32, 1.1]	p=0.093
	No	338	21[a] [16.7, 5.3]	1.0	
Vet distance	-	-	- -	0.9 [0.86, 0.96]	p<0.001
***R. a.* on farm**	Yes	157	31.8[a] [24.5, 39.1]	3.3 [2, 5.4]	p<0.001
	No	283	12.4[b] [8.6, 16.2]	1.0	

Legend for Table 5.8: [a], [b], Values with different superscript letters are significantly (p<0.05) different for levels of each variable along the column of comparison; Values with the same superscript letters are not significantly different (p > 0.05) for levels of each variable along the column of comparison; [c]Freq. of calf tick control**:** number of times acaricide is applied to calf by 6 months of age.; Others[d]: Other drugs such as dewormers, antibiotics and vitamin supplements; OR: Odds ratio; CI: Confidence interval; *R. a.* on farm: *R. appendiculatus* found on farm; Vet distance: distance traveled by farmer to the nearest veterinary clinic, [x], n<440 as one farm reported not to apply acaricides at all, [y], n<440 as not all farms had calves in preceding year.

Table 5.9: Area-level risk factor-stratified seroprevalence, 95% CI and univariate analysis for exposure to *T. parva* in cattle sampled from Mbeere District, Kenya, March 2007

Variable	Level	*n*	Prevalence % [95% CI]	Crude OR [95%CI]	p-value
NDVI		-	-	1.28 [1.17, 1.4]	p<0.001
Division					p<0.001
	Siakago	102	3.9[a] [0.14, 7.6]	0.18 [0.06, 0.54]	
	Gachoka	133	9.8[b] [4.7, 14.9]	0.48 [0.22, 1.02]	
	Evurore	103	18.4[c] [10.9, 25.9]	1.0	
	Mwea	102	48[d] [38.3, 57.7]	4.1 [2.17, 7.7]	
AEZ					p<0.001
	LM4	278	24.1[a] [19.1, 29.1]	1.0	
	L5	162	11.1[b] [6.2, 15.8]	0.39 [0.22, 0.69]	

[a, b, c, d], Values with different superscript letters are significantly ($p < 0.05$) different for levels of each variable along the column of comparison ; CI: Confidence interval; OR: Odds ratio; NDVI: Normalized Difference Vegetation Index; LM4: Lower Midlands 4; L5: Lowlands 5

5.3.5. Multivariable analyses

5.3.5.1. Multivariable model before adjusting for correlation

Before adjusting for correlation at herd level, the significant variables included division, frequency of calf tick control by 6 months of age, herd size and presence of *R. appendiculatus* on the farm (Table 5.10). On comparison of estimates between univariate and multivariate models, approximately similar estimates for variables 'frequency of calf tick control by 6 months of age' and 'presence of *R. appendiculatus* on farm' were obtained. However, the estimates for certain categories of 'Division' and 'herd size' predictors changed substantially probably indicating that they were acting as confounders in the model (Table 5.11). However, none of the possible interaction terms were significant.

Table 5.10: Fixed-effect estimates from multivariable model (before accounting for correlation) used to estimate risk for *T. parva* seroprevalence in the cattle population of Mbeere District, March 2007

Risk factor	Level	Adjusted OR (SE)	Adjusted OR 95% CI
Division	Siakago	0.19 (0.643)	[0.054, 0.67]
	Gachoka	0.31 (0.422)	[0.14, 0.71]
	Mwea	2.5 (0.355)	[1.25, 5]
	Evurore	1.0	-
[a]Freq. of calf tick control	>5	2.3 (0.345)	[1.17, 4.5]
	≤ 5	1.0	-
Herd size	1 to 5	1.0	-
	6 to 10	2.1 (0.372)	[1.19, 4.9]
	Over 10	0.91 (0.440)	[0.38, 2.16]
***R. appendiculatus* seen on farm**	Yes	2.5 (0.318)	[1.34, 4.7]
	No	1.0	-

OR: Odds ratio; SE: Standard error; [a] Freq. of calf tick control**:** number of times acaricide is applied to calf by 6 months of age.

Table 5.11: Comparison of crude and adjusted odds ratios (ORs) in univariate and multivariate analyses and description (%) of change in the odds ratios

Risk factor	Level	Crude OR	Adjusted OR	% change in ORs
Division	Siakago	0.18	0.19	3%
	Gachoka	0.48	0.31	60%
	Evurore	1.0	1.0	-
	Mwea	4.1	2.5	35%
[a]Freq. of calf tick control	≤ 5	1.0	1.0	-
	>5	2.6	2.3	13%
Herd size	1 to 5	1.0	1.0	-
	6 to 10	2.3	2.1	11%
	Over 10	1.5	0.91	123%
***R. appendiculatus* found on farm**	Yes	3.3	2.5	23%
	No	1.0	1.0	-

OR: Odds ratio; [a] Freq. of calf tick control**:** number of times acaricide is applied to calf by 6 months of age.

5.3.5.2. Multivariable model accounting for correlation at the herd level

On accounting for correlation at herd level, fixed effect estimates for some predictors changed substantially (Table 5.12). Comparable estimates for Siakago and Gachoka Divisions were obtained as before accounting for correlation but the odds of some categories in division and herd size variables changed substantially. Overall, Siakago and Gachoka Divisions were associated with lower risk of having *T. parva* antibodies. Cattle from Siakago and Gachoka Divisions were 3 and 5 times less likely to have *T. parva* antibodies compared to those from Evurore division. On the other hand, Mwea Division was associated with higher risk of having *T. parva* antibodies. Cattle from Mwea Division were 5 times more likely to have *T. parva* antibodies compared to those from Evurore Division (Table 5.12). Cattle from herds whose calves' tick control application frequency was more than 5 times by 6 months of age were associated with higher risk of having *T. parva* antibodies as they were 4 times more likely to have *T. parva* antibodies compared to those whose application frequency was less than 5 times (Table 5.12). Cattle from herd size category '6 to 10 cattle' per herd were associated with higher risk of having *T. parva* antibodies as they were 2.7 times more likely to have *T. parva* antibodies compared to cattle from the '1 to 5 cattle' category. Herd size category 'Over 10 cattle' per herd was associated with lower risk of having *T. parva* antibodies as they were 1.1 times less likely to have *T. parva* antibodies compared to cattle from the '1 to 5 cattle' category (Table 5.12). Cattle from herds in which the vector tick, *R. appendiculatus,* had been found were associated with higher risk of having *T. parva* antibodies as they were 4 times more likely to have *T. parva* antibodies compared to those in which the tick vector had not been found (Table 5.12).

The herd variance component from this model was estimated at 2.7. The intra-herd correlation coefficient for these data was computed to be 0.45 which was relatively higher compared to the one obtained by ANOVA method (0.3) (section 5.3.3.2.)

5.3.5.3. Multivariable model without variable 'Division'

As divisional boundaries are administrative in nature, other attributes in the district were investigated for their importance in explaining the *T. parva* seroprevalence variability in the district. A multivariable model without 'Division' was, thus, built with all univariate significant variables with the objective of investigating any additional significant effects which could have been masked by effects of division. The outcome of this model is shown in Table 5.13. Three additional effects became significant ($p<0.05$) and these included, initial calf age tick control, NDVI and AEZ. However, the model in section 5.3.5.2 (Table 5.12) was chosen as the most parsimonious (i.e. explained the greatest proportion of variation in *T. parva* seropositivity) for these data.

Table 5.12: Fixed-effect and variance component estimate from a generalized linear mixed model used to estimate risk for *T. parva* seroprevalence in the cattle population of Mbeere District, March 2007

Variable classification	Variable	Level	Adjusted OR (SE)
Fixed effects			
	Division	Siakago	0.3
		Gachoka	0.21
		Evurore	1.0
		Mwea	5.1
	[a]Freq. of calf tick control		
		≤ 5	1.0
		>5	3.9
	Herd size	1 to 5	1.0
		6 to 10	2.7
		Over 10	0.95
	***R. appendiculatus* found on farm**		
		Yes	3.8
		No	1.0
Random effect	**Herd**	Variance estimate (SE)	2.7 (0.8140)

OR: Odds ratio; SE: Standard error; [a] Freq. of calf tick control**:** number of times acaricide is applied to calf by 6 months of age.

5.3.6. Multilevel models

In the random effects models, the size of the herd variance component reduced almost by half when 'sub-location' variable was added (Table 5.14). However, upon addition of 'division', the herd variance component did not change whereas that of 'sub-location' reduced from 2.4 to 0.393 (Table 5.14). The absolute values of variance components of full random effects model with all the four hierarchical levels were as follows: herd (2.314) (47.6%), sub-location (0.393) (8.1%) and division (2.151) (44.2%). Substantial variation was, therefore, deemed to rest at herd and division levels (Table 5.14).

