A STUDY OF FACTORS INFLUENCING ENDEMIC STABILITY AND INSTABILITY TO THEILERIOSIS AND BABESIOSIS ON DAIRY PRODUCTION IN MURANG'A DISTRICT, KENYA

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A thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

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

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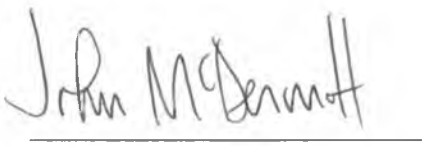

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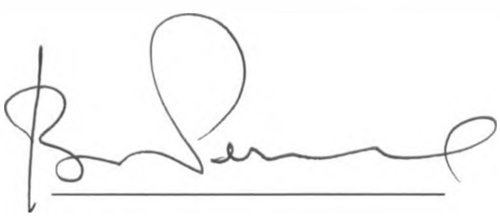

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DEDICATION

To

God be the Glory Great things He has Done

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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ABSTRACT

This thesis describes a study of the epidemiology of theileriosis on smallholder dairy farms in Murang'a District situated in central highlands of Kenya. The main objectives were: 1) to characterise different areas within the district as to their risks for tick-borne diseases (TBDs), in particular infection due to *Theileria parva*, and to classify the potential endemically stable and unstable areas. 2) Estimate health and productivity parameters such as infection rates, morbidity and mortality rates, dynamics of infection and growth patterns in contrasting grazing systems and agro-ecological zones (AEZs) in Murang'a District, Kenya. 3) To study the potential risk factors associated with *T. parva* infections in cattle in smallholder dairy farms in Murang'a District of Kenya.

The study was conducted in two phases. The first phase was a cross-sectional study to estimate the prevalence to tick-borne infections and was conducted between March and June 1994. The cross-sectional serological study was carried out on 750 smallholder dairy farms in Murang'a District, selected in a stratified random sampling method. One hundred and fifty farms were studied from three administrative sublocations in each of the five AEZs. These five AEZS were: Lower Highlands 1 (LH 1), (tea-dairy; altitude, 1730-2130m; mean annual temperature, 15-18^{0}C; annual rainfall, 1700-2400mm); Upper Midlands 1 (UM 1), (coffee-tea; altitude, 1670-1800m; mean annual temperature, 18.0-18.8^{0}C; annual rainfall, 1700-1900mm); UM 2 (main coffee; altitude, 1500-1670m; mean annual temperature, 18.8-19.7^{0}C; annual rainfall, 1300-1620mm); UM 3 (marginal coffee; altitude, 1340-1500m; mean annual temperature, 19.7-20.7^{0}C; annual rainfall, 900-1350mm); and UM 4 (sunflower-maize; altitude, 1340-1520m; mean annual temperature, 19.5-20.7^{0}C; annual rainfall, 850-950mm).

The farms had a total of 362 calves (148 males and 214 females) aged between 6-18 months. Prevalence of serum antibodies to three tick-borne parasites, that is *T. parva, T. mutans* and *Babesia bigemina,* were determined using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Antibody prevalence values significantly differed across the AEZs. The ranges of means for the antibody prevalence were: *T. parva* (18-72%), *T. mutans* (1.5-28%) and *B. bigemina* (12-49%). There were significant differences in serum antibody prevalence for the different TBD parasites across the fives AEZs ($p<0.05$).

The factors that were significantly associated with variations in antibody prevalence are described below. For *T. parva,* Zebu breeds and their crosses with Taurine breeds were significantly ($p<0.05$) associated with higher prevalences than Taurine breeds. Higher prevalence was significantly ($p<0.05$) associated with calves on open grazing than those on zero-grazing. For *T. mutans*, Zebu calves and their crosses with Taurine breeds had significantly ($p<0.05$) higher antibody prevalence than Taurine breeds. For *B. bigemina,* LH 1 and UM 1 were significantly ($p<0.05$) associated with higher prevalence than the other three AEZs. Higher prevalences were also significantly ($p<0.05$) associated with calves grazed freely, when compared to those partially or completely confined. Significantly ($p<0.05$) higher prevalences were seen in older calves and male calves than in younger and female calves. Calves that reportedly had never received any acaricide treatment had significantly ($p<0.05$) higher prevalence than those that had received acaricide treatment, regardless of the method used. The above results served as indicators of possible existence of endemic stability in some AEZs for some parasites.

The second phase was a longitudinal (prospective) study to estimate incidences of *T. parva* infections, such as sero-conversion to *T. parva* and calf morbidity, as well as calf mortality and growth rates associated with different AEZs and grazing systems. The

longitudinal study was conducted over a period of one-and-a-half years between March 1995 and August 1996 in three agroecological zones (AEZs) namely, Upper Midlands 1, 2 and 4, (UM 1, UM 2 and UM 4). The three AEZs were selected based on the serum antibody prevalence results from the cross-sectional study shown below. Two of the AEZs studied were identified as having highest (UM 4 – above 70%) and lowest risk (UM 1 – below 40%) of *T. parva* infections and for morbidity and mortality to ECF while the third (UM 2) was an intermediate zone. Study farms were also stratified by grazing pattern (restricted versus unrestricted) for the high and low risk areas. A total of 188 smallholder dairy farms were selected purposively from which a total of 225 female calves were recruited (also purposively) and were visited within the first two weeks of life and thereafter at biweekly intervals up to the age of 6 months. The mean number of cattle in these smallholder farms was 2.6. The 225 female calves were distributed as follows: 76 in UM 1, 50 in UM 2 and 99 in UM 4. In UM 1, 35 and 41 calves were from farms which practised restricted and unrestricted grazing respectively. In UM 4, 51 and 48 calves were from farms which practised restricted and unrestricted grazing respectively. Both exotic and indigenous breeds of cattle and their crosses were present, with the former predominating. All farms in UM 2 (the intermediate zone) practised zero (restricted) grazing.

The crude ECF-morbidity rates were: 20.7%, and 33.0%, while ECF-fatality rates were: 8.3%, and 13.2% in UM 1 and UM 4 (low and high risk) respectively. When further stratified by grazing management, zero-grazing farms had lower ECF morbidity rates than open-grazing farms, 2.9% versus 18.4% in UM 1 and 11.1% versus 24.7% in open and zero-grazing farms in UM 4 and ECF-mortality, 0% versus 8.3% and 2.3% versus 11.2% in the UM 1 and UM 4 respectively.

The risk of exposure to *T. parva*, estimated by determining the incidence of sero-conversion was not significantly different across the three AEZs; (35.7%, 39.5 % and 45.4% in UM 1, UM 2 and UM 4 respectively ($p>0.05$). The factors significantly associated with sero-conversion to *T. parva* in a multi-variate Glimmix model are briefly described below. Zebu breeds and their crosses with exotic breeds were associated with lower risks of sero-conversion to *T. parva* than the exotic breeds ($p<0.05$). Calves whose dam antibodies were high and positive were significantly associated with higher risk of sero-conversion to *T. parva* as were older calves than the young ones ($p<0.05$). Calves that were reported to have been washed with acaricides to control ticks were associated with higher risks of sero-conversion while calf morbidity was significantly ($p<0.05$) associated with higher risk of seroconversion. Calves that acquired ECF were associated with significantly ($p<0.05$) lower sero-conversion risk rates that those which did not sero-convert. The presence of nymphs (total) and nymph (engorged) *R. appendiculatus* on calves was associated with significantly lower and higher risk of sero-conversion to *T. parva* respectively ($p<0.05$).

Calf mean daily weight gains were mainly associated with calf level factors as described below from a multi-variate mixed model. Older calves were associated with lower mean daily weight gains than young calves, while calves with higher *T. parva* antibody titres were associated with lower mean daily weight gains than those with lower *T. parva* antibody titres ($p<0.05$). Calves that received concentrate feed supplements were associated with higher mean daily weight gains than those not receiving, while Zebu and their crosses were associated with lower mean weight gains than the Taurine breeds ($p<0.05$). Calves that experienced any morbidity were associated with lower mean daily weight gains at the time of morbidity than non-affected calves ($p<0.05$). Calves that

experienced ECF-morbidity were associated with higher mean daily weight gains at the time of morbidity than non-affected calves ($p<0.05$). The presence of female (non-engorged) *R. appendiculatus* on calves was associated with lower mean daily weight gains in calves ($p<0.05$).

CHAPTER 1

INTRODUCTION

In Kenya, the vast majority of the dairy farms are owned and run by smallholder farmers. Smallholder dairy farms are those that practice mixed farming. In addition to keeping dairy cattle, food and cash crops are grown for local consumption and for sale. Between one and ten milking cows are kept in these farms that range in size from two to ten acres. The dairy industry has grown significantly through the upgrading of the local Zebu breed with Taurine dairy breeds by artificial insemination and by the introduction of high producing dairy breeds into the country. Smallholder dairy farms are estimated to produce 75-90% of the milk sold in Kenya (Mbogoh, 1984a, b; Goldson and Ndeda, 1985; Ministry of Livestock Development, 1989). Approximately 65% of dairy cattle in the smallholder farms are stall-fed for the greater part of the year (Gitau *et al.*, 1994a). In 1990, the estimated dairy cattle population was three million (Ministry of Livestock Development, 1989). Of that population, a quarter was made up of calves less than one year old. Dairy cattle in smallholder dairy farms are estimated to be 80% of the total dairy herd in Kenya (Ministry of Agriculture, Livestock Development and Marketing, 1996). Dairy farming provides income through the sale of milk and meat and is a more consistent source of income than many other farming enterprises, particularly in the medium to high agro-potential areas.

A number of factors limit the development of the dairy industry, including diseases, poor management, inadequate nutrition and lack of farm inputs (Annual Report, International Laboratory for Research on Animal Diseases (ILRAD), 1984; Goldson and

Ndeda, 1985). A number of production and infectious diseases are reported to be associated with different types of production losses in different agro-ecological zones (AEZs) (Winrock International, 1992). The most important animal disease constraints to livestock productivity in sub-Saharan Africa are parasitic and viral diseases (Winrock International, 1992). In the group of the most important parasitic diseases were tick-borne diseases (TBDs) which result in great production losses (de Haan and Nissen, 1985; Provost, 1991; Mukhebi *et al.*, 1992). It is assumed that vaccination to prevent these diseases can substantially reduce these losses.

An epidemiological study on ECF and other TBDs is an important step in the understanding of its distribution and impact on smallholder cattle population and a prerequisite to the application of any disease control strategies. A hypothesis that has been developed during years of observations on ECF and other TBDs in the field is the concept of endemic stability (Perry *et al.*, 1992; Perry and Young, 1995). The term endemic stability has been defined by Norval *et al.*, (1992) as "a climax inter-relationship between host, vector and environment in which all coexist with the virtual absence of clinical disease while endemic instability means an incomplete inter-relationship between host, vector and environment in which clinical disease occurs." Endemic stability due to *T. parva*, is defined as "the state in a cattle population (farm, agroecological zone, district, etc.) in which the large majority of that population becomes immune by six months of age and little or no clinical disease occurs." In such a state, the disease is naturally controlled.

Although a lot of data have in the past been accumulated on tick-borne diseases, very little have been obtained from well designed and structured epidemiological studies. Most of the available information in Kenya on TBDs has been gathered from passively-derived data (such as annual reports) through the Veterinary Department, originating

primarily from the District Veterinary Offices. Though passively-derived data may serve to indicate the presence of the disease in a given area, other important measures of disease (such as incidence, distribution, seasonality, etc.) and animal-level factors (such as breed, age, survival rate, impact on productivity, etc.) are usually absent (Norval *et al.*, 1992). Other vital information required is the distribution of the disease by region (e.g. AEZ or administrative location) or by management systems (e.g. grazing management at farm-level, regional level, etc.). Passively-derived data usually do not indicate the true disease status of the population and is thus not useful as baseline for disease monitoring and control strategies. As stated by Norval *et al.*, (1992), both epidemiologists and researchers in general require a more detailed understanding of the occurrence of both infection and clinical disease since the impact and the control of theileriosis are dependent on a number of factors.

With the virtual collapse of government sponsored dipping schemes, farmers are currently employing alternative tick control methods such as handspraying and handwashing; that are applied in either a strategic or haphazard manner. In addition, there is an increasing need for devising alternative methods for the control of ECF and other TBDs. One method that is currently receiving strong consideration is immunisation against ECF.

The continued menace of TBD to the cattle industry in particular the dairy sector in Kenya calls for further studies on the epidemiology and control of the diseases. A study was therefore conducted in Murang'a District of Central Province Kenya which has a wide variation in agroecological zones (AEZs) and high cattle population. The area has five major agroecological zones (AEZs) defined according to altitude, rainfall levels and agricultural activities (Jaetzold and Schmidt, 1983). The district has a high density of

smallholder dairy farms representing a wide range of cattle types, grazing practices and husbandry systems. The density of smallholder dairy farms, distributions of cattle types and grazing management systems varied across the AEZs. Thus, this district provided an opportunity to study the epidemiology and impact of ECF and other TBDs under very different environmental and farming conditions. The main view was on improving the planning and targeting of TBD control programmes, and especially the ECF immunisation.

It is in this context that this study was designed. The general objective was to define appropriate target cattle populations for ECF control strategies, particularly immunisation. To satisfy this general objective, a number of specific objectives were developed as: 1) to characterise different areas within the district as to their risks for TBDs, in particular infection with *T. parva*, and to classify the potential endemically stable and unstable areas; 2) to estimate health and productivity parameters such as infection rates, morbidity and mortality rates, dynamics of infections and growth patterns in contrasting production systems and agro-ecological zones (AEZs); and 3) to study the potential risk factors associated with ECF and *T. parva* infections in different areas. The potential risk factors included were agro-ecological (rainfall and altitude); management (grazing management and tick control practices) and host (breed, age, innate, passive and active resistance).

In order to achieve the above objectives, a two-phase study was initiated. The first was a cross-sectional study to estimate the unknown prevalence of *T. parva* and other TBDs and a second, a longitudinal (prospective) study to estimate incidences of *T. parva* infections, and morbidity, mortality and productivity patterns in defined cohorts of calves under different AEZs and grazing systems. It is hoped that the results from this study will

provide baseline data on the dynamics of *T. parva* infections in the different AEZs and grazing management systems in Murang'a District and probably other highland districts in Kenya. The results will also help to shape ECF control strategies for different AEZs and grazing management systems in other areas.