Table 5.13: Fixed-effect estimates from multivariable model without variable 'Division' used to estimate risk for *T. parva* seroprevalence in the cattle population of Mbeere District, March 2007

Variable	Level	Adjusted OR (SE)	Adjusted OR 95% CI
Initial calf age tick control	<3 months	1.0	-
	3 to 6 months	2.9 (0.391)	[1.34, 6.2]
	> 6 months	3.6 (0.8)	[0.75, 17.2]
Herd size	1 to 5	1.0	-
	6 to 10	2.4 (0.371)	[1.14, 4.9]
	Over 10	0.9 (0.431)	[0.39, 2.1]
Frequency of calf tick control by age 6 months	≤ 5	1.0	
	>5	6 (0.398)	[2.8, 13.2]
NDVI	-	1.22 (0.0586)	[1.1, 1.4]
***R. append.* found on farm**	Yes	2.6 (0.3)	[1.5, 4.7]
	No	1.0	-
AEZ	LM4	1.0	-
	LM5	0.47(0.348)	[0.24, 0.94]

OR: Odds ratio; CI: Confidence interval; NDVI: Normalized Difference Vegetation Index; *R. append.: Rhipicephalus appendiculatus;* AEZ: Agro-ecological zone; Frequency of calf tick control by age 6 months: (number of acaricide applications) of calf tick control by age 6 months.

Presence of fixed effects into the random-effect models usually decreased the size of the previously-estimated variance components under the random-effects mdels (Tables 5.14 and 5.15). The relative size of the variance components for different levels of organization sometimes changed substantially in the presence of fixed effects. However, the herd variance component did not change substantially on addition of higher random effects controlling for fixed effects (Table 5.15) with the variance due to herd being larger than due to geographical areas under all circumstances. The absolute values of variance components of full mixed effects model with all the four hierarchical levels were as follows: herd (2.2185) (43%), sub-location (0.7103) (13.8%) and division (1.6878) (32.9%). Similarly, on inclusion of fixed effects, substantial variation, therefore, rested at herd and division levels in that order (Table 5.15).

Table 5.14: Variance component estimates from random-effects models (without fixed effects) used to evaluate sources of variation in *T. parva s*eropositivity in the cattle population of Mbeere District, March 2007

	2-level model (SE)	3-level model (SE)	4-level model (SE)
Variance due to error	0.52 (0.0385)	0.535 (0.0392)	0.532 (0.0391)
Herd	4.05 (0.9430)	2.234 (0.6636)	2.314 (0.6804)
Sub-location	-	2.4 (1.5289)	0.393 (0.5992)
Division	-	-	2.151 (2.1265)

SE: Standard error

Table 5.15: Variance component estimates from mixed models (both random and fixed effects) used to evaluate sources of variation in *T. parva* seropositivity in the cattle population of Mbeere District, March 2007

	2-level model (SE)	3-level model (SE)	4-level model (SE)
Variance due to error	0.517 (0.0378)	0.520 (0.0379)	0.519 (0.0379)
Herd	2.7011 (0.8140)	2.3566 (0.7703)	2.2185 (0.7261)
Sub-location		0.8084 (1.5289)	0.7103 (0.9161)
Division			1.6878 (1.9594)

SE: Standard error

5.4. Discussion

This study offered a population-structured quantitative baseline cross-sectional study on *T. parva* antibody prevalence, with the objectives of quantifying the relationships between risk factors and prevalence of *T. parva* infection and assessing the epidemiological status of the infection in the cattle population of Mbeere District.

The concept of assessing the epidemiological status of *T. parva* infection in a given cattle population arises from the observations that endemic stability is a possibility where *T. parva* antibody prevalence is high (>70%) in the calf population aged below six months of age, while the antibody prevalence is usually low (<30%) in the endemically unstable state (Norval *et al.,* 1992; Perry and Young, 1995). However, prevalence alone is not an adequate measure to make concrete conclusions on *T. parva* infection endemicity status. For the detection of *T. parva* antibodies, the indirect ELISA test was used in this study. This assay has demonstrated a sensitivity of 99% and a specificity of between 94 and 98% in experimental and field sera (Katende *et al*., 1998).

In Mbeere District, the overall *T. parva* seroprevalence was 19.3% and this varied widely from 3.9% to 48% across divisions. The overall prevalence is comparable to *T. parva* seroprevalence reported in certain AEZs in the Kenyan coastal lowland (Deem *et al.,* 1993; Maloo *et al.,* 2001a) and central Kenya highlands (Gitau *et al.,* 1997), regions characterized by varying AEZs. If antibody prevalence was used as an indicator of endemic stability, the finding in this study indicated that endemic instability existed in Mbeere District. Defining the endemic state on the basis of seroprevalence alone implied that in Mbeere district, only a low proportion of animals were supposedly immune and that the rest of the cattle population (80%) was susceptible to clinical disease. This phenomenon could have arisen out of very low infection challenge, probably due to low levels of vector abundance and distribution and /or low tick infection rates among other reasons.

However, reliable data on tick populations in the district are unavailable. Moreover, a single cross-sectional study can only give one an indicator of the probability of endemic status based on prevalence which can vary quite substantially with climatic conditions and over time (Gitau *et al.,* 1999). Other factors that have been reported to influence *T. parva* transmission and prevalence include host population size and density, habitat modification, vector control programs and the social environments (Olwoch *et al.,* 2008).

Out of the animal-level factors (age, sex, breed and body condition) screened for association with *T. parva* seroprevalence, breed was the only significant variable under univariate analysis and it was no longer associated with the prevalence after controlling for the herd and area-level factors. Furthermore, the odds ratio estimation obtained for breed was not statistically significantly different from unity (value of 1). This could be due to the fact that the majority of animals sampled (92%) in the district were indigenous breeds and their crosses which were classified as 'indigenous' and were reared under various forms of open grazing. Similar association between indigenous breed and open grazing system has been found in other districts with varying AEZs in East Africa (Gitau *et al.,* 1997; Gitau *et al.,* 1999; Maloo *et al.,* 2001a, b; Rubaire-Akiiki *et al.,* 2006; Swai *et al.,* 2007). Animals raised under open grazing management are frequently exposed to infected ticks, and thus, are more likely to develop circulating antibodies to the parasite.

In this study, the few exotic animals sampled were found to be mainly reared under zero grazing management, a practice associated with minimal exposure to ticks (Gitau *et al.,* 1997, Gitau *et al.,* 1999). These findings corroborate with observations that in lower AEZ elevations, grazing management may be important in explaining the variation of *T. parva* seroprevalence in an area (Gitau *et al.,* 1997, Gitau *et al.,* 1999, Swai *et al.,* 2005a, Rubaire-Akiiki *et al.,* 2006).

Age as a risk factor, though not significantly related to *T. parva* seropositivity, was categorized into broad classes (calves, yearlings and adults). Calves less than 4 months were not sampled to minimize the possibility of detecting maternal antibodies (Norval *et al.,* 1992; Deem *et al.,* 1993; Gitau *et al.,* 2000). In this study, there were no significant differences in *T. parva* seroprevalence between the different age classes implying that the infection challenge was likely to be inadequate to induce population immunity even over time or the cattle were not exposed to infected ticks. Under such conditions, animals of all age groups would be at risk of clinical ECF (Lawrence *et al.,* 2004) leading to direct economic effects in the area. Other studies have reported higher prevalences in younger animals than adults by PCR in Uganda (Oura *et al.,* 2005), in Rwanda (Bazarusanga *et al.,* 2007; Bazarusanga *et al.,* 2008) and in Sudan (Salih *et al.,* 2007), while others have reported increasing age being associated with increased *T. parva* seroprevalence (Moll *et al.,* 1986; O' Callaghan, 1998; Gitau *et al.,* 1999, Maloo *et al.,* 2001b; Swai *et al.,* 2005a, Rubaire-Akiiki *et al.,* 2006).

Additional information collected in longitudinal studies and relating age-seroprevalence profiles and forces of infection should be able to investigate patterns of age-specific *T. parva* seroprevalences over time in an endemic unstable area.

In this study, a herd referred to a group of animals under the same management system in a given farm. Animals in a herd were assumed to share common risk factors for the disease. All individual herd factors (herd size, recent use of acaricides, initial age of calf tick control, frequency of calf tick control, calf mortality in the preceding year of the study, common drugs used in the farm and whether cattle in farm had experienced ECF syndrome in the preceding years, the presence of *R. appendiculatus* vector tick on farm and distance to the nearest veterinary clinic) except grazing type were significantly associated with *T. parva* seroprevalence. Similar significant relationships between herd management factors and *T. parva* seroprevalence in endemic unstable areas have been reported by Deem and co-workers (1993). In the current study, a further observation was that majority of the herd-level variables were also significantly related with the area-level variables. For example, the highest percentage of farmers in a division who used acaricides most and who started calf tick control measures at an early age came from Mwea, Evurore, Gachoka and Siakago divisions in that order, and similarly, the *T. parva* seroprevalence levels declined following the same order, most likely because of the level of awareness of disease occurrence.

Further, the significant association between whether suspected ECF syndrome had been reported in a farm or not with *T. parva* sero-prevalence and with all the area-level variables suggested that farmers from areas with the least seroprevalence had only rarely observed the syndrome in their animals while those from areas with higher seroprevalence were possibly well aware of the disease.