CHAPTER 2

LITERATURE REVIEW

2.1 History of theileriosis

Theileriosis, particularly East Coast fever (ECF), is a bovine disease syndrome caused by *Theileria parva* and transmitted by the brown ear tick, *Rhipicephalus appendiculatus* (Norval *et al.*, 1992). Theileriosis caused by *T. parva* in cattle is thought to have originated from buffalo populations in eastern and central Africa (Uilenberg, 1981; Young, 1981; Grootenhuis *et al.*, 1987). The disease was first reported in Kenya after 1904 but may have been present long before that date in the Lake Victoria Basin and the coastal regions (Norval *et al.*, 1992). In other parts of Central Kenya such as Machakos, Kitui, Kiambu and in Naivasha, cases of ECF increased as ox transport became a popular mode of transport in various parts of the country between 1901-1910 (Anon, 1910 cited in Norval *et al.*, 1992). Reports of the disease increased as cattle from disease free areas were brought into disease endemic areas by colonial settlers (Anon, 1910 cited in Norval *et al.*, 1992).

2.2 General epidemiologic patterns of theileriosis

Theileriosis is one of the tick-borne infections of livestock in Africa that presents a constraint to livestock development (Perry and Young, 1995). In Kenya, *T. parva* is associated with severe diseases in cattle (that is ECF and Corridor disease). The other *Theileria* species, *T. taurotragi, T. mutans* and *T. velifera* cause benign diseases (Kariuki, 1990) and can occur in the same areas as *T. parva*. The disease organisms are transmitted

transstadially by an Ixodid tick *R. appendiculatus* by passage of infection from larval to nymphal or nymphal to adult stages. The infected nymph or adult tick then transmits the organisms to a susceptible animal during feeding. The distribution of theileriosis follows two broad patterns: in areas where cattle graze close to, or interact with, the Cape buffalo (*Syncerus caffer*), buffalo-derived theileriosis (commonly known as Corridor disease) is predominant, while cattle-derived theileriosis occurs in areas without wildlife contact (Norval *et al.*, 1992).

The study of the epidemiology of theileriosis in Kenya has become of great interest due to two main reasons. First, the desire to import higher producing exotic cattle from disease free zones and second, the improvement of diagnostic techniques that have made further exploration of the disease easier (Norval *et al.*, 1992).

Past studies have revealed that a number of factors are involved, in a complex manner, in influencing the occurrence of theileriosis. Some important variables found to be associated with the different epidemiological states of the disease are: tick variables (distribution, numbers, etc), host variables (innate variation in susceptibility); parasite variables (virulence of the parasite stocks); animal husbandry variables (grazing management and tick control) and ecological variables (climatic suitability of tick vector), (Norval *et al.*, 1992; Perry *et al.*, 1992; Perry and Young, 1995). In Kenya for example, ECF has been maintained under communal type of grazing systems whereas it has been restricted in some farming systems such as the zero-grazing units (Norval *et al.*, 1992).

Computerised techniques have been applied to map out the distribution of the tick vector for theileriosis and to study the epidemiology of the disease (Perry *et al.*, 1990; Lessard *et al.*, 1990). Perry *et al.* (1990) applied the climate-matching model CLIMEX

(Sutherst and Maywald, 1985), to calculate the likely environmental suitability of *R. appendiculatus* based on climatic factors.

2.3 Host dynamics on theileriosis

Theileria parasites are maintained in carrier mammalian host animals; the most important being cattle and the buffalo from where they are transmitted by ticks from one animal to another (Norval *et al.*, 1992). In order to study theileriosis effectively, it is important to understand the host and the vector dynamics since these play a crucial role in the life cycle of *Theileria* species.

The presence or absence of the hosts determine whether *Theileria* parasites can be found in a certain locality. For many decades, cattle have been moved from one area to another following new human settlements or in search of pastures. The wildlife has migrated from one area to another due to encroaching human settlement in their natural habitation or in search of pastures. The resulting migration has assisted in the introduction of the disease in formerly disease free areas either by introducing the infected tick or the parasite of the disease mainly through carrier animals. The introduction of the disease in the formerly disease free areas has in the past resulted in heavy mortalities especially in southern Africa (Lawrence, 1992).

A number of studies carried out in Africa have shown that indigenous breeds of cattle such as Zebu and Sanga are more resistant to ECF than Taurine breeds and their crosses (Norval *et al.*, 1989; ILRAD 1990; Jongejan *et al.*, 1989; Fivaz and Norval, 1990; Spickett *et al.*, 1989). In Uganda, a study by Stobbs (1966) demonstrated variation in susceptibility to ECF between two breeds of Zebu and further showed that these breeds were different in their ability to resist tick infestations. Tick resistance and the innate

ability of local breeds to withstand virulent infection by *T. parva* allow these animals to survive in *T. parva* infested areas where the Taurine breeds fail to survive (Moll *et al.*, 1984, 1986).

2.4 Vector dynamics on theileriosis

Another crucial component to the understanding of theileriosis is the vector dynamics, that is the presence and abundance of the tick vector in a certain area. There is variation in the tick population with time (season to season and year to year) and space (among habitats and ecological zones) (Norval *et al.*, 1992). For adequate tick populations to be maintained and sustain the disease parasite, the factors that contribute to the survival, development and behaviour of the different life cycle stages need to be favourable. A number of studies have shown that all stages of *R. appendiculatus* wait to attach to the potential host on the vegetation (Short and Norval, 1981; Short *et al.*, 1989a, 1989b). It is therefore important that the various climatic or microclimatic conditions, the most important being temperature and humidity, be suitable for the survival and development of the tick populations.

Sutherst *et al.*, (1978) lists the factors that influence the success of ticks to host finding as: (1) host density, activity and coverage of habitat, (2) longevity of ticks and (3) daily and seasonal activity patterns of the ticks. In Kenya, the seasonal occurrence of *R. appendiculatus* has been studied in certain localities (Food and Agricultural Organisation (FAO), 1975; Newson, 1978; Newson and Punyua, 1978). The tick population is reported to be high following the rainy season which provides an ideal environment for tick multiplication. These findings led to the conclusion that the seasonal patterns of tick abundances are set by the adults that are active (host finding, engorging and mating) only under certain conditions of climate (temperature and rainfall) (Newson and Punyua,

1978).. In addition to the environmental factors, host density is also crucial (Norval *et al.*, 1992), in that in a given environment, the tick can only become established if there are enough hosts to sustain it. This is true especially in open grazing management systems where cattle are constantly moved from one area to another.

2.5 Endemic stability and instability of theileriosis

An important concept that has been developed by several workers during years of observations on theileriosis in the field is that of endemic stability (Perry *et al.*, 1992). This state can be found in a numbers of situations: i) the cattle possess a low innate susceptibility to the effects of *T. parva* infection (Morzaria *et al.*, 1988a). ii) *R. appendiculatus* is present virtually throughout the year (Yeoman, 1966a, b). iii) the large majority of young calves are exposed to a low *T. parva* challenge. In endemically stable areas, the ticks become infected from carrier animals as few clinical cases occur and the infection rates in ticks are low (Leitch and Young, 1981).

Useful indicators that can be used for determining whether endemic stability exists are: herd antibody prevalence, disease incidence, age group clinically affected and case fatality rate (Norval *et al.*, 1992). In endemically stable situations, these indicators can be measured in animals under six months of age (as they are unexposed soon after birth to *T. parva*) while in endemically unstable situations, they need to be measured in all age groups.

The antibody prevalence is high and the disease incidence is very low or absent in endemically stable areas (in all age groups) whereas the antibody prevalence is low and the disease incidence is high in endemically unstable areas (Norval *et al.*, 1992; Perry *et al.*, 1992). In endemically stable situations most calves less than six months of age become infected but the case-fatality rate is low whereas in endemically unstable

situations, all age groups are affected by the disease and the case-fatality is high (Norval *et al.*, 1992; Perry and Young, 1995). However, there are situations where the relationships between antibody prevalence and disease incidence are not known and where both prevalence and incidence might be low. An understanding of endemic stability is important as it has been a useful criteria (animals risk to disease is known) while mapping out and implementing disease control strategies.

2.6 Diagnosis of theileriosis

The most widely used method in the diagnosis and reporting of theileriosis in Kenya and the rest of the African regions is the use of clinical signs. This method has been widely applied for two main reasons: i) the lack of adequate facilities or equipment for laboratory diagnosis and ii) unavailability of easily applicable and reliable methods of diagnosis for use in the field. However, when clinical diagnosis is applied alone, it is important to recognise that mixed infections with other tick-borne diseases such as anaplasmosis and babesiosis often occur (Norval *et al.*, 1992).

Diagnosis (confirmatory) became possible when a number of reliable and sensitive laboratory diagnostic tests for the detection of *Theileria* parasites and antibodies in cattle and other livestock were developed in the mid-1980s (Irvin and Mwamachi, 1983; FAO, 1984 and Young, 1987). The most effective and cheap laboratory or field method for parasite detection in the mammalian host to date is the staining and examination of lymph node biopsy smears. This method detects schizonts in the lymph node smear and is widely used in conjunction with a clinical assessment (Norval *et al.*, 1992). A limitation is that the detection of *Theileria* schizonts in the lymph node smear may merely demonstrate the presence of a carrier state, but not necessarily confirm a

clinical infection. However, the main disadvantage of direct staining techniques is that various strains cannot be differentiated (FAO, 1984).

The indirect fluorescent antibody test (IFAT) is the most commonly used serum antibody assay for *T. parva* and *T. annulata* (Burridge and Kimber, 1972; Goddeeris *et al.*, 1982). The performance of the technique has been described in FAO (1984). The IFAT has been extensively used to estimate the prevalence of the infection and in monitoring experimental infections. The disadvantages of using the test is that it is cumbersome to carry out and that it lacks specificity (Burridge and Kimber, 1972; Burridge *et al.*, 1974a, b; Williamson *et al.*, 1990). For these reasons, alternative tests have been developed.

Enzyme-linked immunosorbent assay (ELISA), has been used in recent years and the disadvantages associated with IFAT were reasonably overcome. The ELISA test is easier to perform and is considered to be more sensitive and specific. With automation of the reading process, a large number of samples can be processed within a short time. The first *Theileria*-based ELISA to be developed was that for *T. mutans* (Katende *et al.*, 1990) and uses monoclonal antibodies (prepared in mice) to detect the *T. mutans* antigens. The circulating antibodies in the host animal are detected by a purified protein antigen (32 kilodalton protein) on a micro-ELISA plate (Katende *et al.*, 1990). Other ELISA tests have been developed recently for *T. parva* (Katende *et al.*, 1997) while those of *T. annulata* and *T. taurotragi* are currently under development. The performance of the ELISA tests for tick-borne pathogens that have been developed at ILRI has recently been evaluated. The sensitivity for ELISA tests for *T. parva* and *T. mutans* has been estimated to be over 99% while that of *B. bigemina* has been estimated to be about 97%, (Katende *et al.*, 1996; Katende, personal communication). The specificity has been estimated to be 97% for

T. parva, 99% for *T. mutans* and 98% for *B. bigemina* (Katende *et al.*, 1997). The main disadvantage of the antibody-based ELISA test is that antibody titres are well below the detectable levels in incubatory or carrier animals (Williamson *et al.*, 1990).

Recent developments in immunological and molecular diagnostic tools for *Theileria* species have been reported (Stiller, 1990; Williamson, *et al.*, 1990; Morzaria, *et al.*, 1995; Toye, *et al.*, 1996). These recent techniques are being perfected for the detection of parasite DNA and RNA. The techniques are proving to be a valuable means of distinguishing species, strains and stocks of pathogenic protozoan parasites which are difficult or impossible to distinguish morphologically or by serological techniques (Norval *et al.*, 1992). Today, other highly sensitive methods such as the polymerase chain reaction (PCR) are either in use and or at various stages of further validation (Saiki *et al.*, 1988). The PCR technique has been applied successfully in the detection of *Theileria* parasites of several stocks in the blood of carrier cattle (Conrad *et al.*, 1987; Allsopp and Allsopp, 1988; ILRAD, 1992; Nene *et al.*, 1992; Bishop *et al.*, 1993).

2.7 Control of theileriosis

The control of theileriosis is directed towards both the host and the vector. The methods that are directed at the host are aimed at preventing the vector from successful transmission of the parasite e.g. by immunisation or treating the already infected and sick cases. Those directed at the vector are aimed preventing the vector coming into contact with the host thus breaking the life cycle and reducing the vector population through direct killing of the vector or destruction of the vector habit.

The major method that has been used in the past to control theileriosis and other TBDs in Kenya is acaricide application using plunge dips and spraying (handpraying or

spray races) (Norval *et al.*, 1992), especially in intensive and highly productive beef and dairy units (Perry and Young, 1995). The use of acaricides for tick control has been reviewed in detail in FAO (1984). The main advantages of acaricide application has been the effectiveness of application (especially plunge dips and spray races) and communal-based application, thus making it cheap. Though widely used and effective, the application of acaricides has been adversely affected by lack of a strict and effective monitoring strategy. Many times, inadequate concentrations of acaricides are used in diptanks or handwashings, resulting in the development of tick resistance to acaricides (FAO, 1984). As it becomes increasingly difficult for the government to maintain the supply of the acaricides in communal diptanks, regular application of the acaricides has not been carried out. Given this uncertainty, acaricide application alone cannot be relied upon in the future and alternatives methods to acaricide application, such as immunisation, need to be exploited.

Other methods that have been used in tick control are: grazing management (e.g. confinement of animals or pasture spelling), the control of livestock movement, breed selection and application of tick decoys.

Strict confinement of animals (zero-grazing) reduces contact between animals and between animals and vectors. This method has helped to reduce TBDs among the smallholder dairy farms but the main disadvantages are that some farmers may not strictly confine the animals and tick-infested fodder for animals may be imported thus introducing ticks into such farms. Pasture spelling (keeping of pasture free of cattle for 15 months) is not practical in intensive grazing areas due to lack of land given the high human population and the poor control of cattle movement in Kenya.

Restriction of livestock movement is less effective than acaricide application. In Kenya, a livestock movement permit is required as part of the department of veterinary services regulations before livestock are moved. However, strict compliance to this regulation, which requires that the animals to be moved must be free of ticks, is questionable or has been implemented haphazardly. Apparently, it is in the disease risk areas that cattle movement and tick control measures are poorly or irregularly implemented (Musisi, 1990). As strict control of livestock movement has not been enforced, the susceptible animals have constantly been exposed to the risk of the disease following the introduction of infected or carrier cattle.

Tick eradication by use of acaricides has proved difficult to achieve, and integrated control strategies are generally advocated (Young *et al.*, 1988). An integrated control strategy is based on the application of several methods for the control of ticks and the various TBDs they transmit (Norval *et al.*, 1992). While implementing an integrated tick control programme, all the important TBDs that are of economic importance in a region need to be considered with the most severe of all being given the priority. As stated by Norval *et al.* (1992), the aims of integrated tick control strategies should be to manage tick populations and tick-borne diseases within economically acceptable limits in which the risks of the disease outbreaks are minimal. In Burundi for example, tick control that involved a strategic dipping programme, where dipping coincided with months of greatest tick population was reported by Kaiser *et al.*, (1988). The programme involved three to four months of intensive dipping to coincide with the feeding period of the adult tick. In Kenya, several trials on such programmes have been carried out particularly on commercial ranch settings (Tatchell *et al.*, 1986). In the above study reported by Tatchell *et al.*, groups of cattle were managed under limited or no acaricide application in Kiboko,

Machakos District, where no obvious differences in productivity (morbidity, mortality, milk yield and growth) were observed. It is possible that an integrated tick control programme would be the choice for the future.

Though Zebu breeds have been upgraded and more Taurine breeds of cattle have been introduced in Kenya, subsequent application of strict management and disease control measures have not been followed. This has resulted in continuous spread of ECF (Norval *et al.*, 1992).

2.8 Treatment of theileriosis

Chemotherapy in the treatment of theileriosis has become popular since the mid-1980's (Dolan, 1981; McHardy, 1984, 1989). Three therapeutic agents namely, parvaquone (Clexon®) and buparvaquone (Butalex®) (Wellcome Pharmaceutical, United Kingdom) and halofuginone (Terit®, Hoechst Pharmaceutical, Germany) were developed, registered and marketed. These have proved effective; however, their effectiveness depends on an early diagnosis of the disease. In Kenya, these chemotherapeutic agents have been used in many of the large scale dairy farming areas with veterinarians reporting differing success rates, probably due to the different timing of treatment during the course of the disease. However, there has been limited use of the three therapeutic agents by smallholder farmers as the drugs are expensive and unaffordable. Late diagnosis of ECF has been associated with poor success rates for all treatments and regimes (Norval *et al.*, 1992).

2.9 Immunity and immunisation against theileriosis

In Kenya, several reports dating as early as the beginning of this century indicate that indigenous and Zebu-Taurine crosses were able to acquire and develop immunity to ECF (Anon, 1910 cited in Norval *et al.*, 1992). Whenever ECF outbreaks occurred after cattle were moved from disease free to disease endemic areas, the animals that survived the epidemic were shown to be immune to challenge when exposed to the disease on farms where the disease was known to be endemic. It was demonstrated that the previously exposed (or "salted") animals were immune. The above observations coupled with other experiments carried out elsewhere later have become the baseline for the understanding and studying of immunity to ECF today.

Different breeds of cattle show variations in immunity to *T. parva* with the local indigenous breeds showing highest resistance while the exotic breeds show the lowest resistance. Barnett (1968) and Moll *et al.*(1984, 1986) have shown that low numbers of Zebu calves die in endemically stable areas due to *T. parva* infection whereas high numbers of improved Taurine cattle die if introduced in these areas. Both carrier states and sterile immunity have been observed in *Theileria*-infected cattle. The carrier state occurs in recovered animals which have the ability to infect ticks which are then able to transmit the parasite successfully to a susceptible animal (Young *et al.*, 1986).

Animals that recover spontaneously from infection with *T. parva* are solidly protected against homologous challenge for up to three and a half years (Burridge *et al.*, 1972). Antibody is not considered to play a important role in the protection seen in recovered animals (Burridge and Kimber, 1972) as transfer of immune serum fails to protect against the disease (Muhammed *et al.*, 1975; Emery, 1981). These observations have led scientists to believe that protection against *T. parva* is as a result of cell-mediated

immune mechanisms. However, sera from cattle that had undergone repeated challenges was shown to neutralise the infectivity of sporozoites in cattle, an observation that led Musoke *et al.*, (1982) to suggest that serum antibodies may still have some protective role in immunity against *T. parva*. Several aspects of the bovine immune system suggest that immunity to *T. parva* infection is directed to the schizont-infected cell (Emery, 1981; McKeever and Morrison, 1990; Norval *et al.*, 1992; Musoke *et al.*, 1993). Recent studies have revealed that protection is likely to be mediated by parasite-specific cytotoxic T-cells (McKeever and Morrison (1990); Morrison (1996)).

Following the demonstration by Neitz, (1953) and Neitz and Jansen, (1957), that administration of tetracycline over a prolonged period resulted in cattle becoming effectively immunised against theileriosis without adverse effect, many trials have been carried out using the "infection and treatment method". A significant breakthrough on the method of infection and treatment was achieved following the production of sporozoite stabilates which allowed cattle to be infected with a particular predetermined dose (Cunningham *et al.*, 1973). Other drugs that have also been evaluated in the "infection and treatment" method are parvaquone (Dolan *et al.*, 1984; Dolan, 1986; Young *et al.*, 1990) and buparvaquone (McHardy and Wekesa, 1985; McHardy, 1989; Mutugi *et al.*, 1988; Mutugi *et al.*, 1991; Young *et al.*, 1990). In Kenya for example, the "infection and treatment" method has been carried out in several areas and immunised animals have been reported to survive reinfection challenges (Dolan, 1985; Morzaria *et al.*, 1988b; Young *et al.*, 1990; Young *et al.*, 1992). A key issue to the method of "infection and treatment" is the occurrence of different *T. parva* strains in different localities which may render immunisation ineffective in some areas. Trials on the "infection and treatment" method

were conducted in Zimbabwe recently and were reported to have achieved significant successes (Pegram *et al.*, 1996).

As the "infection and treatment" method involves the introduction of live parasites in cattle and thus the risk of development of severe disease or carrier states, the use of a mild form of *T. parva* parasite may overcome the problem (Mbogo *et al.*, 1996). Other problems associated the "infection and treatment" method are the requirement of a cold chain for delivery, severe reactions following immunisation, introduction of new strains of parasites and potential danger of other spreading other organisms contaminating the vaccines (Morzaria, 1996).

In the last few years, workers at ILRI (former ILRAD) have embarked on research towards the development of a vaccine against ECF (ILRAD, 1991, 1992). One type is a sporozoite-based vaccine (designated p67-vaccine) derived from an antigen which is found only on the surface of the sporozoite and has a molecular mass of 67 kilodaltons (Musoke *et al.*, 1993). This antigen has been shown to induce antibody production in cattle that neutralise the ability of sporozoites to infect lymphocytes in vitro (ILRAD, 1990, 1991; Musoke *et al.*, 1993). The p67-vaccine has been used recently to immunise cattle and these animals have been shown to resist subsequent experimentally-induced challenge with lethal doses of heterologous cattle-derived *T. parva* parasite (Nene *et al.*, 1996). This confirms that the p67 molecule has potential use as a broad-spectrum vaccine antigen for the control of *T. parva* infection.

Another *T. parva* protein identified at ILRAD (ILRAD, 1990, 1991) that will be exploited for its potential as a vaccine (or diagnostic antigen) appears on both sporozoites and intracellular schizonts. The antigen is called the polymorphic immunodominant molecule (PIM). The schizont is the pathogenic stage of *T. parva* and it is this form that

parasitises host lymphocytes and causes them to proliferate excessively leading to the development of the disease (ILRAD, 1991). The death or recovery of an animal depends greatly on its ability to control the rapidly dividing infected lymphocytes. Another important aspect in the vaccine development is the identification of a suitable delivery system that will not interfere with induction of immunity and will prevent vaccinated cattle from succumbing to tick-transmitted challenge in the field.

2.10 Productivity and economic impact of theileriosis

Cattle are raised for meat and milk production which are either consumed in the household or sold to provide a source of income. Any constraint to the health and performance of cattle will lead to an imbalance in food production and availability, and to economic losses to the individual farmer and the country as a whole. It has been noted that since the mid-1970s, the supply of meat and meat products in the African continent has dropped and this has been marked by an increased importation of meat and milk and their products (Sarma and Yeung, 1985).

For many decades, it was perceived that tick-borne diseases took a heavy toll on the Taurine cattle introduced in many areas in the tropics, such as Kenya, and this was considered to have retarded the development and improvement of livestock production in these areas (Callow, 1983). There are various categories of production losses that are experienced both at the farm and national levels. Direct losses due to theileriosis arise through morbidity and mortality (Mukhebi *et al.*, 1992). Cattle that survive the disease experience reduction in milk yield and may also show diverse weight gains. Other losses include lowered growth rates in calves, infertility, delayed oestrus, abortions and decreased provision of draught power (Mukhebi *et al.*, 1992). The indirect production

losses arise from the disease being a constraint to livestock production and improvement. The disease renders the raising of exotic and high producing animals difficult or impossible due to the risk of the disease.

Although direct and indirect economic losses attributed to ECF are one of the main sources of economic loss in dairy cattle, very little specific information is available to quantify the actual losses. Some few case studies show that the losses due to ECF could be substantial although the methods used to make the estimates assume uniform distribution of disease in a region and also rely on passively-derived data . Miller *et al.*, (1977) estimated that about one million cattle die annually from ECF in Kenya, Tanzania and Uganda. Onchoke (1993) estimated that the loss due to ECF (morbidity, mortality, etc.) was approximately Ksh. 1.1 billion in Uasin Gishu District in Kenya in 1992.

The cost incurred in the control of the disease through the extensive tick control programmes and the treatment of clinical cases has been an enormous burden to the farmer and to the economy of the country. In 1987, Kenya spent about US$ 10 million (Young *et al.*, 1988) through the provision of dipping services and curative services. It is no doubt that though the tick control services are for all tick-borne diseases, the main disease prompting the use of acaricides in Kenya is theileriosis (Cunningham, 1977).

2.11 Impact of theileriosis on dairy production

The smallholder dairy sector comprises the major milk producing sector within the overall dairy sector in Kenya. The vast majority of the dairy farms are owned and run by smallholder farmers who are estimated to produce 75-90% of the milk sold in the country (Mbogoh, 1984a, b; Goldson and Ndeda, 1985; Ministry of Livestock Development, 1989). The majority of the cattle raised in these smallholder dairy farms are the much

valued improved dairy breeds that are also highly susceptible to tick-borne diseases particularly ECF. These improved breeds have been shown to have higher economic returns in milk production than the local breeds (Mukhebi *et al.*, 1992).

The most important constraints to smallholder dairying include diseases, poor management, inadequate nutrition and lack of farm inputs (Annual Report, ILRAD, 1984; Goldson and Ndeda, 1985). Tick-borne diseases, in particular theileriosis, are the important group of infectious and parasitic diseases that are reported to be associated with different types of production losses in different agro-ecological zones (AEZs) and dairy production systems (Winrock International, 1992). Tick-borne diseases have been reported to contribute to great production losses among the smallholder farms (de Haan and Nissen, 1985; Provost, 1991).

Other constraints such as inadequate nutrition and poor management, exert different production losses within and between AEZs (Omore *et al.*, 1996; van Schaik *et al.*, 1996). The study by van Schaik *et al.* (1996) showed that nutrition was the single most important input in the smallholder dairy farming that was associated with high milk yield. Farms that supplemented animals with commercial concentrate feeds recorded significantly higher levels of milk yield and higher benefit-cost-ratio (BCR) than those that did not feed concentrate feeds. The high human population density in areas where smallholder farms predominate has resulted to intensive farming and the land available for fodder production for cattle is small (Winrock International, 1992). Further increases in production and productivity have been limited by the general inadequate farm inputs such as technology, fertilisers, source of power, high yielding forages and feed crops, extension and veterinary services - including breeding services and poor infrastructure particularly road networks (Winrock International, 1992).

Tick-borne diseases have in the past been considered to be a major constraint to improving the dairy industry in Kenya (Kariuki, 1990). Among the diseases, ECF is one of the major constraints in the group of the important TBDs, not only in Kenya but in the entire region of eastern and central Africa (Bram, 1983; Norval *et al.*, 1992). Due to the perceived importance of TBDs, in particular ECF, a number of workers have attempted to quantify ECF production losses using the available poor quality and unverified data.

Most of the workers that have quantified the estimated production losses due to ECF have attempted to do so at the country and regional levels. These estimates, which are based on data from the veterinary department records, assume universal distribution of ECF in all the agro-ecological zones and production systems and are in many cases of low quality and not verified. It is estimated that about half of cattle losses from mortality in sub-Saharan Africa is as a result of diseases transmitted by external parasites, especially ticks (de Haan and Bekure, 1991). Mortality due to theileriosis in eastern, central and southern Africa has been estimated to be 1.1 million cattle, a loss estimated to cost US$ 168 million per year (Mukhebi *et al.*, 1992). Miller *et al.*, (1977) estimated a regional mortality of one million cattle annually from ECF in the three East African. The figure estimated by Miller *et al.*, (1977) appears very high compared with the recent findings (O'Callaghan *et al.*, 1994) which show that mortality due to ECF is very low and may perhaps indicate that the times of high ECF mortality may be gone. Onchoke (1993) estimated that the loss (such as morbidity and mortality) due to ECF was approximately Ksh. 1.1 billion (about US $ 18 million) in Uasin Gishu district in 1992. Mortality from ECF in indigenous breeds of cattle ranges from zero to approximately 50% (Staak, 1981; Moll *et al.*, 1984, 1986; Ngulo, 1985; Ngulube *et al.*, 1985; Berkvens *et al.*, 1989; Otim, 1989). In highly susceptible breeds of cattle (*Bos taurus* and some indigenous cattle from

ECF free zones), mortality can be as high as 100% (Cunningham, 1977; Hooke, 1981; Julla, 1985; Morzaria *et al.,* 1988b). In addition to mortality, productivity losses are experienced during the course of the disease and over the recovery period (Moll *et al.,* 1984). In order to understand the importance of ECF in smallholder dairy production, estimates on measures such as morbidity, mortality, impact on growth rate and cost of control and treatment are required from well designed and structured studies.