Although there was just marginal association between the variable 'calf mortality in the preceding year' and *T. parva* seroprevalence, there was no statistical difference in seroprevalence between animals in herds which had experienced crude calf mortality or not. The proportion of herds that had experienced crude calf mortality within the year prior to this study was only 34%. The association between this variable and division suggested that differential crude calf mortality levels across the divisions existed. A longitudinal study can help establish the relative importance of ECF-morbidity and mortality relative to other diseases in such an area as Mbeere District.

In this study, area-level variables (division, Normalized Differential Vegetation Index (NDVI) and AEZ) had the most important and large effects associated with *T. parva* seroprevalence. Vegetation in this study was inferred through the use of NDVI which is indirectly related to relative humidity and rainfall. Thus, NDVI served as a surrogate measure of any environmental differences in tick suitability habitats in the district. Other studies have similarly used NDVI as an area-level variable (Kadohira *et al.,* 1996).

The NDVI values have also been successfully utilized in predicting tick distributions based on climatic suitability and eco-climatic indices in Africa (Cumming, 2000). In the current study, NDVI values were significantly associated with division and AEZ. Higher NDVI values were obtained in sub-locations, divisions and AEZ that had higher *T. parva* seroprevalence. Higher NDVI values are normally associated with higher levels of healthy vegetation cover (Chen and Brutsaert, 1998) and, therefore, the increased risk of having *T. parva* antibodies associated with higher index values supported the proposition that tick suitability habitats differed across the divisions and AEZs zones resulting in the geographical variability in the *T. parva* seroprevalence observed. Similar findings have been reported with *R. appendiculatus* field infestation levels and *T. parva* seroprevalence varying across administrative districts and AEZs in East Africa (Deem *et al.,* 1993; Gitau *et al.,* 1997; Gitau *et al.,* 1999; Maloo *et al.,* 2001a, b; Rubaire-Akiiki *et al.,* 2006; Bazarusanga *et al.,* 2007; Bazarusanga *et al.,* 2008) and clearly reflect different levels of exposure to *T. parva* infection. In one of these studies, the hypothesis of varying levels of vector competence amongst ticks from different ecological areas (Ochanda *et al.,* 1998) was also a likely explanation of geographical variability (Bazarusanga *et al.,* 2007).

Cattle were sampled from two out of the three AEZs in the district. The lower and significantly different *T. parva* seroprevalence in cattle from L5 compared to those from LM4 could be attributed to the ecological differences between the two zones particularly the short length of plants' growing period and the short grass savannah rangelands that

predominate on overgrazed and eroded areas in L5 zone (these conditions are unsuitable for the vector tick) (Norval *et al.,* 1992). This finding further corroborates the proposition that there could be significant differential ecological and climatic variability in vector suitability habitats in the district.

A distinct pattern between *R. appendiculatus* distribution and *T. parva* seroprevalence in Mbeere District was found and this has been reported for other areas by other investigators both in cross-sectional studies (Deem *et al.,* 1993; Fandamu, *et al.,* 2005) and in longitudinal studies (Yeoman, 1966; Swai *et al.,* 2005b; Rubaire-Akiiki *et al.,* 2006). The significance of the variable 'presence of the tick vector' was expected, as biologically, the geographic distribution of ECF is strictly associated with the distribution of *R. appendiculatus* (Norval *et al.* 1992).

For the variable herd size, farmers with large herd sizes came from areas with the least seroprevalence (Siakago and Gachoka Divisions) while it was vice versa in Mwea and Evurore Divisions. The relationship between herd size and *T. parva* seroprevalence could not, however, be explained by the information available. Whether the relationship between herd size and *T. parva* seroprevalence arose from mortality constraints due to ECF needs to be investigated in a longitudinal study.

The other significant herd-level factor that had large and important effect was 'frequency of calf tick control'. The finding that frequent application of acaricides to calves was associated with higher *T. parva* seroprevalence would normally be unexpected.

However, as noted above, farmers from areas with the least *T. parva* seroprevalence (Siakago and Gachoka Divisions) applied acaricides the least number of times and started off calf tick control measures when calves were much older compared to farmers from areas with higher *T. parva* seroprevalence (Evurore and Mwea Divisions). These findings highlighted distinct differential herd tick control management strategies across the district and further implied that geographical (environmental) determinants were related to the differences in herd tick control management (This was found to be statistically true from the various significant relationships between herd-level and area-level risk factors). The differences in herd tick control management could have arisen out of possible disparity in perceptions of importance of ECF in their respective areas. This finding corroborates with the results of the questionnaire that ECF as a constraint was ranked first only in Mwea Division (*T. parva* seroprevalence of 48%) whereas none of the farmers from Siakago Division (*T. parva* seroprevalence of 3.8%) ranked ECF either in the first or second position. The difference in perceptions of occurrence and importance of ECF could have been related to possible differential levels of tick burdens on their animals, presence of ECF clinical signs and/or mortality of their animals as seroconversion has recently been strongly associated with clinical signs of ECF (particularly lymph node enlargement) (Magona *et al.,* 2008).

The variable 'Division' when modeled as a fixed effect was suspected to be a confounder variable (related to both independent variables and the outcome variable). The geographical and administrative location has been found to be a confounder in a similar study (Swai *et al.,* 2006). On the other hand, it could have been due to collineality with the other independent variables (statistical dependence of independent variables). However, the latter concept could have been detected during modelling. When the variable 'Division' was omitted from the model its area-level counterparts (NDVI and AEZ) became significant, further supporting the importance of area-level variables in explaining the variation in *T. parva* seroprevalence in the district. To add on to similar observations by Deem et al. (1993) that in endemically unstable areas herd management factors were more important than in endemic stable areas, findings in this study suggested that differential environmental effects may indirectly explain variability of *T. parva* seroprevalence at the herd level in endemically unstable areas and subsequent differential herd disease management practices. This observation, however, needs to be further confirmed in a longitudinal study.

Multilevel models are used to analyze data possessing a nested hierarchy (Edmond *et al.,* 2006). In this study, cattle could be viewed as nested within herds, herds nested within sub-locations, and sub-locations nested within divisions according to the multistage sampling approach adopted.

By using multilevel models (both mixed-effects and random-effects models), patterns of clustering of *T. parva* seropositivity in the cattle population of Mbeere District were determined using Schall's algorithm (Schall, 1991). The principle advantage of Schall's algorithm is that it can be extended to account for correlations at multiple levels of organization (Schall, 1991). During the modeling process, the addition of sub-location and division variance components decreased the size of the herd-level variance component. The latter finding suggested that (probably under ECF endemic instability), accounting only for herd-level clustering may result in an overestimate of herd variability. This indicated the importance of sub-location and division (environmental) effects in explaining *T. parva* seroprevalence variation in Mbeere District. Furthermore, as only single-level clustering is accounted for in sampling in many studies (Dohoo *et al.,* 2003), this may be inadequate in situations where TBDs occur under endemic instability and where responses cluster at multiple levels as seen in this study.

The mixed model analyses further demonstrated that variance component estimates in Schall's algorithm can change substantially if compared between models with random effects only and mixed-effect models, indicating the importance of the fixed effects in explaining *T. parva* seroprevalence variation in this study. All the fixed effects that were incorporated into the mixed models were the significant herd-level risk factors. Presence of these fixed effects reduced overall variance but changed the distributions of the variance components only slightly, leaving values at herd level unaffected (and large) but

reducing the values at division level. This further supported the importance of both herd and area effects in the study.

From the analyses of the two types of multilevel models, it was clear that factors that vary from herd to herd and from division to division were by far, the most important source of variation in *T. parva* seroprevalence in Mbeere District. As highlighted above, the large variation at the herd level appeared to be related to tick control management possibly arising out of differential perceptions of importance of ECF. It also indicated that outcomes within a herd had a common cause and/or strong dependency in terms of parasite exposure. The latter consideration could have been indirectly related to differences in herd vector presence/burdens and subsequent carrier state levels that may constitute a potential risk factor for within-herd disease transmission (Bishop *et al.,* 1992; Kariuki *et al.,* 1995). The large geographical variation obtained in this study also supports the possibility of occurrence of differential ecological and climatic variability in vector suitability habitats in the district. Thus, multilevel modeling approach provided more information. Such spatial variations in *T. parva* seropositivity have also been reported (Deem *et al.,* 1993; Rubaire-Akiiki *et al.,* 2006; Swai *et al.,* 2006; Swai *et al.,* 2007) though they were not quantified. The results of these models imply that both among-farm and among-division transmission factors were important in designing disease control strategies in this district. The findings thus strongly suggest that intervention factors directed at the farm- and division- levels are likely to have the greatest impact.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

- Traditional mixed crop-livestock farming system is an important enterprise in Mbeere District. Overall, 90% of the households kept only indigenous cattle breeds mainly under open grazing management systems. Overall, feed and water availability, and livestock diseases were ranked as the most important livestock production constraints in all divisions. Anaplasmosis, ECF and foot lameness were ranked as the most common disease constraints in that order.

- The overall *T. parva* seroprevalence was 19.3% (range: 3.9% to 48% across divisions) in the district [95%CI: 13.7%, 24.9%]. Regression analysis found four major factors (fixed effects) associated with seropositivity: presence of the vector tick on the farm, frequency (number of times) of calf tick control before 6 months of age, herd size and division.

- On evaluation of sources of variation in *T. parva* seropositivity in the cattle population of Mbeere District utilizing both random effects models (incorporating random effects only) and mixed models (incorporating both random effects and the significant fixed effects), substantial variation rested at herd and division levels though the variance due to herd was larger than that due to geographical areas in both models. The large variation at the herd level appeared to be related to

tick control management possibly arising out of differential perceptions associated with ECF occurrence and distribution. It also indicated that outcomes within a herd had a common cause and/or strong dependency in terms of parasite exposure. The large geographical variation obtained could be related to possible differential ecological and climatic variability in vector suitability habitats in the district. The results of these models imply that both among-farm and among-division transmission factors were important in designing disease control strategies in Mbeere District. From the findings, intervention factors directed at the farm- and division- levels are likely to have the greatest impact.

6.2. Recommendations

- Further research on characteristics of crop-livestock systems in Kenya should adopt a multi-focused approach addressing production-specific research and innovative and promotional strategies for crop and livestock research knowledge and technologies such as development and promotion of dual-purpose food-feed crops, improved and enlightened use of available production systems-based feed resources for ruminants and development and promotion of fodder conservation strategies.

- In designing strategies to control ECF in Mbeere District, perhaps differential disease control strategy is an area that needs to be investigated further in a

longitudinal study as the cross-sectional study does not seem to indicate a relationship on their use.

- In circumstances where both farm managemental and environmental effects are associated with *T. parva* seroprevalence, further information on both environmental suitability and environment-specific farm management factors is required for planning disease control programs.

- As the limits of *R. appendiculatus* distribution may expand or contract with changes in climatic and ecological suitability in areas of low endemicity, the geographical distribution and relative suitability of the different circumstances for *R. appendiculatus* need to be defined in Mbeere District, preferably by use of the relatively new predictive distribution models so that the level of challenge presented to cattle in each environment can be clearly and effectively determined**.** This may mitigate some of the disadvantages that goes with general chemical control and may also save both the government and farmers' time and resources because varying actions will be targeted to the relevant risk levels depending on the intensity of the problem. This information needs to be investigated in longitudinal studies and incorporated in future control strategies by divisional veterinary authorities and thereafter disseminated to farmers so that they can design and implement better and more economical disease control strategies.

REFERENCES

Bazarusanga, T., Vercruysse, J., Marcotty, T., Geysen, D. 2007. Epidemiological studies on Theileriosis and the dynamics of *Theileria parva* infections in Rwanda. *Veterinary Parasitology,* **143,** 214–221.

Bazarusanga, T., Geysen, D., Vercruysse, J., Marcotty, T. 2008. The sensitivity of PCR and serology in different *Theileria parva* epidemiological situations in Rwanda. *Veterinary Parasitology,* **154,** 21-31.

Billiouw, M., Mataa, L., Marcotty, T., Chaka, G., Brandt, J., Berkvens, D. 1999. The current epidemiological status of bovine theileriosis in eastern Zambia. *Tropical Medical International Health,* **4,** A28 - A33.

Billiouw, M., Brandt, J., Vercruysse, N., Speybroeck, J., Marcotty, T., Mulumba, M., Berkvens, D. 2005. Evaluation of the Indirect Fluorescent Antibody Test as a diagnostic tool for East coast fever in Eastern Zambia. *Veterinary Parasitology,* **127,** 189-198.

Bishop, R.P., Sohanpal, B.K., Kariuki, D.P., Young, S., Nene, V., Baylis, H., Allsopp, B.A., Spooner, P.R., Dolan, T.T., Morzaria, S.P. 1992. Detection of a carrier state in *Theileria parva*-infected cattle by the polymerase chain reaction. *Parasitology,* **104**, 215-232.

Blalock, H.M. Jr. 1964. Causal inferences in non-experimental research. New York: Norton; Chap. 1.

Central Bureau of Statistics (CBS), 2001. 1999 Population and Housing Census. II.Socio-economic profile of the population. Ministry of Finance and Planning, Republic of Kenya. Central Bureau of Statistics.

Chema, S., Waghela, S., James, A.D., Dolan, T.T., Young, A.S., Masiga, W.N., Irvin, A.D., Mulela, G.H.M., Wekesa, L.S. 1986. Clinical trial of parvaquone for the treatment of East Coast fever in Kenya. *Veterinary Record*, **118,** 588-589.

Chen, D., Brutsaert, W. 1998. Satellite-sensed distribution and spatial patterns of vegetation parameters over a tall grass prairie. *Journal of the Atmospheric Sciences,* **55,** 1225-1238.

Collett, D. 2002. Modeling binary data. 2nd Edition. Chapman and Hall/CRC Boca Raton, Florida, pp. 24.

Cumming, G.S. 2000. Using habitat models to map diversity: pan-African species richness of ticks (Acari: Ixodidae). *Journal of Biogeography,* **27,** 425-440.

Deem, S.L., Perry B.D., Katende J.M., McDermott, J.J., Mahan, S.M., Maloo, S.H., Morzaria, S.P., Musoke, A.J., Rowlands, G.J. 1993. Variations in prevalence rates of tick-borne diseases in Zebu cattle by agro-ecological zone: Implications for the East Coast fever immunization. *Preventive Veterinary Medicine,* **16**: 171-187.

Department for International Development, Animal Health Programme (DFID). 2001. East coast fever in sub-Saharan Africa. Poster. DFID (AHP). 1pp.

Di Giulio, G., Lynen, L., Medley, G., O'Callaghan, C.J., Kiara, H.K., McDermott, J.J. 2003. Characterisation of theileriosis in pastoral production systems in northern Tanzania. Abstract submitted to the Tenth International Symposium of Veterinary Epidemiology and Economics, held in Chile, 17-21 November 2002.

Dohoo, I.R., Tillard, E., Stryhn, H., Faye, B. 2001. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Preventive Veterinary Medicine,* **50,** 127-144.

Dohoo, I., Martin, W., Stryhn, H. 2003. Veterinary Epidemiologic Research. *In*: S.M. Mcpike (Ed.). A comprehensive text for the discipline. AVC Inc. Charlottetown, Prince Edward Island, Canada.

Dolan, T.T. 1986. Chemotherapy of East Coast fever: Treatment of infection induced by isolates of *Theileria parva* with halofuginone. *Acta Tropica,* **43,** 165-173.

Dolan, T.T., Injairu, R., Gisemba, F., Maina, J.N., Mbadi, G., Mbwiria, S.K., Mulela, G.H.M., Otieno, D.A.O. 1992. A clinical trial of buparvaquone in the treatment of East Coast fever. *Veterinary Record*, **130,** 536-538.

Edmond S.W. Ng., Carpenter, J.R., Goldstein, H., Rasbash, J. 2006. Estimation in generalised linear mixed models with binary outcomes by simulated maximum likelihood. *Statistical Modelling,* **6**, 23–42.

Eriks, I. S., Stiller, D., Palmer, G. H. 1993. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *Journal of Clinical Microbiology,* **31**, 2091-2096.

Fandamu, P., Duchateau, N., Speybroeck, N., Marcotty, T., Mbao, V., Mtambo, J., Mulumba, M., Berkvens, D. 2005. *Theileria parva* seroprevalence in traditionally kept cattle in southern Zambia and El Niño. *International Journal for Parasitology,* **35,** 391-396.

FAO (Food and Agriculture Organization). 1984. *Ticks and Tick-Borne Disease Control. A Practical Field Manual.* Vol. I and II. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (Food and Agriculture Organization). 2003. *FAOSTAT*, Website: http://www.faostat.fao.org/default.htm.

Fellner, W.H. 1986. Robust estimation of variance components. *Technometrics*, **28,** 51-60.

GenStat Release 4.24DE, 2005. GenStat for windows, discovery 2nd edn. Lawes Agricultural Trust, Rothamsted Experimental Station, UK.

Georghiou, G.P. and Lagunes-Tejeda, A. 1991. The occurrence of resistance to pesticides in arthropods. AGPP/MISC/91-1, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Gitau, G.K., McDermott, J.J., Waltner-Toews, D., Lissemore, K.D., Osuma, J.M., Muriuki, D. 1994. Factors influencing calf morbidity and mortality in smallholder dairy farms in Kiambu District, Kenya. *Preventive Veterinary Medicine,* **21,** 167-177.

Gitau, G.K., Perry, B.D., Katende, J.M., McDermott, J.J., Morzaria, S.P., Young, A.S. 1997. The prevalence of tick-borne infections in small-holder farms in Murang'a District, Kenya: a cross-sectional study. *Preventive Veterinary Medicine,* **30**, 95 – 107.

Gitau, G.K., Perry, B.D., McDermott, J.J. 1999. The incidence, calf morbidity and mortality due to *Theileria parva* infections in smallholder dairy farms in Murang'a District, Kenya. *Preventive Veterinary Medicine,* **39,** 65-79.