Some retrospective studies on theileriosis/East Coast fever carried out in Kenya by Kariuki (1990), Mulei and Rege, (1989) and Kyule, (1989) on ECF cases reported annually by the Veterinary Department between 1969-1987 showed that the cases increased annually. However, it may be hard to associate the increase with an absolute increase in the ECF cases as this may have been attributed to improved reporting due to available chemotherapy from the mid-1980's (Dolan, 1981; McHardy and Wekesa, 1985) or due to the problem of a diagnostic criteria which may have classified some cases as ECF when they were actually not ECF. The studies by Mulei and Rege, (1989) and Kyule, (1989) attempted to look at the patterns of ECF in Kenya on the basis of passively-derived data. Mulei and Rege (1989) used cases of ECF reported to and seen by the Ambulatory Clinic of the University of Nairobi's Department of Clinical Studies. Both studies attempted to calculate annual disease incidences based on assumed cattle populations and that all cases of ECF in the locality were reported to the ambulatory clinic. A cross-sectional study by Deem *et al.,* (1993), estimated the likely extent of theileriosis and prevalence of the important TBDs in the coastal area in Kenya. This cross-sectional study defined agro-ecological zones, and was an example of how serology might be useful in characterising areas. Significant differences in prevalence rates were found across the various agro-ecological zones. When infection prevalence information is

known, additional information such as morbidity and mortality rates, grazing management, tick control and cattle types raised provide a more robust picture of the epidemiology of ECF in an area.

Recent studies from the smallholder dairy farms in central highlands in Kenya (Gitau *et al.*, 1994a,b,c; O'Callaghan *et al.*, 1994; van Schaik *et al.*, 1996) have described smallholder dairying, the types of production constraints and losses associated with general farm management and diseases such as ECF. The general farm management and production constraints among the smallholder dairy farms showed greater variations among AEZs, grazing management and farming systems and by other factors such as nutrition. These studies showed that past studies on production losses due to ECF and other TBDs among the smallholder farms in the Kenyan highlands have tended to overestimate the losses (O'Callaghan, 1994). The higher ECF figures and production losses have probably resulted from the type and precision of questions asked, variability of ECF across AEZs and the changing ECF pattern over the years.

Estimates on incidence of ECF morbidity and mortality and impact on growth rate, cost of control and treatment from these recent studies indicate that ECF is not the major constraint in smallholder dairy farms in the Kenyan highlands. Further, production losses from ECF vary significantly between zero and open grazing management systems among the smallholder dairy farms with higher production losses in the open grazing management system (Maloo *et al.*, 1994). The studies from the smallholder dairy farms in central highlands in Kenya (O'Callaghan *et al.*, 1994) indicate that high production losses due to ECF are not universal but vary by AEZ, grazing, farming system and other factors. This will require refining TBD programmes based on good epidemiologic studies in priority (target) production systems.

CHAPTER 3

MATERIALS AND METHODS

The study was conducted in two phases: a cross-sectional study and a longitudinal observational study. First, a cross-sectional study was performed to characterise the risk of TBD infections in the cattle population according to agro-ecological zones and grazing management systems for three months between March and June 1994. Second, a longitudinal observational study, to investigate in detail risk differences in contrasting low, intermediate and high risk zones based on the initial cross-sectional study was carried out for a period of one-and-a-half years between March 1995 and August 1996. In addition, the longitudinal study also estimated parameters such as the incidence of *T. parva* infection, crude and cause-specific morbidity and mortality losses, and identified risk factors associated with these parameters. The detailed description of the two study components is given below.

3.1 STUDY DESIGN FOR THE CROSS-SECTIONAL STUDY

3.1.1 Description of the study area

The study was conducted in Murang'a District, Central Province, Kenya (Figure 3.1), an area with a high density of smallholder dairy farms estimated to be approximately 50,000 (Ministry of Agriculture, Livestock Development and Marketing, 1996). The district covers approximately 1,600 km^2 and has an estimated human population of 858,600 persons (Ministry of Planning and National Development , 1989). There are various administrative areas within the district, the smallest being sublocations. The sublocation

area comprises a number of smallholder farms (also large holder farms where applicable) and occupy a mean area of 10-20 km^2 depending on human population density. Those sublocations tend to be smaller in size in areas with high human population density while those in low density areas are larger as the sub-divisions are based on human population.

Five major agro-ecological zones (AEZs), defined according to altitude, rainfall levels and agricultural activities, have been identified (Jaetzold and Schmidt, 1983). These are: Lower Highlands 1 (LH 1), (tea-dairy; altitude, 1730-2130m; mean annual temperature, 15-18^{0}C; annual rainfall, 1700-2400mm); Upper Midlands 1 (UM 1), (coffee-tea; altitude, 1670-1800m; mean annual temperature, 180-18.8^{0}C; annual rainfall, 1700-1900mm); UM 2 (main coffee; altitude, 1500-1670m; mean annual temperature, 18.8-19.7^{0}C; annual rainfall, 1300-1620mm); UM 3 (marginal coffee; altitude, 1340-1500m; mean annual temperature, 19.7-20.7^{0}C; annual rainfall, 900-1350mm); and UM 4 (sunflower-maize; altitude, 1340-1520m; mean annual temperature, 19.5-20.7^{0}C; annual rainfall, 850-950mm).

Cattle are raised in all five AEZs; Taurine (*Bos taurus*) breeds are raised mainly in LH 1 and UM 1-3, while in UM 4 both Taurine and Zebu breeds (*Bos indicus* - principally the East African Zebu) and their crosses are raised. Most smallholder dairy farmers in Murang'a District raise the improved Taurine breeds of dairy cattle in zero-grazing units or small paddocks, especially in LH 1 and UM 1-3, which are the main milk producing zones. Zero-grazing is a management system in which cattle are confined permanently in houses or sheds and feeds (especially cut fodder) and water are delivered to the animal.

Most of the smallholder farmers in these three zones are members of dairy co-operative societies. These dairy co-operative societies collect the farmers' milk at various collecting centres and market the milk on behalf of the farmers either locally or deliver it to a central milk marketing body, the Kenya Co-operative Creameries Limited. In UM 4, the

driest AEZ of the district, an open or paddock grazing management system is practised. In the majority of the farms, the cattle are released to graze on individually or communally owned pastures with communal watering points shared in some areas. Zero-grazing management is also used in this zone.

Agro-ecological, cattle management and calf-level factors were important potential explanatory and risk factors that were expected to be associated with differences in seroprevalence rates to TBDs in Murang'a District. Antibody prevalence estimates were expected to be different across the five AEZs. The important cattle management factors expected to be associated with different seroprevalence rates were grazing management (zero- versus open-grazing), tick control practices, calf housing, source of forage feeds and feeding practices. The calf factors that were considered to be potentially and significantly associated with differences in seroprevalence were sex, breed and calf age (Table 3.1).

3.1.2 Selection of sublocations and farms

Sublocations and farms were selected by a stratified random sampling selection procedure. The stratification was by sublocations (area) and farm (Figure 3.1). All the five AEZs were included in the study and provided a baseline for the selection of areas and for more intensive scrutiny in the subsequent longitudinal study. In the first stage, all sublocations in the district were classified by AEZ. Three sublocations from each AEZ strata were selected randomly, using random number tables. Some sublocations were found in two AEZs. To equalise the chances of selection for all sublocations, sublocations which straddled an AEZ boundary were listed twice (once in each AEZ), while sublocations only in one AEZ were listed twice under that AEZ.

Relatively few sublocations per AEZ were selected as the average seroprevalence by sublocation was expected to be relatively constant within an AEZ. The latter was due to the expected uniform exposure to the parasites and similar grazing management practices within an AEZ. In some AEZs, farm-to-farm variation for some sublocations was expected. Most of the farms were expected to have only one calf.

In the second stage, 50 farms per selected sublocation were randomly sampled (using random number tables) from a list frame of farms. The list frames were obtained from dairy co-operative societies in the western part of the district (Kangema Division) located in LH 1 and UM 2, and from diptank registers in all other areas (Figure 3.1). The inability to use the same list frame was because dairy co-operatives were still being established in some regions. However, in these regions complete registry lists for communal dipping existed. Most farmers had joined dairy co-operatives in Kangema Division because of the construction of a modern milk cooling plant in the area, thus giving many farmers easy access to the market for their milk. Except for the mode of milk marketing, other aspects of the farm management and the type of animals raised within each AEZ did not appear to be different between members and non-members of dairy co-operatives.

The sample size initially estimated, using simple random sampling and assuming a 50% seroprevalence and a 10% allowable error, was 100 farms per AEZ. The formula ($n = 4PQ/L^2$), which was used to estimate the sample size, was obtained from Martin *et al.* (1987). Assuming some clustering of seroprevalence, especially by sublocation and farm, the sample size was inflated by 50% giving a total of 150 farms per AEZ. An overall total of 750 farms were identified for sampling.

3.1.3 Data and sample collection

For each of the farms visited, a questionnaire (Appendix 1.1) was administered to the farm owner to gather some specific farming activities, for example the proportion of the farm area allocated to each farming activity, number of animals raised and their breeds (verified by the interviewer), animal management practices, grazing management system and specific tick control practices. A separate questionnaire (Appendix 1.2) was administered for all calves between six and 18 months old. For each calf, the following were recorded: breed, age, sex, tick control history (including method used), initial age of first application, frequency of application, and past cause-specific morbidity. All questions were presented in a closed format and responses further verified by inspection of the farm. To maintain consistency, all interviews were conducted in the local Kikuyu dialect by the author.

Blood samples, for sera preparation, were taken during the farm visit. Blood was collected from each calf in two 10-ml plain vacutainer tubes (Becton Dickinson Vacutainer Systems-England) by jugular venipuncture. The tubes were labelled and labelling verified before drawing the blood from the calves. After collection, blood samples were stored in ice boxes until they could be refrigerated (usually within 2-6 hours). The next day, the sera were separated by centrifugation at 3000 *g* for 20 minutes and divided into four aliquots of approximately 0.5 mls and stored in dry ice (solid CO_2 at approximately -160^{0}C) until they could be transferred to freezers (-20^{0}C) in the laboratory at the International Livestock Research Institute (ILRI). Laboratory analysis for the parasites followed later as described in section 3.3.

3.1.4 Data handling and storage

Data files of responses to questionnaires and results of laboratory assays were prepared in Dbase IV Plus (Ashton-Tate Corporation, Torrance, CA, USA). Separate files were prepared for the farm and individual-calf questionnaires.

3.1.5 Data analysis

3.1.5.1 Descriptive analysis

The statistical analyses were conducted in Statistical Analysis Software for DOS (SAS for DOS) version 6.04 (SAS Institute Inc., Cary, NC, USA). Descriptive analyses were used to classify farm and calf categories (e.g. breed, housing, etc) and to compute associations by AEZ, grazing management, tick control and calf breed. The differences in the estimated antibody prevalence to the TBD parasites were compared across the AEZs using the Mantel-Haenszel chi-square. Confidence limits for the binomial proportions (Snedecor and Cochran, 1989) for each AEZ and grazing system were generated for all antibody prevalence estimates. As most farms had only one calf, clustering between farms was ignored while calculating the confidence since most variation in antibody prevalence estimates was expected between calf observations.

3.1.5.2 Analysis of factors associated with sero-prevalence estimates

The potential explanatory and risk factors associated with antibody prevalence were listed in section 3.1.1 and on Table 3.1. Both linear (least squares) and non-linear (logistic) regressions were performed on the antibody prevalence data for *T. parva, T. mutans* and *B. bigemina*, using percent positivity (PP) values as the outcome variable (see section 3.3.2) against the potential risk factors (dependent variables) mentioned on section 3.1.1.

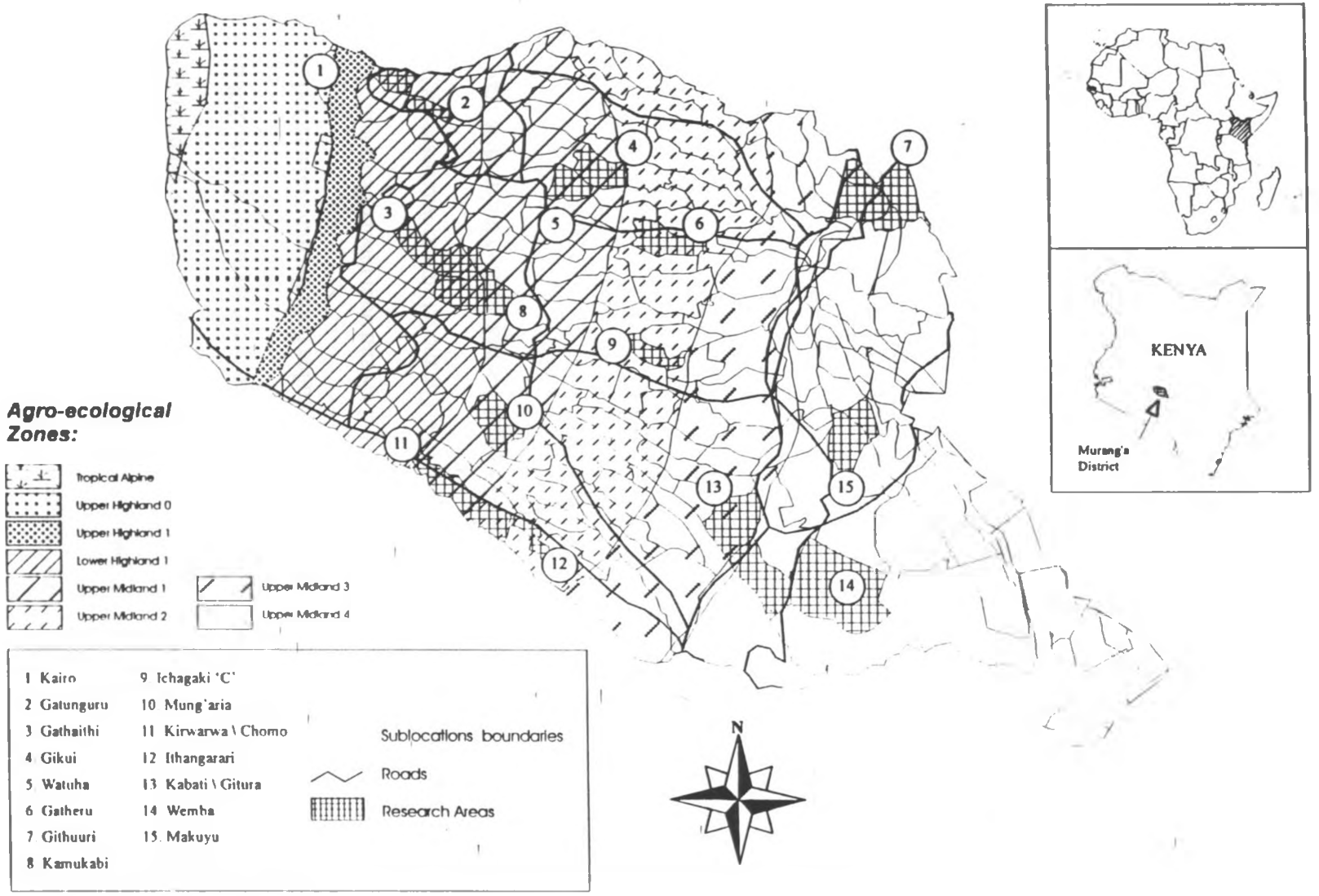


Figure 3. 1 Map of Murang'a District Kenya showing the agro-ecological zones and the sublocations selected for the cross-sectional tick-borne disease study (March - June 1994). Source: Geographical Information System (GIS) database for Murang'a District at ILRI (*Arcview Software, ESRI, 1994, USA*).