Gitau, G.K., McDermott, J.J., Katende, J.M., O' Callaghan, C.J.O., Brown, R.N., Perry, B.D. 2000. Differences in the epidemiology of theileriosis on smallholder dairy farms in contrasting agro-ecological and grazing strata of highland Kenya. *Epidemiology and Infection,* **124,** 325-335.

GoK (Government of Kenya), 1994. Government of Kenya Annual Report for the Development of Livestock Production, Ministry of Agriculture and Livestock Development, Nairobi, Kenya.

GoK (Government of Kenya), 1997. Government of Kenya, National Development Plan, 1997–2001, Government Printers, Nairobi, Kenya. pp 254.

GoK (Government of Kenya), 2001. *Mbeere District Development plan, 1997-2001. Office of the vice president and ministry of planning and national development.* Government printer, Nairobi.

GoK (Government of Kenya), 2006. Economic survey 2006. Ministry of Economic planning and National Development, Nairobi, Kenya.

Henderson, C.R. 1963. Selection index and expected genetic advance. National Research Council Publ. No. 892, National Academy of Science, Washington, DC, pp. 141- 163.

Irvin, A.D., Mwamachi, B.M. 1983. Clinical and diagnostic features of East Coast fever (*Theileria parva*) infection in cattle. *Veterinary Record,* **113**, 192-198.

Irvin, A.D. 1987. Characterization of species and strains of *Theileria. Advances in Parasitology,* **26**, 145-197.

Jaetzold, R., Schimidt, H. 1983. Farm management handbook of Kenya. Volume II. Natural conditions and farm management information. Ministry of Agriculture, Kenya.

Jahnke H.E. 1982. *Livestock production systems and livestock development in tropical Africa*. Kieler Wissenschaftsverlag Vauk, Kiel, Germany. 253 pp.

Kadohira, M., McDermott, J.J., Shoukri, M.M., Thorbum, M.A. 1996. Assessing infections at multiple levels of aggregation. *Preventive Veterinary Medicine,* **29,** 161-177.

Kamau, P. 2004. Land Use Impacts and Dynamics (LUCID) Project Working Paper 36. Nairobi, Kenya: International Livestock Research Institute.

Kangara, J.N.N., Kariuki, I.W., Mbaka, J., Muthanga, G.W. 1996. Forage production of different varieties of Kenaf in marginal and low potential areas. *In:* Fungoh, P.O. and Mbadi, G.C.O. *Focus on agricultural research for sustainable development in a changing Economic Environment*. Proceedings of the 5th KARI Scientific Conference, 14th-16th October, 1996. KARI, pp 76-81.

Karanja, A.M., 2003. The Dairy Industry in Kenya: The Post-Liberalization Agenda. Paper presented at a dairy industry stakeholders workshop, 27th August, 2002, Nairobi Kenya.

Kariuki, D.P., Young, A.S., Morzaria, S.P., Lesan, A.C., Mining, S.K., Omwoyo, P., Wafula, J.L.M., Molyneux, D.H. 1995. *Theileria parva* carrier state in naturally infected and artificially immunised cattle. *Tropical Animal Health & Production,* **27**, 15-25.

Katende, J.M., Goddeeris, B.M., Morzaria, S.P., Nkonge, C.G., Musoke, A.J. 1990. Identification of *Theileria mutans* — specific antigen for use in an antibody and antigen detection ELISA. *Parasite Immunology,* **12**, 419-433.

Katende, J.M., Morzaria, S.P., Toye, P., Skilton, R.A., Nene, V., Nkonge, C., Musoke, A.J. 1998. An enzyme-linked immunosorbent assay for the detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. *Parasitology Research,* **84**, 408-416.

Konnai, S., Imamura, S., Nakajima, C., Witola, W.H., Yamada, S., Simuunza, M., Nambota, A., Yasuda, J., Ohashi, K., Onuma, M. 2006. Acquisition and transmission of *Theileria parva* by vector tick, *Rhipicephalus appendiculatus. Acta Tropica.* **99,** 34–41.

Lawrence, J.A., de Vos, A.J., Irvin, A.D. 1988. East Coast fever. In: Thomson, G.R., Tustin, R.C. (Eds.), *Infectious diseases of livestock with special reference to Southern Africa*. Cape Town Oxford University Press, Cape Town. 1605pp.

Lawrence, J.A., Perry, B.D., Williamson, S.M. 2004. East coast fever. *In:* J.A.W. Coetzer and R.C. Tustin (eds). *Infectious Diseases of Livestock.* Vol. I. Oxford University Press Southern Africa, Cape Town.

Lenné, J. M., Thomas, D. 2005. Opportunities for increasing productivity and reducing poverty through crop-livestock integration in sub-Saharan Africa. *In*: D. Harris, J. I. Richards, P. Silverside, A. F. Ward, and J. R. Witcombe, (eds). Pathways out of Poverty. *Aspects of Applied Biology*, **75**, 99-108.

Lessard, P., 'Eplattenier, R.L., Norval, R.A., Kundert, K., Dolan, T.T., Croze, H., Walker, J.B., Irvin, A.D., Perry, B.D. 1990. Geographical information systems for studying the epidemiology of cattle diseases caused by *Theileria parva*. *Veterinary Record*, **126,** 255-262.

Magona, J.W., Walubengo, J., Olaho-Mukani, W., Jonsson, N.N., Welburn, S.C., Eisler, M.C. 2008. Clinical features associated with seroconversion to *Anaplasma marginale, Babesia bigemina* and *Theileria parva* infections in African cattle under natural tick challenge. *Veterinary Parasitology,* **155,** 273–280.

Maloo, S.H., Thorpe, W., Ngumi, P.N., Perry, B.D. 1994. Systemic epidemiological studies to identify and resolve health constraints to smallholder dairy production in coastal lowland Kenya. *Kenya Veterinarian,* **18,** 359-361.

Maloo, S.H., Thorpe, W., Kioo, G., Ngumi, P., Rowlands, G.J., Perry, B.D., 2001a. Seroprevalences of vector-transmitted infections of smallholder dairy cattle in coastal Kenya. *Preventive Veterinary Medicine,* **52**, 1-16.

Maloo, S.H., Rowlands, G.J., Thorpe, W., Gettinby, G., Perry, B.D., 2001b. A longitudinal study of disease incidence and case-fatality on smallholder dairy farms in coastal Kenya. *Preventive Veterinary Medicine*, **52**, 17-29.

Marcotty, T., Brandt, J., Billiouw, M., Chaka, G., Losson, B., Berkvens, D. 2002. Immunisation against *Theileria parva* in eastern Zambia: influence of maternal antibodies and demonstration of the carrier status. *Veterinary Parasitology,* **110**, 45-56.

Martin S.W., Meek, A.H., Willeberg, P. 1987. *Veterinary Epidemiology: Principles and methods*. Iowa State University Press.

Matete, G.O., Kanyari, P.W.N., Ngatia, T.A., Kariuki, D.P., Ndung'u, S.G. 2004. Characterization of *Theileria parva* isolates from Kiambu District, Kenya. *Veterinary Parasitology,* **121,** 247-253.

Mbwambo, H.A., Sudi, F.F., Mkonyi, P.A., Mfinanga, J.M., Mella, E.S., Ngovi, C.J. 2002. Comparative studies of the efficacy of parvaquone and parvaquone-plus-frusemide in the treatment of *Theileria parva* infection (East Coast fever) in cattle *Veterinary Parasitology*, **108**, 195-205.

Mbwambo, H.A., Magwisha, H.B., Mfinanga, J.M., 2006. Evaluation of buparvaquone (BUTA-Kel^{TM} KELA, Belgium) as a treatment of East coast fever in cattle in the peri-urban of Dar es Salaam city Tanzania. *Veterinary Parasitology,* **139,** 67-73.

McDermott, J., Schukken, Y., 1994. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. *Preventive Veterinary Medicine,* **18**, 155–176.

McDermott, J.J., Schukken, Y.H., Shoukri, M.M., 1994. Study design and analytical methods for data collected from clusters of animals. *Preventive Veterinary Medicine,* **18,** 175-192.

McDermott, J.J., Randolph, T.F., Staal, S.J. 1999. The economics of optimal health and productivity in smallholder livestock systems in developing countries. *Rev. Sci. Tech. Off. Int. Epizoot.* **18**, 399–424.

McHardy, N., Haigh, A.J. and Dolan, T.T. 1976. Chemotherapy of *Theileria parva* infection. *Nature,* **261,** 698-699.

McHardy, N. and Morgan, D.W.T. 1985. Treatment of *Theileria annulata* infection in calves with parvaquone. *Research in Veterinary Science*, **39,** 1-4.

McHardy, N. 1989. Buparvaquone–the new anti-theilerial: A review of its efficacy and safety. In: T.T. Dolan (ed.). *Theileriosis in Eastern, Central and Southern Africa*: Proceedings of a workshop on East Coast Fever Immunisation held in Lilongwe, Malawi, 20-22 September, 1988. pp. 155-165.

Ministry of Environmental Conservation (MEC). 1999. *Sessional paper No. 6 on environment and development.* Nairobi, Kenya.

Ministry of Livestock and Fisheries Development (MoLD). 2003. Mbeere District Veterinary Office report, Siakago, Mbeere.

Minjauw, B., McLeod, A. 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

Moll, G., Lohding, A., Young, A.S. 1984. Epidemiology of theileriosis in calves in the Trans-Mara Division, Kenya: Husbandry and disease background and preliminary observation on theileriosis in calves. *Preventive Veterinary Medicine,* **2,** 801-831.

Moll, G., Lohding, A., Young, A.S., Leitch, B.L. 1986. Epidemiology of theileriosis in calves in an endemic area of Kenya. *Veterinary Parasitology,* **19,** 255–273.

Mukhebi, A.W. 1992. The economic impact of theileriosis and its control in Africa. *In:* Norval, R.A.I., Perry, B.D., Young, A.S. (Eds). The Epidemiology of Theileriosis in Africa. Academic Press, Harcourt Brace Jovanovich Publishers, pp 380-402.

Mulei, C.M., Rege, J.E.O., Kiptoon, J.C. 1989. Prevalence and seasonal occurrence of East Coast fever in Kiambu District Kenya. *Bulletin of Animal Health and Production in Africa*, **37,** 35-39.

Muraguri, G.R., Kiara, H.K., McHardy, N. 1999. Treatment of East coast fever: a comparison of parvaquone and buparvaquone. *Veterinary Parasitology,* **87,** 25-37.

Muraguri, G.R., McLeod, A., McDermott, J.J., Taylor, N. 2005. The incidence of calf morbidity and mortality due to vector-borne infections in smallholder dairy farms in Kwale District, Kenya. *Veterinary Parasitology*, **130,** 305–315.

Muraguri, G., Ngumi, P., Wesonga, D., Ndungu, S., Wanjohi, J., Bang, K., Fox, A., Dunne, J., McHardy, N. 2006. Clinical efficacy and plasma concentrations of two

formulations of buparvaquone in cattle infected with East Coast fever (*Theileria parva* infection). *Research in Veterinary Science*, **81**, 119 – 126.

Ndamukong, K.J.N. 1993. The seasonal variation of tick infestation of sheep and goats under two management systems at Mankon, Cameroon. *Bulletin of Animal Health and Production in Africa,* **41,** 277-283.

Ndegwa, R. 2005. Livestock vaccine development and the case of ECF vaccine research. Presentation given at a workshop on "Public-Private Partnerships in Agricultural Research: Finding Common Ground for Common Good," convened by the International Food Policy Research Institute, the International Maize and Wheat Improvement Center (CIMMYT), and the Kenya Institute of Agricultural Research (KARI), Nairobi, Kenya, June 21, 2005.

Ndung'u, S.G., Brown, C.G.D., Dolan T.T. 2005. *In vivo* comparison of susceptibility between *Bos indicus* and *Bos taurus* cattle types to *Theileria parva* infection. *Onderstepoort Journal of Veterinary Research.* **72,** 13–22.

NEPAD (The New Partnership for Africa's Development). 2005. Integrating livestock, forestry and fisheries sub-sector into the CAADP. Companion document to the Comprehensive Africa Agriculture Development Programme, NEPAD, Nairobi, Kenya.

Ngumi, P.N., Young, A.S., Lampard, D., Mining, S.K., Ndungu, S.G., Lesan, A.C., Williamson, S.M., Linyonyi, A., Kariuki, D.P. 1992. Further evaluation of the use of buparvaquone in the infection and treatment method of immunizing cattle against *Theileria parva* derived from African buffalo (*Syncerus caffer*). *Veterinary Parasitology,* **43**, 15-24.

Norval, R.A.I. Lightfoot, C.J. 1982. Tick problems in wildlife in Zimbabwe. Factors influencing the occurrence and abundance of *Rhipicephalus appendiculatus*. Zimbabwe. *Veterinary Journal*, **13,** 11-20.

Norval, R.A.I., Perry, B.D., Young, A.S., 1992. The Epidemiology of Theileriosis in Africa. Academic Press. London, UK.

O'Callaghan, C.J. 1998. A study of the epidemiology of theileriosis on smallholder dairy farms in Kiambu District, Kenya. PhD. Thesis, University of Guelph, Canada. pp. 145.

Ochanda, H., 1994. *Factors affecting the population dynamics of Theileria parva in rhipicephalid ticks*. PhD Thesis, University of Warwick.

Ochanda, H., Young, A.S., Medley, G.F., Perry, B.D., 1998. Vector competence of 7 rhipicephalid tick stocks in transmitting 2 *Theileria parva* parasite stocks from Kenya and Zimbabwe. *Parasitology* **116**, 539–545.

Odongo, D.O., Oura, C.A.L., Spooner, P.R., Kiara, H., Mburu, D., Hanotte, O.H., Bishop, R.P. 2006. Linkage disequilibrium between alleles at highly polymorphic mini- and micro-satellite loci of *Theileria parva* isolated from cattle in three regions of Kenya. International Journal for Parasitology, 36, 937–946.

Ogden, N. H., Gwakisa, P., Swai, E., French, N.P., Fitzpatrick, J., Kambarage, D., Bryant, M. 2003. Evaluation of PCR to detect *Theileria parva* in field-collected tick and bovine samples in Tanzania. *Veterinary Parasitology,* **112**, 177-183.

Okuthe, O.S. Buyu, G.E. 2006. Prevalence and incidence of tick-borne diseases in smallholder farming systems in the western-Kenya highlands. *Veterinary Parasitology,* **141,** 307-312.

Olwoch, J.M., Reyers, B., Engelbrecht, F.A., Erasmus, B.F.N. 2008. Climate change and the tick-borne disease, Theileriosis (East Coast fever), in sub-Saharan Africa. *Journal of Arid Environments,* **72**, 108–120.

Onduru, D.D., Gachumbi, L., Maina, F., Muchena, F.N., de Jager, A. 2002. Sustaining agricultural production in the semi-arid areas of eastern Kenya: A case study of Mbeere District. INMASP Report No. Ke-03: ETC-EA, KARI (NARL) and LEI-DLO.

Oura, C.A.L., Asiimwe, B.B.,Weir,W., Lubega, G.W., Tait, A., 2005. Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. *Molecular and Biochemical Parasitology*. **140**, 229–239.

Paling, R.W., Mpangala, C., Luttikhuizen, B., Sibomana, G. 1991. Exposure of Ankole and cross bred cattle to theileriosis in Rwanda. *Tropical Animal Health and Production,* **4**, 203 – 214.

Peeler, E.J., Omore, A. 1997. Manual of Livestock Production systems in Kenya. 2nd Edition.

Perry, B.D., Deem, S.L., Medley, G.F., Morzaria, S.P., Young, A.S. 1992. The ecology of *Theileria parva* infections of cattle and the development of endemic stability. *In*: U.G. Munderloh and T.J. Kurtti (eds.): The Host-Vector Interface: An Agenda for Research. Proceedings of the First International Conference on Tick-borne Pathogens. University of Minnesota, College of Agriculture, Department of Entomology and Minnesota Extension Services, Saint Paul, Minnesota, USA.

Perry, B.D., Young, A.S. 1995. The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: The case of theileriosis. *Preventive Veterinary Medicine,* **25,** 107-120.

Radley, D.E., 1981. Infection and treatment method of immunisation against Theileriosis. In: Irvin, A.D., Cunningham, M.P., Young, A.S. (Eds.), Advance in the Control of Theileriosis. Martinus Nijhoff Publishers, The Hague, pp. 227–237.

Radley, D.E., Brown, C.G.D., Cunningham, M.P., Kimber, C.D., Musisi, F.L., Payne, R.C., Pumell, R.B., Stagg, S.M., Young, A.S., 1975. East Coast fever 3. Chemoprophilactic immunisation of cattle using oxytetracycline and a combination of theilerial strains. *Veterinary Parasitology.* **1**, 35–45.

Rothman, K.J., 1990. A sobering start for the cluster buster's conference. *American Journal of Epidemiology,* **132,** s6-s13.

Rubaire-Akiiki, C.M., Okello-Onen, J., Musunga, D., Kabagambe, E.K., Vaarst, M., Okello, D., Opolot, C., Bisagaya, A., Okori, C., Bisagati , C., Ongyera, S., Mwayi, M.T. 2006. Effect of agro-ecological zone and grazing system on incidence of East Coast Fever in calves in Mbale and Sironko Districts of Eastern Uganda. *Preventive Veterinary Medicine*, **75**, 251–266.

Salih, D.A., El Hussein, A.M., Seitzer, U., Ahmed, J.S. 2007. Epidemiological studies on tick-borne diseases of cattle in Central Equatoria State, Southern Sudan. *Parasitology Research*, **101,** 1035–1044.

Schall, R. 1991. Estimation in generalized linear models with random effects. *Biometrika*, **78,** 719-727.

Schukken, Y.H., Grohn, Y.T., McDermott, B., McDermott, J.J. 2003. Analysis of correlated observations: background, examples and solutions. *Preventive Veterinary Medicine*, **59**, 223-240.