The absolute PP values were entered in the linear regression model as the response (dependent) variable. For the logistic regression procedure, values were coded as positive or negative using a fixed cut-off of 15 PP (see section 3.3.2). In both regression procedures, a final model was chosen by backward elimination with non-significant main effects removed and previously removed main effects assessed for re-inclusion at each step. Several positive interaction terms were created from the main effects that were found significant through the backward elimination procedure. A final overall model included both the main effects and the interaction terms. The procedures were performed in SAS and both main effects and interaction terms were considered significant at ($p<0.05$). The model fitness was assessed by Wald's tests (SAS/STAT User's Guide Manual). The baseline risk AEZ in the models was LH 1.

3.2 STUDY DESIGN FOR THE LONGITUDINAL STUDY

3.2.1 Selection of study areas

Though four indicators (antibody prevalence, disease incidence, age group clinically affected and case-fatality rate) may be applied to define epidemiologic state of tick-borne infections in a population (Norval *et al.*, 1992), only antibody prevalence was applied in this case for the selection of three contrasting epidemiologic states to be studied in the longitudinal study. The other indicators can only be reliably estimated in a longitudinal study. The three contrasting AEZs chosen were , UM 1, UM 2 and UM 4 (Figure 3.1).

The three study areas were purposively selected on the basis of different antibody prevalence rates to *T. parva* estimated from the cross-sectional study. Agro-ecological

zone UM 4 was classified as high risk area as it had approximately 70% antibody prevalence. The other 4 AEZs (LH 1, UM 1, 2 and 3) were classified as low risk areas as they had antibody prevalence below 40%. One high-risk (UM 4) and one low-risk (UM 1) were purposively selected for the longitudinal study. In each of these two AEZs, grazing management was stratified into zero (restricted) and open (unrestricted) grazing systems. In a third AEZ (UM 2), cattle were raised exclusively under zero-grazing management system and antibody prevalence was below 40%. This third AEZ was included as it was the area with the highest number of zero-grazing smallholder dairy farms in Murang'a. It was important to characterise the risk of TBD infection for farms in this zone with a view to future planning of possible TBD vaccination programme. The details on each of the three selected AEZs are described below.

The high-risk AEZ for *T. parva* exposure selected was UM 4, the AEZ with the lowest elevation (approximately 1,100 - 1,500 metres above sea level). The majority of cattle were Zebu and their crosses while only a few were Taurine purebreeds cattle. Most cattle, particularly the Zebu and Zebu crosses were allowed to graze on either communal pastures or private paddocks, while the few Taurine cattle were often kept in zero-grazing enclosures within smallholder mixed farms.

The low-risk AEZ for *T. parva* exposure selected was UM 1, the AEZ with the highest elevation (approximately 1,800 - 2,100 metres above sea level). The majority of cattle were Taurine purebreeds. Most cattle were raised under improved management on small to medium holder farms on zero-grazing enclosures or were allowed to graze on private paddocks within the farm.

The third AEZ was UM 2, with a medium elevation (approximately 1,600 - 1,700 metres above sea level). This AEZ was selected for two reasons; first, as low-risk AEZ to

T. parva exposure (antibody prevalence below 40%) and second, as an AEZ where cattle were raised exclusively under zero-grazing management. In this AEZ, Taurine purebreeds were predominant.

3.2.2 Selection of study farms

In each selected area, farms on which female calves were born between March 1995 and August 1996 and in which the farmers were willing to participate in the study were purposively recruited (Figure 3.2). The recruitment of farms was carried out progressively during the study period as calving was spread throughout the year. This prolonged recruitment helped to avoid confounding between time of recruitment and seasonal effects. The choice to only recruit female calves was based on results from a study that showed that significant differences occurred in the management of male and female calves (Gitau *et al.*, 1994b,c). Female calves were much more valued and were given more attention than male calves (Gitau *et al.*, 1994b,c). Female calves were of principle interest in this dairy production system since farmers took more care in raising them as the future replacements in their dairy herd. Most farmers were not interested in male calves since feed resources were too limited.

Since AEZ and grazing system were confounded, and grazing system was found to be important in the cross-sectional study, special efforts were made to recruit farms with contrasting grazing management in each study AEZ. With the exception of UM 2 which had only zero-grazing farms, special efforts were made to recruit zero- and open-grazing farms in the other two AEZs. In UM 1 and UM 4, 100 female calves were targeted, 50 from zero-grazing and 50 from open-grazing, while in UM 2, the target number was 50 calves from the zero-grazing farms. In UM 4 the number of zero-grazing

farms were fewer and a number of farms were recruited from zero-grazing farms along the border of UM 3 and UM 4. The sample size of 50 calves per grazing system was considered a reasonable sample size for statistical analysis purposes (due to various levels of stratification) and was the number that could be covered adequately during the regular visits given the available resources such as finances, personnel, and frequency of visits.

3.2.3 Data and sample collection

The selected farms were visited once every two weeks. Two veterinarians were hired and assisted in farm visits, and in data and sample collection. One veterinarian worked in UM 1 and 2 while the other worked in UM 4. Calves were observed by these veterinarians from recruitment to six months of age. Calves with complete follow-up were sampled biweekly for six calender months resulting in 14 observations. As much as possible, the first visit and sampling were done within the first two weeks of life. Data on routine farm management practices such as tick control procedures and access to pastures/grass were collected during the biweekly visits and were recorded in a closed format questionnaire (Appendix 1.3). The calf identity, visit number and date of visit were recorded. In addition, data on morbidity and mortality were collected on every visit. Farmers usually called the veterinarians for any morbidity or mortality cases that occurred between visits. The specific causes of calf morbidity were established by the veterinarians through clinical diagnosis. Post-mortem was carried out to determine the cause of mortality while ECF was confirmed by serology. Tick infestation was estimated by counting the number of *Rhipicephalus appendiculatus* nymphs (total and engorged) and adult ticks (males, females – non-engorged and engorged) on the body of each calf

(Horak, 1982). The weights of calves were measured and recorded on every visit using a Salter Scale (Avery Kenya Limited, Nairobi, Kenya).

Blood samples, for serum preparation, were taken on every visit. Blood was collected from each calf in two 10-ml plain vacutainer tubes (Becton Dickson Vacutainer Systems-England) by jugular venipuncture. The tubes were labelled and this was verified before drawing the blood from the calves. After collection, blood samples were stored in ice boxes until they could be refrigerated (usually within 2-6 hours). The next day, the sera were separated by centrifugation at 3000 *g* for 20 minutes and divided into four aliquots of approximately 0.5 mls and stored in dry ice (solid CO_2 at approximately -160^{0}C) until they could be transferred to freezers (-20^{0}C) in the laboratory at ILRI in Nairobi. Later in the laboratory at ILRI, ELISA assays were performed on the sera samples as described in section 3.3.

3.2.4 Location of study farms

In order to accurately locate the geographical position of the study farms, a Magellan global position system (GPS) device was used (Magellan Systems Corporation, 1991, 1995, California USA). Using the GPS hand-held receiver, locations for each farm (latitude and longitude) were taken and recorded. The receiver was positioned at the most appropriate point within the homestead, clear of buildings and vegetation so as not to block the satellite signals. A position was calculated within 3-4 minutes. The recorded farm locations were later transferred to the Geographical Information System (GIS) database for Murang'a District at ILRI (Arcview Software, ESRI, 1994, USA) (Figure 3.2).

3.2.5 Data handling and storage

Data files of questionnaires and laboratory results were prepared in Dbase IV Plus (Ashton-Tate Corporation, Torrance, CA, USA). Separate files were prepared for the individual-calf questionnaires and for the ELISA results and all were classified by AEZ and, farm and calf identity. The statistical analyses were conducted in Statistical Analysis Software for Windows (SAS for Windows) Version 6.11 (SAS Institute Inc., Cary, NC, USA). Before the data analysis was initiated, the separate data files were transferred into SAS and merged by AEZ, and farm and calf identity.

3.2.6 Data analysis

3.2.6.1 Measures of calf health and *T. parva* infection

The measures of calf health and *T. parva* infection that were estimated in this study are defined on Table 3.2. Table 3.2 further defines the denominators used for the computation of the measures of calf health. Calf morbidity was defined as any calf sickness that had a recognisable clinical manifestation while calf mortality was defined as any death. Initially, crude calf morbidity and mortality rates were estimated from the morbidity and mortality events recorded and then cause-specific morbidity and mortality were estimated from the diagnoses. The proportional ECF-specific morbidity and mortality rates were also calculated. The ECF morbidity events were initially based on clinical assessment and subsequently confirmed by sero-conversion.

All visits and other outcome events were recorded by the two-week visit interval. Crude and cause-specific risk rates (RR) were calculated for all the outcome events of interest first for the two-week visit intervals and also as overall outcome events for the

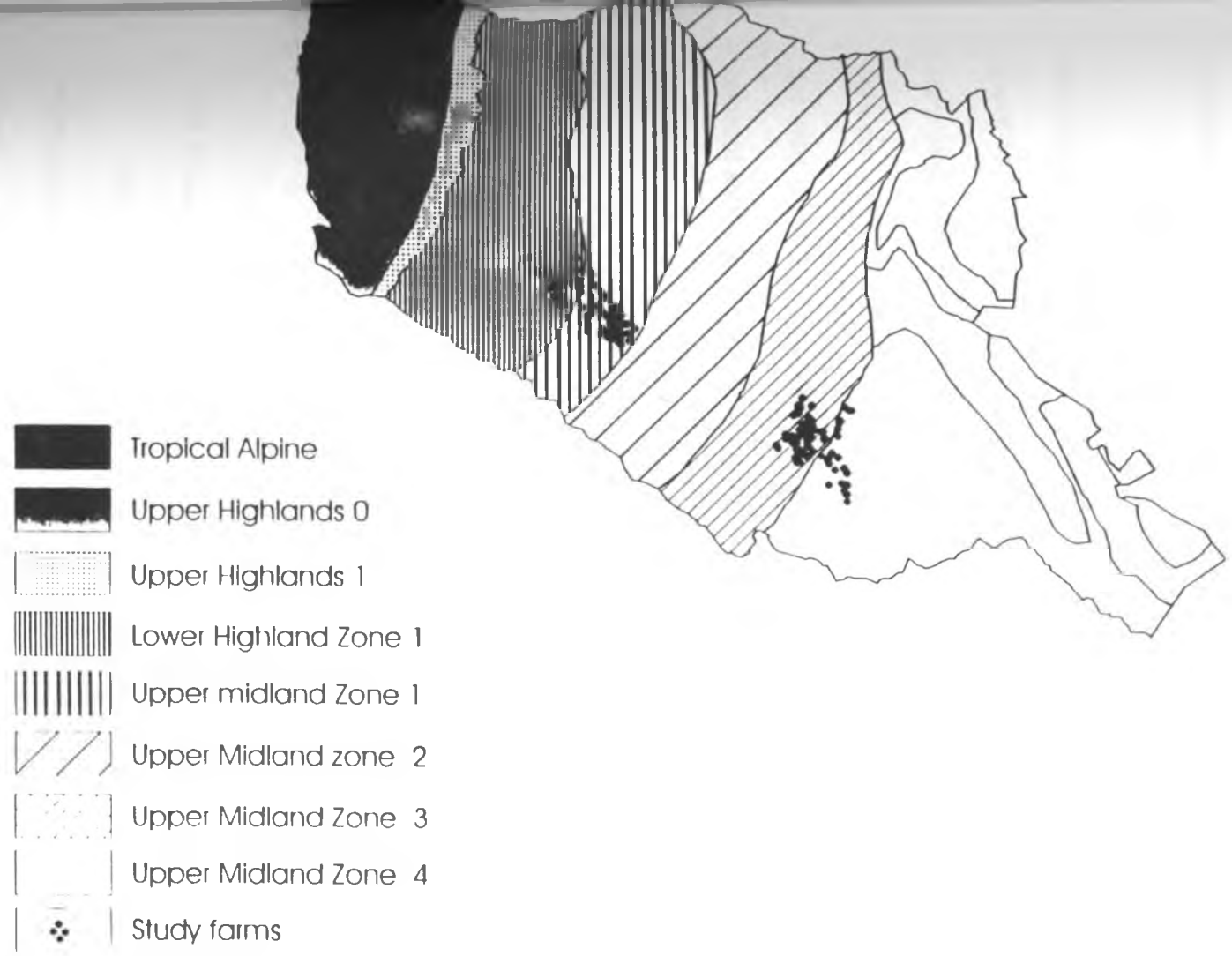


Figure 3.2 Map of Murang'a District Kenya showing the agro-ecological zones and the farms selected for the longitudinal study (March 1995 - August 1996). Source: Geographical Information System (GIS) database for Murang'a District at ILRI (Arcview Software, ESRI, 1994, USA).

entire study period. Risk rates were calculated as the number of events at a given time interval divided by the number of calves at risk at the beginning of the period minus half the number of withdrawals at that period (Table 3.3). For ECF morbidity and mortality, RRs were first calculated for all calves diagnosed as suffering from ECF based on clinical diagnosis and then for calves confirmed as suffering from ECF on ELISA test.