Seifert, H.S.H. 1996. Tropical animal health. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Short, N. J., Norval, R. A. I. 1981. The seasonal activity of *Rhipicephalus appendiculatus* Neumann, 1901 (Acarina: Ixodidae) in the highveld of Zimbabwe Rhodesia. *Journal of Parasitology*, **67,** 77-84.

Simpson, J.A., Weiner, E.S. (eds). 2000. *The Oxford English Dictionary. Second Edition.* Oxford University Press, Oxford, UK.

Skilton, R.A., Bishop, R.P., Katende, J.M., Mwaura, S., Morzaria, S.P. 2002. The persistence of *Theileria parva* infection in cattle immunized using two stocks which differ in their ability to induce a carrier state: analysis using a novel blood spot PCR assay. *Parasitology,* **124,** 265-276.

Stendel, W., Hamel, H.D. 1990. Flumethrin pour-on and cyfluthrin pour-on for ectoparasite control in cattle and sheep. *Medicamentum, Germed, Dresden*, 10-13.

Swai, E.S., French, N.P., Karimuribo, E.D.,. Fitzpatrick, J.L., Bryant, M.J., Brown, P.E., Ogden, N.H. 2005a. Spatial and management factors associated with exposure of smallholder dairy cattle in Tanzania to tick-borne pathogens. *International Journal for Parasitology*, **35,** 1085–1096.

Swai, E.S., French, N.P., Beauchamp, G., Fitzpatrick, J.L., Bryant, M.J., Kambarage, D., Ogden, N.H. 2005b. A longitudinal study of sero-conversion to tick-borne pathogens in smallholder dairy young stock in Tanzania. *Veterinary Parasitology*, **131**, 129–137.

Swai, E.S., Karimuribo, E.D., Rugaimukamu, E.A., Kambarage, D.M. 2006. Factors influencing the distribution of questing ticks and the prevalence estimation of *T. parva* infection in brown ear ticks in the Tanga region, Tanzania. *Journal of Vector Ecology,* **31**, 224-228.

Swai, E.S., Karimuribo, E.D., Kambarage, D.M., Moshy, W.E., Mbise, A.N., 2007. A comparison of seroprevalence and risk factors for *Theileria parva* and *T. mutans* in smallholder dairy cattle in the Tanga and Iringa regions of Tanzania. *The Veterinary Journal,* **174**, 390–396.

Tatchell, R.J. & Easton, E. 1986. Tick (Acari: Ixodidae) ecological studies in Tanzania. *Bulletin of Entomological Research,* **76**, 229–246.

Thaiya, A.G., Chege, J.N., Muriuki, S.K., Wekesa L.S. 1993. Efficacy of buparvaquone in the treatment of ECF cases in Kabete area of Kiambu District of Kenya. Preliminary results. *Bulletin of Animal Health and Production in Africa*, **41**, 333-335.

Thornton, P. K., Krusska, R. L., Henninger, N., Kristjanson, P., Reid, R. S., Atieno, A. N., Ndegwa, T. 2002. Mapping Poverty and Livestock in the Developing World. ILRI, Nairobi, Kenya.

Thorpe, W., Chabari, F., Maloo, S.H., Muinga, R.W., Mukhebi, A., Mullins, G., Muriethi, J., Mussukuya, E., Nyambaka, R. 1993. Smallholder dairy cattle production in coastal Kenya: Resource base assessment and constraint identification. Proceedings of the meeting on animal production in developing countries, Ashford, UK 1991. British Society of Animal Production, Penicuik, Scotland, UK, pp. 167–168.

Toye, P.G., Goddeeris, B.M., Iams, K.P., Musoke, A.J. Morrison, W.I. 1991. Characterisation of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva. Parasite Immunology,* **13,** 49-62.

Toye, P.G., Nyanjui, J., Goddeeris, B.M., Musoke, A.M. 1996. Identification of neutralization and diagnostic epitopes on PIM, the polymorphic immunodominant molecule of *Theileria parva*. *Infection and Immunity*, **64**, 1832-1838.

Ueti, M.W., Palmer, G. H., Kappmeyer, L.S., Statdfield, M., Scoles, G. A. Knowles, D. P. 2005. Ability of the vector tick *Boophilus microplus* to acquire and transmit *Babesia equi* following feeding on chronically infected horses with low-level parasitaemia. *Journal of Clinical Microbiology,* **43**, 3755-3759.

Uilenberg, G., 1999. Immunization against diseases caused by *Theileria parva*: a Review. *Tropical Medicine and International Health*, **4**, pp a12–a20.

Velthof, G.L., Bannink, A., Oenema, O., van der Meer Autor(s), H.G. 2000. Relationships between animal nutrition and manure quality. A literature review on C, N, P, and S compounds. Wageningen, Alterra, Green World Research. Alterra-Document 1. 44 blz. 8 fif.; 4 tab.; 90 ref.

Waithaka, M.M., Herrero, M., Thornton, P.K., Shepherd, K.D., Ndiwa, N., 2004. Characterizing farming systems in Vihiga in western and Kilifi in coastal Kenya. Project Paper, ILRI, Nairobi.

Wanjohi, J.M., Muraguri, G.R., Rumberia, R.M., Kariuki, D.P. and McHardy, N. 1995. Chemotherapy of East Coast fever: Buparvaquone treatment of field cases occurring in Kikuyu and Limuru Divisions of Kiambu District, Kenya. *East African Agricultural and Forestry Journal*, **60**: 131-135.

Wilkie, G.M., Brown, C.G.D., Kirvar, E., Thomas, M., Williamson, S.M., Bell-Sakyi, L.J., Sparagano, O. 1998. Chemoprophylaxis of *Theileria annulata* and Theileria parva infections of calves with buparvaquone. *Veterinary Parasitology*, **78,** 1-12.

Winrock International, 1992. Assessment of Animal Agriculture in sub-Saharan Africa. Winrock International Institute for Agricultural Development. Morrilton, Arkansas, USA. Pp. 1 -27.

World Bank, 2003. *World Development Indicators 2003* (CD-ROM), World Bank, Washington, DC.

Wright, P.F., Nilsson, E.A.M., Vanrooij, M., Lelenta Jeggo, M.H. 1993. Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. *Rev. Sci. Tech. Off. Int. Epizootique,* **12,** 435-450.

Yeoman, G.H. 1966. Field vector studies of epizootic East coast fever. I. A quantitative relationship between *R. appendiculatus* and the epizooticity of East coast fever. *Bulletin of Epizootic Diseases in Africa.* **14,** 5-27.

APPENDIX 1:

Multistage cattle sample and target population structure used in cross-sectional study of the seroprevalence of *T. parva* infection in Mbeere District, Kenya.

Division	Sub-location population	Herd no.	Herd sample	Herd population	AEZ
Siakago	**Kerie**	1	3	7	LM 4
,,	,,	2	5	11	,,
,,	,,	3	4	8	,,
,,	,,	4	6	23	,,
,,	,,	5	4	9	,,
,,	,,	6	4	11	,,
,,	,,	7	6	12	,,
,,	,,	8	11	24	,,
,,	,,	9	3	3	,,
,,	,,	10	4	8	,,
Total (Kerie sub-location)			**50**	**116**	
,,	**Mutitu**	11	7	14	L5
,,	,,	12	3	7	,,
,,	,,	13	5	10	,,
,,	,,	14	3	3	,,
,,	,,	15	9	18	,,
,,	,,	16	4	10	,,
,,	,,	17	7	14	,,
,,	,,	18	5	10	,,
,,	,,	19	7	14	,,
,,	,,	20	2	2	,,
Total (Mutitu sub-location)			**52**	**102**	
Total (Siakago Division)			**102**	**218**	
Gachoka	**Njigo**	21	3	3	LM 4
,,	,,	22	4	8	,,
,,	,,	23	12	24	,,
,,	,,	24	7	9	,,
,,	,,	25	5	11	,,
,,	,,	26	10	20	,,
,,	,,	27	4	4	,,
,,	,,	28	15	30	,,
,,	,,	29	2	2	,,
,,	,,	30	10	20	,,
Total (Njigo sub-location)			**72**	**131**	
,,	**Kombo Munyiri**	31	2	2	L5
,,	,,	32	6	6	,,