The risk of exposure of calves to *T. parva* was estimated by the sero-conversion incidence rate. Any calf that showed a positive rise in antibody titre of 15PP units and above and sustained this rise for at least two consecutive visits (except those experiencing acute deaths or those which showed a rise in antibody for a single visit only) was considered to have sero-converted and thus had been exposed to *T. parva*. For calves that had initially shown positive antibody titres (presumed to be due to maternally-derived colostral antibodies), any rise of about 15PP units and above from the declining maternally-derived antibody profile was considered as sero-conversion. The risk of *T. parva* exposure (sero-conversion rate) by AEZ, grazing system and age were calculated as the number of calves sero-converting divided by number at risk at the beginning of the interval minus half the withdrawals. The proportional ECF-specific morbidity and mortality in relation to all calves that sero-converted were calculated as number of ECF cases divided by number of calves that sero-converted.

3.2.6.2 Descriptive statistics

The potential explanatory variables and risk factors associated with change in antibody titres, ECF incidence and mean weight gains are shown on Table 3.3. Descriptive statistics were used to classify farm- and calf-related outcomes and to compute associations between these farm- and calf-related outcomes in relation to AEZ, grazing

management, tick control, tick populations, antibody titres and mean "weight gains classes" where applicable. T-tests for paired comparisons were used to compare mean weight gains and antibody titres for calves in same age group (determind by visit). For other comparisons with categorical responses, chi-square (χ^2) tests were used to compare the potential associations by use of cross-tabulations. All the descriptive analysis procedures were performed in SAS.

Mean weight gains were generated from the biweekly weights taken for each calf during each visit. The mean weight gains were calculated as the difference between two consecutive biweekly weights divided by the number of days between these two visits. Scatter plots for mean daily weight gains were generated against the mean age of calves, between two consecutive weights, in days (Excel®, Microsoft Corporation, USA). These plots were created for all calves stratified by AEZ.

The proportion of variations attributed to AEZ, farm, calf and within calf measurements for antibody titres (PP values) and mean daily weight gains were estimated using the Variance Components Estimating Procedure (PROC VARCOMP in SAS).

3.2.6.3 Survival analysis

There were a number of time-to-event outcome measurements of interest and these were, time to decline of maternal antibodies and age to sero-conversion, age to morbidity and mortality and age to development of ECF. Time to decline of maternal antibodies was the age when the maternal antibodies declined below the cut-off level. The age to sero-conversion was age when the antibody titre moved above the cut-off level (or a threshold of 15PP from declining maternal antibodies). The age to morbidity, mortality

and ECF was the age when a calf acquired any of these events. The time interval for the survival analysis was the two-week visit intervals.

The survivorship analysis for age to decline of maternal antibodies and sero-conversion to *T. parva* were estimated using a non-parametric data analysis procedure, the survival analysis. For these two outcomes, the number of calves with the event of interest (and visit when it occurred), those at risk and those censored were entered as separate columns as an input dataset in SAS. The survival times and distribution curves were generated using the PROC LIFETEST procedure in SAS. The survivorship analysis for crude morbidity and mortality and ECF-specific morbidity and mortality rates for the cohort of female calves were calculated using follow-up life table approach (Armitage and Berry, 1988). The first visit was taken as the beginning interval for all calves that were at risk during recruitment, while the second visit was the beginning interval for the group of calves at risk during the second interval and so on. Risk rate for each group of calves in an age interval was calculated as the number of calves with an outcome event of interest divided by number of calves at risk during the beginning of interval minus half the number of withdrawals. Confidence limits (95%) for each interval (age group) were generated using standard errors calculated for each interval. Survivorship curves for each outcome of interest were plotted and confidence limits fitted.

For all the outcomes, the number of calves developing the event of interest or those withdrawn were first recorded for all the AEZs and were then stratified by AEZ. The generalised Wilcoxon Test was used for testing the homogeneity of the survival curves (for PROC LIFETEST procedure) across the three AEZs. This was to establish whether the survival curves and times were significantly different.

3.2.6.4 Simple and multi-variate regression analysis

As the data collected were clustered in time and space, a regression technique for correlated data was used for continuous variables. The Mixed Model Analysis (PROC MIXED), a multi-level regression data analysis was used in the analysis of the continuous repeated measures data in this study. Secondly, data with binary/binomial outcome and multiple variables, were first subjected to the Multiple Logistic Regression (PROC LOGISTIC) and then to the Glimmix Procedure (GLIMMIX - a macro that runs in PROC MIXED). All these procedures were performed in SAS for Windows® (version 6.12).

The Mixed Model Procedure was first used to explore the associations between continuous outcome (dependent) variables and the explanatory (independent or potential risk factors) variables in multi-level models, taking into account both spatial and temporal dependencies. The Mixed Model Procedure estimates the variance components associated with random-effects variables and uses them in estimating the fixed effects parameters (Prescott and Brown, 1994; Littel *et al.*, 1996).

Calf observations were clustered and correlated with time within the individual calf measurements. For the mixed procedure, data were classified by both the farm and calf identity. These variables were considered as the random-effects factors. However, for the mixed models procedures involving one AEZ alone, data were classified by farm identity and calf identity (as AEZ was only in one level) and these two variables were used as random-effects factors in the respective models. The fixed-effect variables entered in the mixed models on data from this study were: AEZ, grazing management, calf housing, breed of calf, dam antibody titres, age of calf, mean weight gains, source of forage, acaricide application, previous acaricide application, East Coast fever, calf sickness and presence of various *R. appendiculatus* tick classes (see section 3.2.3) .

The details on data modelling that were applied in this study in the PROC MIXED procedure are described below. Mixed models were prepared and performed on several data classes in this study. First, associations between each of the continuous outcome variable (antibody titres and mean daily weight gains) and each explanatory (potential risk factor) factor were explored in uni-variate models in PROC MIXED. A normal distribution link function was specified for these two outcome variables.

For all the uni-variate models, data were classified by farm and calf identity and these variables were used as random-effects factors. For the Multi-variate models, one overall mixed model was initially prepared and performed on all the data in all AEZs and classified by farm identity and calf identity. One mixed model was prepared each for UM 1, UM 2 and UM 4. They were also classified by farm and calf identity. Associations between the outcome variables and the explanatory variables were considered significant at $p< 0.1$.

For data with binary outcome, data for calf categories were first prepared for use in the PROC LOGISTIC and GLIMMIX data analysis procedures. When calves acquired the event of interest the first time, that is sero-conversion or ECF, they were classified as sero-converted and ECF categories (coded 1) while those that did not acquire the event of interest were classified as non sero-converted and non-ECF categories (coded 0). For sero-converted and ECF categories, the set of covariates (independent variables) during the visit of event were entered in the model. For the non sero-converted and non-ECF calf categories, one visit was randomly selected and the set of covariates for the visit entered in the model.

The multiple-logistic regression analysis was first used to model the risk factors associated with exposure to *T. parva,* (sero-conversion) and acquisition of ECF. The logistic procedure models a qualitative (binary) outcome variable (such as diseased or not diseased)

against a set of risk factors (independent variables) which may take quantitative or qualitative values. The procedure relates the risk of the outcome variable (e.g. risk of a disease) which is defined as the random variable to a group of risk factors also known as covariates or confounders. The logistic procedure thus defines the probability of a certain outcome measure in relation to the known risk factors (Hosmer and Lemeshow, 1989). As exposure to *T. parva* is generally a sequel to the development of clinical ECF, it was important therefore to model the risk or probability of exposure of the calves to *T. parva* (sero-conversion) and to acquisition of ECF against a set of known covariates (farm and calf-level factors) (Table 3.2) that were considered to be the most important potential risk factors.

A logistic regression model was prepared for all calves in all AEZs. The association between the outcome variables, sero-conversion and ECF and all the potential risk factors were explored together in a multi-variate model. The variable AEZ was forced in the model as the dummy variable for the intercept with UM 1 as the baseline risk AEZ. All the logistic regression models were fitted by backward elimination procedure. All risk factors were considered significant at the level of $p<0.05$.

In the second analysis, the Glimmix Procedure was applied to model the factors associated with sero-conversion and ECF. Unlike the logistic regression procedure, the Glimmix Procedure is a multi-level mixed model procedure for outcome variables with a binomial distribution. This procedure accounts for both the random and fixed effects variables in the analysis and estimates the variance components associated with random-effects variables and uses them in estimating the fixed effects parameters (Prescott and Brown, 1994; Littel *et al.*, 1996).

A Glimmix model was prepared for all calves in all AEZs. The association between the outcome variables, sero-conversion and ECF and all the potential explanatory

factors were explored together in a Multi-level model. Data were classified by farm and calf identity and these two variables were used as random-effects factors in the model. All risk factors were considered significant at the level of $p<0.1$.

3.3 LABORATORY DETERMINATION OF ANTIBODY TITRES TO *T. PARVA*

3.3.1 Enzyme-linked-immunosorbent assays

The enzyme-linked-immunosorbent assay (ELISA) technique was applied to evaluate the level of antibodies to certain tick-borne parasites in order to estimate prevalence and sero-conversion. In the cross-sectional study, the prevalence of antibodies to *T. parva, T. mutans* and *Babesia bigemina* were evaluated, while in the longitudinal study, the incidence of *T. parva* antibodies was estimated. All the serology work was carried out at the ILRI laboratories in Nairobi, Kenya.

The ELISA test was carried out using the method of Katende *et al.*, (1990, 1997). Briefly, polysorb micro-ELISA plates (Polysorb, Nunc, Denmark) were coated with recombinant antigens at a concentration of 50 ng/well and kept frozen. The specific recombinant antigens were used as follows: *T. parva*, the polymorphic immunodominant (PIM) antigen, *T. mutans*, p32 kiloDalton antigen and *B. bigemina*, p200 kiloDalton antigen. Excess antigen solution was discarded and the uncoated sites on wells of the microtitre plate and the non-specific sites on the antigen were blocked by adding 0.25% casein and incubating at 37^{0}C for two hours. Test sera, diluted 1:200 (*T. parva*) and 1:100 (*T. mutans* and *B. bigemina*) in Dulbeco's phosphate buffered saline (DPBS) pH 7.4, containing 0.1% Tween 20 and 5% skimmed milk, were added to wells of the micro-ELISA plate in two replicates (100 µl per well). Control sera (strong positive, weak positive and

negative control) were diluted as for the test sera. These and the conjugate control (DPBS alone) were added to the plate in four replicates. The antibodies were allowed to bind to the antigen by incubating the plate for 25 minutes at room temperature (RT) with continuous gentle agitation on a micro-agitator (Heidolph, France). The unbound antibodies were removed by washing the plate extensively with DPBS before adding to each well 100 µl anti-bovine Ig horse radish peroxidase (HRP) conjugate, diluted 1:5000 in DPBS containing 0.1% Tween 20 and 2.5% skimmed milk. The plate was incubated at 25^{0}C at room temperature and washed as before. The reaction was developed by addition of 100 µl sodium citrate buffer pH 4.0 containing 1% hydrogen peroxide as a substrate and 40 mM 2,2'-azino-*bis* (3-ethylbenz-thiazoline-6-sulphuric acid), diammonium salt (ABTS) as chromogen and incubated for 1 hour in the dark. During incubation, the micro-ELISA plate was shaken for five minutes every 15 minutes to ensure maximum colour development. The optical density (OD) was determined in a Multiscan MCC/340 spectrophotometer (Biological Diagnostic Supplies Ltd., (BDSL), UK).

Optical density (OD) readings from the reference highly-positive control sera were used to compute the percent positivity for the test sera (Wright *et al.*, 1993). Percent positivity (PP) for test serum was expressed as follows: test serum OD divided by the mean OD from the strong positive control serum and expressed as percent (simply expressed: (OD of test/OD of strong positive) x 100)). These were obtained from the linear curve of OD against the reciprocal of serial dilutions. (Wright *et al.*, 1993; Katende *et al.*, 1997). All the results were expressed as percent positivity (PP).

Percent positivity (PP) values were preferred to optical density readings as the PP values were adjusted for variations associated with inconsistent background activity while

performing the ELISA tests (Wright *et al.*, 1993). The test error may arise from varying laboratory conditions such as handling of sera and preparation of sera dilutions.

For the purpose of data analysis, any reading of 15 PP or above was considered a positive test for antibodies to *T. parva* PIM recombinant antigen. For *T. mutans* and *B. bigemina*, a sample was considered positive if the PP value was 20 or above.

Table 3.1 Potential explanatory and risk factors associated with antibody prevalence to *T. parva, T. mutans and B. babesia* from the cross-sectional study in Murang'a District, Kenya (March 1994 - June 1994.

Geographical factors
Agro-ecological zone: Lower Highlands 1, Upper Midlands 1, 2, 3, 4
Calf-related factors
Breed of calf or dam or sire: Friesian\Guernsey\Ayrshire\Jersey\Zebu\Cross (exotic*exotic)\Cross (exotic*Zebu)
Method of insemination used: artificial insemination/natural service (bull)
Age of calf: in months during the visit
Sex of calf: male/female
Calf sickness: no/yes
History East Coast fever: no/yes
History of anaplasmosis: no/yes
History of babesiosis: no/yes
Number of tick (all types): numbers (numerical counts)
Calf management factors
Age calf weaned: months
Where calf kept before weaning: indoors/outdoors/combination of indoors and outdoors
Where calf is currently kept: indoors/outdoors/combination of indoors and outdoors
Forage offered to calves presently: pastures (grazing)/cut fodder
Source of forage: owners farm/from outside the farm/combination of both
Method of tick treatment: none/dipping/spraying/handwash
Age of first tick treatment: in months
When calf last treated for ticks: < 7 days/1 - 2 weeks/>2 - 4 weeks/> month
Number of tick treatment times last 6 months: none/1 - 5/6 - 10/> 10

Table 3.2. Definitions of specific measures of outcome for calf health and *Theileria parva* infection.

Outcome measure	Definition
Morbidity	Any disturbance in normal health that had a clear clinical manifestation, initially reported by the farmer and verified by the veterinarian.
Mortality	Any calf death, irrespective of cause.
East Coast fever	Calf morbidity with the following clinical syndrome: fever (41.0^0C or above), enlarged superficial lymph nodes (especially parotid and prescapular), petechiation on oral mucous membranes, harsh lung sounds (breathing) and sometimes coughing; positive antibody results on ELISA except cases with acute death before antibody response.
Cause-specific morbidity	Calf morbidity due to a defined or established cause.
Cause-specific mortality	Calf death due to a defined or established cause.
Sero-conversion	A persistent rise in antibody level to *T. parva* above the cut-off point of 15PP or above for at least two consecutive visits. For calves showing presence of maternal antibodies, a positive rise was considered after the maternal antibodies had declined. For calves showing no maternal antibodies, any rise at any point was considered sero-conversion.
Animals-at-risk	Number of calves at risk from a specified cause for the study period or AEZ or grazing system or age group.
Number at the beginning	Number of calves at risk at the beginning of a specified interval period (visit here defined as interval)
Risk rates	number of calves with an event of interest / number at risk at the beginning of period - 1/2 withdrawals

Table 3.3 Potential explanatory and risk factors associated with incidence of antibodies to *T. parva*, ECF and mean daily weight gain from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Geographical factors

Agro-ecological zone: Upper Midlands 1\Upper Midlands 2\Upper Midlands 4

Calf-related factors

Breed of calf or dam or sire: Friesian\Guernsey\Ayrshire\Jersey\Zebu\Cross (exotic*exotic)\Cross (exotic*Zebu)
Age of calf: in days at first visit and during subsequent visits
Calf sickness (any) since birth or last visit: no/yes
East Coast fever diagnosis (clinically)?: no/yes
East Coast fever diagnosis (confirmation)?: no/yes
Calf withdrawal from study: no/died/withdrawn/sold
Weight of calf: weight in kgs during current visit

Calf management factors

Grazing system: zero-grazing/open-grazing
Where calf is kept currently: indoors/outdoors/combination of indoors and outdoors
Feed given to calf presently: forage/concentrates/minerals/legumes/milk
Source of forage: owners farm/from outside the farm/combination of both
Method of tick treatment: none/dipping/spraying/handwash
Was calf treated for ticks since last visit: no/yes

Rhipicephalus appendiculatus tick count information

Adult males: numbers counted on each calf
Adult females total: numbers counted on each calf
Adult females engorged: numbers counted on each calf
Total nymphs: numbers counted on each calf
Total engorged nymphs: numbers counted on each calf

CHAPTER 4

RESULTS

4.1 THE CROSS-SECTIONAL STUDY

4.1.1 Farm Demographics

The highest mean percent of the land was devoted to subsistence farming (growing of food crops for home consumption) irrespective of the agro-ecological zone (Table 4.1). The sampling frame included all farms that were either members of the dairy societies or were registered with communal diptanks. Of these farms, 90% (678/750) had subsistence farming as part of the farming activity while 82% (612/750) had some portion of the land devoted to dairy farming, either as grazing paddocks or as cultivated fodder. During the time the study was conducted, 93% (699/750) of the farms had at least one bovine animal on the farm. Seven percent (51/750) of the farms without cattle cited recent death or sale of animals as reasons of not having them, suggesting that livestock ownership was approximately 100%. In 87 farms, cattle were present though land was devoted to grazing or cultivated fodder hence 612 farms recorded that a portion of land was devoted to dairying while 699 farm recorded presence of cattle. In these 87 farms, zero-grazing system was used and all fodder was brought from outside the farm or the owners used communal grazing lands.

The mean number of cattle (all ages) for farms that had at least one bovine animal was 2.6 (1848 cattle in 699 farms) with 69% (1270/1848) being adult cattle over 18 months old (Table 4.2). Approximately 13% (236/1848) of the cattle were calves up to six months old. At the time of carrying out the study, only 41% (305/750) of the farms had calves aged between 6-18 months on the premises. The largest number of calves was found

Table 4. 1. Distribution of farming activities among the 750 smallholder farms from the cross-sectional study in Murang'a District, Kenya (March 1994 - July 1994).

Farming activity	No. of farms (%)	Mean % farm activity	Median	Std Dev	Std error	Range	Confidence Limits (95%)
Dairy	612 (82)	23.7	20	11.4	0.462	5-90	22.8, 24.6
Coffee	465 (62)	30.1	30	14.9	0.689	2-80	28.8, 31.5
Tea	189 (25)	35.6	35	17.2	1.267	1-90	33.1, 38.1
Horticulture	11 (1.5)	29.1	25	23.5	7.097	5-80	15.2, 43.0
Subsistence Farming	678 (90)	44.9	40	19.4	0.746	5-95	43.4, 46.4

Table 4.2. Distribution of cattle by breed and age (number of farms in brackets) among the 750 smallholder dairy farms participating in the cross-sectional study in Murang'a District, Kenya (March 1994 - July 1994).

Breed[a]	< 6 months	6-18 months	> 18 months	Total Cattle	Breed (%)
Friesian	32(31)	65(63)	327(228)	424(312)	22.9
Guernsey	27(25)	39(36)	193(161)	259(222)	14.0
Ayrshire	17(14)	25(23)	85(69)	127(106)	6.9
Jersey	0(0)	3(3)	11(10)	14(13)	0.8
Zebu	16(14)	24(15)	141(64)	181(93)	9.8
Cross (Exo*exo)[b]	103(92)	141(112)	250(192)	494(396)	26.7
Cross(Exo*loc)[c]	41(34)	62(52)	242(111)	345(197)	18.7
Other breeds[d]	0(0)	3(3)	1(1)	4(4)	0.2
Total	236(194)	362(305)	1250(655)	1848(699)	100

[a]Breed identity based on phenotypic classification by the author
[b]Exo*exo means crosses between various exotic breeds of cattle
[c]Exo*loc means crosses between exotic and local (Zebu) breeds of cattle
[d]Other breeds found in very small numbers were Sahiwal and Boran.

in UM 4 (24.9%) while the smallest number was found in UM 3 (17.7%) (Table 4.3). Most farms without calves aged between 6 and 18 months reported no calving on the farm and about 10-15% reported recent deaths of calves. A total of 362 calves were found in various AEZs and sublocations (Table 4.3). About 86% of the farms had only one calf aged between 6-18 months, while 12% had two and the remaining 2% had 3 and 4 calves. The mean number of calves in these farms in that age group was 1.2 (362/305).

4.1.2 Response rate

Of the original 750 farmers initially selected, 35 could not be located, 13 were found to be in other subdivisions and three had moved (Table 4.4). This gave a potential farm participatory rate (by farm location) of 92.8% (696/750). However, the 35 farmers which were not located initially were all replaced by the next farms appearing on the lists giving a total of 750 farmers targeted to participate. During the actual farm visits, three farmers declined to participate. A final voluntary participatory rate of 99.6% (747/750 farms) was thus achieved.

4.1.3 Distribution of potential risk factors

The distribution of cattle types in the study area were: Taurine 73.5% (266/362), Taurine-Zebu 20.4% (74/362) and Zebu 6.1% (22/362) with the latter present only in UM 4 (Table 4.5). In this study, calves were defined as any bovine between 6-18 months old. The mean age for all the calves studied was 10.9 months (standard deviation 3.6).). There were 148 (41%) males and 214 (59%) females. The mean age for females was 11.3 months and for males 10.4 months.

Table 4.3. Number of calves by sub-location, Division and AEZ from the cross-sectional Study in Murang'a District, Kenya (March 1994 - July 1994).

AEZ	Sub-location	Division	Number no. of calves	Total calves(%)	Number of farms	Total no. of farms
LH 1	Gatunguru	Kangema	21		19	
	Kairo	Kangema	25	67(18.5)	19	57
	Kamukabi	Kigumo	21		19	
UM 1	Gathaithi	Kiharu	28		24	
	Mungaria	Kandara	23	75(20.7)	20	66
	Rwegetha	Gatanga	24		22	
UM 2	Gikui	Kangema	18		16	
	Watuha	Kangema	21	66(18.2)	21	62
	Ithang'arari	Gatanga	27		25	
UM 3	GatheruKiharu		16		15	
	Ichagaki `C	Kigumo	27	64(17.7)	24	54
	Kabati	Kandara	21		15	
UM 4	Githuuri	Kiharu	37		26	
	Wemba	Makuyu	32	90(24.9)	23	66
	Makuyu	Makuyu	21		17	
Total				362		305

Key:

LH 1 = Lower Highland Zones (Tea and Dairy)
UM 1 = Upper Midland Zones (Coffee and Tea)
UM 2 = Upper Midland Zones (Main Coffee)
UM 3 = Upper Midland Zones (Marginal Coffee)
UM 4 = Upper Midland Zones (Maize and Sunflower)

Table 4.4. Response rate by sub-location and AEZ from the cross-sectional study in Murang'a District, Kenya (March 1994 - July 1994).

AEZ	Sub-location	Initial Voluntary	Initially Unavailable	Initial Refusal	Total farms
LH 1	Gatunguru	47	3	0	50
	Kairo	50	0	0	50
	Kamukabi	45	5	0	50
UM 1	Gathaithi	48	2	0	50
	Mung/Mukangu	49	0	1	50
	Rweg/Chomo	47	3	0	50
UM 2	Gikui	50	0	0	50
	Watuha	45	5	0	50
	Ithang'arari	45	4	1	50
UM 3	Gatheru	45	5	0	50
	Ichagaki 'C	39	11	0	50
	Kabati	48	1	1	50
UM 4	Githuuri	48	2	0	50
	Wemba	42	8	0	50
	Makuyu	48	2	0	50
Total		696	51	3	750

Initial potential participatory rate (by farm location) = 92.8% (696/750)
Final voluntary participatory rate = 99.6% (747/750)
35 - not traced originally
13 - different sublocation (recent demarcation)
3 - moved to different localities (outside the sublocation)
3 - refused to participate (did not risk animals to be bled)

Table 4.5. Characteristics of 362 selected calves in Murang'a District Kenya, in terms of location, breed, age, sex, housing, feeding and tick control.

Category	Number of calves	Percentage
Agro-ecological zone		
Lower Highlands 1	67	18.5
Upper Midlands 1	75	20.7
Upper Midlands 2	66	18.2
Upper Midlands 3	64	17.7
Upper Midlands 4	90	24.8
Breed		
Exotic (Bos taurus)	266	73.5
Zebu and crosses (Bos indicus)	96	26.5
Sex		
Male	148	40.9
Females	214	59.1
Calf kept before weaning		
Indoors	230	63.5
Outdoors	132	36.5
Calf currently kept		
Indoors	181	50.0
Outdoors	181	50.0
Forage given presently		
Open grazing	159	43.9
Cut fodder or grass	203	56.1
Source of forage feed		
Owners farm	162	44.8
Brought outside the farm	262	55.2
Tick treatment method		
None	203	56.1
Any method	159	43.9
Last tick treatment		
≤ 2 weeks	89	24.6
> 2 weeks	273	75.4
No. of times (last 6 months)		
≤ 5	205	31.2
> 5	157	47.1

4.1.4 Patterns of serum antibody prevalence to *T. parva, T. mutans* and *B. bigemina*

The mean antibody prevalence (with 95% confidence limits for the binomial proportions) for *T. parva, T. mutans* and *B. bigemina* by AEZs and grazing systems are shown in Table 4.6 and Figures 4.1 and 4.2. There were significant ($p<0.05$) differences in serum antibody prevalence for the different TBD parasites across the AEZs as indicated on Table 4.6. As the main interest and focus on the study was *T. parva*, the details on its prevalence by sublocation and AEZ are shown in Table 4.7. The antibody prevalence to *T. parva* in UM 4 was significantly different ($p<0.05$) from the other AEZs. There were further significant ($p<0.05$) differences in *T. parva* antibody prevalence across some sublocations within an AEZ for each AEZ except UM 4 (Table 4.7).

Three factors were significantly associated with variations in antibody prevalence to *T. parva* from the linear regression model and these were: agro-ecological zone, breed of calf ($p<0.05$) and grazing system ($p<0.1$) (Table 4.8). Zebu breeds and their crosses with Taurine breeds were significantly ($p<0.05$) associated with higher prevalences than Taurine breeds. Higher prevalence was significantly ($p<0.05$) associated with calves on open grazing than those on zero-grazing. The interaction terms between AEZ, breed and grazing were not significant ($p>0.05$).

For *T. mutans*, only the breed of the calf was significantly ($p<0.05$) associated with differences in prevalence. Zebu calves and their crosses with Taurine breeds had significantly ($p<0.05$) higher antibody prevalence than Taurine breeds.

For *B. bigemina,* the factors significantly associated with antibody prevalence were: AEZ, grazing system, age of calf, tick control procedure ($p<0.05$) and sex of calf ($p<0.1$). Agro-ecological zones LH 1 and UM 1 were significantly ($p<0.05$) associated with higher prevalence than the other three AEZs. Higher prevalences were significantly ($p<0.05$)

associated with calves grazed freely, when compared to those partially or completely confined. Significantly ($p<0.05$) higher prevalences were seen in older calves and male calves than in younger and female calves. Calves that reportedly had never received any acaricide treatment had significantly ($p<0.05$) higher prevalence than those that had received acaricide treatment, regardless of the method used. The interaction terms between AEZ, breed and grazing were not significant ($p>0.05$).

The results from the logistic regression model are shown on Table 4.9. Zebu breeds and their crosses were twice as likely to have the risk of having positive antibody titres to *T. parva* than Taurine breeds (odds ratio = 2.0).

For *T. mutans*, the only significant ($p<0.01$) factor associated with variation in prevalence was AEZ. Calves raised in UM 4 were at twice the risk of being associated with a positive antibody titre than those raised in other AEZs.

Various factors were significantly ($p<0.05$) associated with positive antibody titres for *B. bigemina*. Three AEZs (LH 1, UM 1 and UM 4) had higher risks of seropositivity compared to other AEZs. Also, calves on a free grazing system had higher positive antibody titres than those confined to zero-grazing units. Males calves were significantly ($p<0.05$) more likely to have a positive antibody titre than female calves. The interaction terms between AEZ, breed and grazing were not significant ($p>0.05$).