Division	Sub-location population	Herd no.	Herd sample	Herd population	AEZ
Gachoka	**Kombo Munyiri**	33	6	6	L5
,,	,,	34	3	4	,,
,,	,,	35	5	5	,,
,,	,,	36	9	9	,,
,,	,,	37	4	4	,,
,,	,,	38	5	5	,,
,,	,	39	4	4	,
,,	,,	40	17	30	,,
Total (Kombo Munyiri sub-location)			**61**	**75**	
Total (Gachoka Division)			**133**	**206**	
Evurore	**Ishiara**	41	4	4	LM4
,,	,,	42	11	11	,,
,,	,,	43	8	8	,,
,,	,,	44	4	4	,,
,,	,,	45	6	6	,,
,,	,,	46	5	5	,,
,,	,,	47	5	5	,,
,,	,,	48	5	5	,,
,,	,,	49	3	3	,
,,	,,	50	3	3	,,
Total (Ishiara sub-location)			**54**	**54**	
,,	**Kamarandi**	51	6	6	L5
,,	,,	52	6	9	,,
,,	,,	53	5	5	,,
,,	,,	54	3	3	,,
,,	,,	55	3	3	,,
,,	,,	56	5	5	,,
,,	,,	57	2	3	,,
,,	,,	58	8	8	,,
,,	,,	59	6	6	,,
,,	,,	60	5	5	,,
Total (Kamarandi sub-location)			**49**	**53**	
Total (Evurore Division)			**103**	**107**	
Mwea	**Wachoro**	61	2	2	LM4
,,	,,	62	5	8	,,
,,	,,	63	5	6	,,
,,	,,	64	3	4	,,
,,	,,	65	4	4	,,
,,	,,	66	6	12	,,
Mwea	**Wachoro**	67	2	3	LM4
,,	,,	68	1	3	,,
,,	,,	69	3	3	,,

Division	Sub-location population	Herd no.	Herd sample	Herd population	AEZ
,,	,,	70	11	20	,,
Total (Wachoro sub-location)			**42**	**65**	
,,	**Karaba**	71	3	3	LM4
,,	,,	72	7	13	,,
,,	,,	73	7	7	,,
,,	,,	74	9	9	,,
,,	,,	75	7	14	,,
,,	,,	76	4	4	,,
,,	,,	77	6	6	,,
,,	,,	78	2	2	,,
,,	,,	79	7	7	,,
,,	,,	80	8	16	,,
Total (Karaba sub-location)			**60**	**81**	
Total (Mwea Division)			**102**	**146**	
Grand Total			**440**	**677**	

AEZ: Agro-ecological zone; LM4: Lowland midlands 4; L5: Lowlands 5

APPENDIX 2

Mbeere District cattle population census (2003) in relation to the sampled cattle in the *T. parva* infection seroprevalence cross-sectional study of March 2007 ((Ministry of Livestock and Fisheries Development (MoLD, 2003)

Breed	**Evurore**	**Mwea**	**Gachoka**	**Siakago**
Dairy cattle	207 (2.5%)	16 (0.22%)	91(0.9%)	480 (5.2%)
Local breeds	8,237 (97.5%)	7,315 (99.78%)	10,063 (99.1%)	8795 (94.8%)
Totals	8,444	7,331	10,154	9,275
Animals sampled (% of population)	103 **(1.2%)**	102 **(1.4%)**	133 **(1.3%)**	102 **(1.1%)**

APPENDIX 3:

Cross-sectional study questionnaire on "Estimating the seroprevalence of *T. parva* infection and determination of associated risk factors in the cattle population of Mbeere District, Kenya".

Section A: Introduction, Location & family data details

Name of interviewer			
DISTRICT			
DIVISION			
LOCATION			
SUB-LOCATION			
VILLAGE			
GPS reading	S	E	Alt.
Name of Household head (head of the family) and gender			
Name of the respondent and gender			
Age of respondent			
Family size			
Level of education attained by household head			
Level of education attained by respondent			

Section B: Farming Activities and gender roles

1. Would you characterize your farming activities as?
   a) Mainly livestock b) Mainly crops c) Both crops and livestock
2. Which farm enterprise is the most important for the subsistence of the household?
   a) Mainly livestock b) Mainly crops c) Both crops and livestock
3. Which farm enterprise is the most important for household cash income?
   a) Mainly livestock b) Mainly crops c) Both crops and livestock
4. What is the land legislation in the area (land tenure)?
   a) Privately owned (b) Group ownership (c) Government owned
   (d) others (specify)

5. What are the cattle breeds on the farm? Indicate numbers in the box.

Cattle	Zebu		Zebu/exotic crosses		Exotic	
	Males	Females	Males	Females	Males	Females
Calves						
Young stock						
Adults						

6. Who owns cattle with regard to gender? ……..
7. Who is mainly concerned with management of cattle on a day to day basis with regard to gender? ……
8. Who is mainly concerned with disease diagnosis (knowing when an animal is sick) with regard to gender? …….
9. Who is mainly concerned with decision making on whether the animal will be treated or not with regard to gender? ……
10. What is the main source of treatment charges in case treatment is sought? …….
11. Fill in the following table appropriately concerning other livestock in the household:

Livestock category	Ownership with regard to gender	Management and decision making with regard to gender
Indigenous sheep and goats		
Dairy goats		
Donkeys		
Pigs		
Rabbits		
Bees		
Dogs		
Others		

12. Mode of cattle husbandry?
(a) Free grazing (b) Tethering
(c) Stall feeding (d) Paddock/improved grazing (d) others (specify)
13. Do sheep and goats accompany cattle to grazing pastures? Yes…… No……
14. Are sheep and goats kept together with cattle at night? Yes…… No……
15. Do donkeys accompany cattle to grazing pastures? Yes…… No……
16. What is the source of labour in the livestock grazing?
a) Family labour (specify……..)
(b) Hired labour (c) Family and hired labour d) others (specify)
17. After how long after birth do calves suckle colostrums?

18. For how long are calves allowed to suckle milk from their dams?
19. What crops do you grow on the farm?
20. Are cattle offered crop residues? Yes…….. No……..
21. If yes, which ones?
22. Reasons for keeping livestock:

Livestock	**Livestock use**							
	Milk for consumption	Milk for sale	Meat for domestic consumption	Sale of animal	Traction	Manure	Eggs	Others
Zebu/Cross								
Exotic cattle								
Indigenous sheep and goats								
Dairy goat								
Poultry								
Donkeys								
Pigs								
Rabbits								
Bees								
Others								

23. What is average milk production/cow/day?.....
24. Milk utilisation and disposal
    a) Household consumption
    (b) Sold locally
    c) Sold to dairy co-operatives
    (d) Sold to middle-men
    (e) Others
25. What is the price of milk.....KShs.
26. Animals brought in the last 12 months…….

Section c: Constraints to production and other risk factors interview

27. Constraints to production scored where 1 is very important, serially, and 0 is completely unimportant). Constraints not listed can be added.

Constraints	**Score**
Diseases	
Feed availability	
Water availability	
Low genetic potential	
Poor fertility, e.g. late puberty, long calving interval, poor heat detection, repeat breeder syndrome	
Labour	
Marketing of livestock and livestock products	
Lack of access to Veterinary services	
Lack of AI	
Others (specify)	
Others (specify)	
Others (specify)	

28. List the most common 5 diseases or disease syndromes that affect cattle in your farm:

29. What are the drugs used in treatment of these diseases?

30. What veterinary drugs do you commonly use on your stock?

……. (Observe empty packages e.g. bottles, packets, sachets)

31. What veterinary personnel are used on this farm?

a) Veterinary officers (VO)

(b) Animal health assistants (AHA)

(c) Quacks using veterinary drugs

(d) Quacks using traditional herbs

(d) None

32. If available/used, what is the distance to the nearest personell? ..... Km.

33. What tick control practices do you use for the following classes of livestock? (Please tick)

Category	Zebu/cross cattle	Exotic cattle	Indigenous sheep and goats	Dairy goat
Acaricides				
Hand picking				
Traditional				
Others				

33. If acaricide is used indicate the method of application for each class of livestock? (Please tick)

Class of Livestock	**Method of Application**				
	Dip	Hand Spray	Hand-wash	Pour-on	Other
Zebu/cross cattle					
Exotic cattle					
Indigenous sheep and goats					
Dairy goats					

34. Have you used acaricides against ticks for each age group in the following classes of livestock, in the last two weeks? (Please tick)

	Zebu/cross cattle	Grade cattle	Indigenous sheep and goats	Dairy goats
Calves				
Young stock				
Adult				

35. At what age do you first treat calves with acaricides? …… (Months)

36. On average, how many times do you apply acaricides on calves up to when they are about 6 months of age? (Please tick)

a) Less or 5 times

b) Greater than 5 times

37. Which of these have you seen on calves, adult cattle and in sheep and goats in your farm?

Tick	Seen on calves (Please tick)	Seen on cattle (Please tick)	Seen on sheep and goats (Please tick)
Brown ear ticks			
Blue ticks			
Bont ticks			

38. Have you experienced clinical cases or syndrome of the following tick borne diseases in your cattle in the last one year? (Interviewer to give a brief overview of clinical syndrome of each disease or local name) (Please tick)

Disease	Occurrence		Species affected	Age category	Mode of treatment	Case recovered or died?
	Yes	No				
ECF Generalized lymphadenopathy, fever, coughing, respiratory distress, and nasal discharges						
Heartwater Fever and nervous signs including head pressing, circling, aimless walking, leg pedaling and convulsions then death; similar signs also seen in sheep and goats						

39. If yes: When did the disease occur? (Please tick)

Disease	**Occurrence**		
	Last one year	Last 3 years	> 3 years
East Coast fever			
Heartwater			

39. Any calf mortality within the last one year?.....
40. If yes what were the signs exhibited?.....
……..
……