Table 4.6. Mean antibody prevalence (%), with 95% confidence limits, to *Theileria parva*, *Theileria mutans* and *Babesia bigemina* by agroecological zones and grazing system (March – June 1994).

	Theileria. parva[a]	*Theileria mutans*[b]	*Babesia bigemina*[c]
Agro-ecological zone			
LH 1	18.0[1] (8.8, 27.2)	4.5[1,2] (0, 20.2)	47.8[1] (35.8, 59.8)
UM 1	26.7[1] (16.7, 36.7)	12.0[1,2] (4.7, 19.4)	49.3[1] (37.9, 60.6)
UM 2	28.8[1] (17.9, 39.7)	1.5[1] (0, 10.8)	12.1[1,2] (4.2, 19.9)
UM 3	37.5[1] (25.6, 49.3)	4.7[1,2] (0, 21.1)	20.3[2] (10.4, 30.2)
UM 4	72.2[2] (62.9, 81.5)	27.8[2] (18.6, 37.1)	21.3[2] (12.7, 29.9)
Grazing system			
Open Grazing	63.2[1] (50.7, 75.7)	35.1[1] (22.7, 47.5)	28.1[1] (16.4, 39.7)
Zero-Grazing	30.5[2] (24.2, 36.9)	5.4[2] (0, 15.3)	23.7[2] (17.8, 29.5)
Combination	41.2[2] (31.6, 50.7)	9.8[1,2] (0, 28.0)	46.1[1] (36.4, 55.8)

[a]Tested for antibodies against Polymorphic Immunodominant Molecule (PIM) recombinant antigen.
[b]Tested for antibodies against Protein 32 Kilodaltons antigen.
[c]Tested for antibodies against Protein 200 Kilodaltons antigen.
[1,2]Antibody prevalence estimates for each parasite within AEZ or grazing system with different superscripts significantly ($p<0.05$) different.

Table 4.7. Antibody prevalence (%) to *Theileria parva* by Sub-location and AEZ from the cross-sectional study (March – June 1994).

AEZ	Sub-location	Number positive	Total calves	% +ve calves	Mean % for AEZ
LH 1	Gatunguru	7	21	33.3^{2}	
	Kairo	3	25	12.0^{1}	17.9^{1}
	Kamukabi	2	21	9.5^{1}	
UM 1	Gathaithi	4	28	14.3^{1}	
	Mungaria/Mukangu	9	23	39.1^{2}	26.7^{1}
	Rwegetha/Chomo	7	24	29.2^{2}	
UM 2	Gikui	8	18	44.4^{2}	
	Watuha	4	21	19.5^{1}	28.8^{1}
	Ithang'arari	7	27	26.0^{1}	
UM 3	Gatheru	2	16	12.5^{1}	
	Ichagaki 'C	9	27	33.3^{2}	37.5^{1}
	Kabati	14	21	66.7^{3}	
UM 4	Githuuri	26	37	70.3^{1}	
	Wemba	27	32	84.4^{1}	72.2^{2}
	Makuyu	13	21	62.0^{1}	

^{1,2}Antibody prevalence estimates for each parasite within sublocation or AEZ with different superscripts are significantly different ($p<0.05$).

Key:

LH 1 = Lower Highland Zones (Tea and Dairy)
UM 1 = Upper Midland Zones (Coffee and Tea)
UM 2 = Upper Midland Zones (Main Coffee)
UM 3 = Upper Midland Zones (Marginal Coffee)
UM 4 = Upper Midland Zones (Maize and Sunflower)

Table 4.8. Variables associated with *Theileria parva*, *Theileria mutans* and *Babesia bigemina* antibody titres from the linear regression model for 362 calves in Murang'a District, Kenya (March – June 1994).

Variable[1]	b	Se(b)	p-value
Theileria parva[a]			
Upper Midlands 3 (UM 3 = 1, other AEZs = 0)	7.58	2.62	0.004
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	11.51	3.90	0.003
Breed (Exotic = 0, Zebu*crosses = 1)	9.64	3.74	0.010
Grazing system (indoors = 1, outdoors = 0)	-3.35	1.99	0.095
Theileria mutans[b]			
Breed (Exotic = 0, Zebu*crosses = 1)	-2.02	0.27	0.0001
Babesia bigemina[c]			
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	6.51	1.66	0.0001
Grazing system (indoors = 1, outdoors = 0)	-3.70	1.45	0.010
Age of calf (absolute value)	0.43	0.19	0.020
Sex (males = 0, females = 1)	-2.25	1.34	0.090
Tick control (none = 1, any method = 0)	6.00	2.32	0.010

[1]Variables considered significant at $p<0.1$.
[a]Tested for antibodies against Polymorphic Immunodominant Molecule (PIM) recombinant antigen.
[b]Tested for antibodies against Protein 32 Kilodaltons antigen.
[c]Tested for antibodies against Protein 200 Kilodaltons antigen.

Table 4.9. Variables associated with *Theileria parva, Theileria mutans* and *Babesia bigemina* antibody titres from the logistic regression model for 362 calves in Murang'a District, Kenya, March – June 1994.

Variable[1]	b	Se(b)	p-value
Theileria parva[a]			
Breed (Exotic = 0, Zebu*crosses = 1)	-2.02	0.27	0.0001
Theileria mutans[b]			
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	-2.29	0.89	0.010
Babesia bigemina[c]			
Upper Midlands 2 (UM 2 = 1, other AEZs = 0)	1.69	0.44	0.0001
Upper Midlands 3 (UM 3 = 1, other AEZs = 0)	1.22	0.37	0.001
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	1.36	0.32	0.0001
Grazing system (indoors = 1, outdoors = 0)	0.61	0.28	0.030
Sex (males = 0, females = 1)	-0.53	0.26	0.040

[1]Variables considered significant at $p<0.05$.
[a]Tested for antibodies against Polymorphic Immunodominant Molecule (PIM) recombinant antigen.
[b]Tested for antibodies against Protein 32 Kilodaltons antigen.
[c]Tested for antibodies against Protein 200 Kilodaltons antigen.

Figure 4.1 Mean serum antibody prevalence (with 95% confidence limits) to *Theileria parva, Theileria mutans* and *Babesia bigemina* by agro-ecological zone from the cross-sectional study in Murang'a District, Kenya (March - June, 1994.

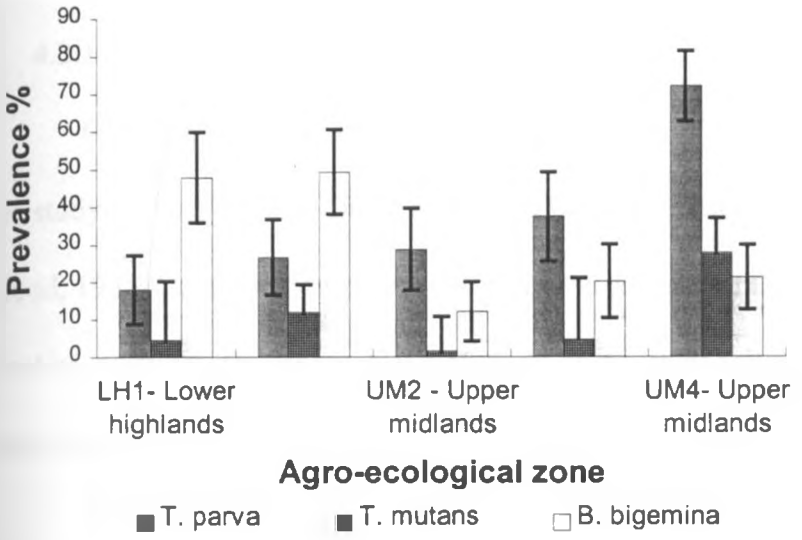
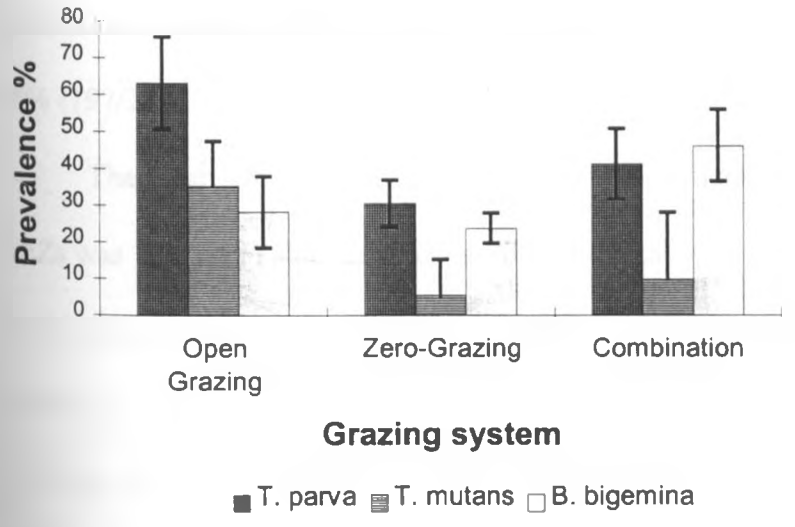


Figure 4.2 Mean serum antibody prevalence (with 95% confidence limits) to *Theileria parva, Theileria mutans* and *Babesia bigemina* by grazing management from the cross-sectional study in Murang'a District, Kenya (March - June, 1994.



4.2 THE LONGITUDINAL STUDY

4.2.1 Calf and farm factors

4.2.1.1 Calf factors

Of the 225 cohort of female calves in the study, 28 (12%) were withdrawn from the study for two reasons. One was that the farmers did not permit the withdrawal of blood, 19 (8%), and the other was that recruited calves were sold, 9 (4%), (Table 4.10). A total of 29 calves did not reach end of study, that is their follow-up was incomplete as the study ended before they had reached 14th visit (Table 4.10).

The cohort of female calves in the three AEZs and two grazing systems were distributed by AEZ as shown in Table 4.11. In UM 1, a total of 76 calves were recruited, 34 (45%) from zero-grazing system and the other 42 (55%) from the open grazing system. In UM 2 all the 50 calves studied were from the zero-grazing system as per the study design. In UM 4, a total of 99 calves were studied, 50 (50.5%) from zero-grazing system and 49 (49.5%) from the open grazing system. The overall follow-up rate was thus 88% (197/225).

The mean age for calves at the entry into the longitudinal study (visit 1) in all AEZs was 10 days (Table 4.10). Over 90% of the calves in the cohort were less than 2 weeks old at the time of recruitment and the age classes during the first visit were as follows: 1-5 days, 56 (25%); 6-10 days, 76 (38%); 11-15 days, 76 (38%); and > 16 days, 17 (8%). The age in the subsequent visits increased uniformly by about 14 days. The mean age of the calves at the end of the study (visit 14) was 192 days (approximately 6.4 months) and the age classes were as follows: 182-191 days, 76 (48%); 192-201 days, 75 (48%); and > 201 days, 7 (4%).

Five calf breeds and their crosses were differentiated through phenotypic identification (Table 4.12). The most prevalent specific cattle breed was Friesian, 18% (41/225) of all calves. The next prevalent breeds were Ayrshire (6.2%, 14/225) and Zebu (3.6%, 8/225) while the rest of the breeds made up less than 1% of the total. Crosses among exotic breeds (*Bos taurus*) comprised the largest category 65% (146/225) while crosses between exotic and Zebu (*Bos indicus*) comprised 6% (13/225). Zebu and their crosses with exotic breeds were found mainly in UM 4.

Table 4.10. Number of calves indicating reasons for withdrawal and deaths in all AEZs by visit (mean age in days in brackets), from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Visit (age in days)	Withdrawal of blood[a]	Calves sold[b]	End of study[c]	Calf deaths[d]
1 (10)	0	0	0	0
2 (24)	1	0	0	1
3 (38)	1	0	1	2
4 (52)	5	0	0	4
5 (66)	2	0	4	2
6 (80)	5	0	2	3
7 (94)	2	0	4	1
8 (109)	0	1	3	1
9 (123)	0	2	3	1
10 (136)	1	2	4	0
11 (152)	1	0	3	0
12 (164)	0	1	2	0
13 (178)	0	2	3	0
14 (192)	1	1	0	0

[a]Calves withdrawn due to withdrawal of blood (6 in UM 1, 1 in UM 2 and 12 in UM 4)
[b]Calves sold (3 in UM 1, 5 in UM 2 and 1 in UM 4)
[c]Calves censored (9 in UM 1, 7 in UM 2 and 13 in UM 4)
[d]Calf deaths (5 in UM 1, 1 in UM 2 and 9 in UM 4)

4.2.1.2 Farm factors

A total of 188 farms were purposively recruited for the longitudinal study and these had a total of 225 female calves. Eighty nine percent (168/188) of the farms had one calf; 9% (16/188) had 2 calves and 2% (4/188) had more than 2 calves (Table 4.11). Table 4.11 further shows the distribution of calf numbers based on age, grazing system and calf rearing system in various AEZs.

Farmers routinely keeping calves under strict confinement were: 24%, 15% and 11% in UM 1, UM 2 and UM 4 respectively (Table 4.11). Those allowing calves access to outdoors were: 22%, 23% and 68% in UM 1, UM 2 and UM 4 respectively. The rest of the farmers raised calves by a combination of both methods. These routine practices of calf housing were significantly ($p<0.001$) different across the AEZs.

Table 4.13 shows the various feeding management practices routinely employed by the farmers during the longitudinal study. Feeding of fodder/forage to the calves was the most popular practice by the farmers and was recorded about 90% of the time in all AEZs except during the first visit, when the calves were presumably still very young. Farmers reported feeding of commercial concentrate feeds to calves less than 20% of the time during the visits in all AEZs. Farmers that were reported to be giving commercial concentrate feeds were: 19%, 17% and 8% in UM 4, UM 2 and UM 1 respectively and this practice was significantly ($p<0.01$) different across the AEZs. Except in UM 4 where 15% of the farmers reported giving mineral supplements to calves, farmers in UM 1 and UM 2 reported less use of mineral supplements (< 1%) and this was significantly ($p<0.01$) different across the AEZs. Supplementing calves with legumes (e.g. sweet potato vines) was rare (less than 1%) in all AEZs.

Most of the forage consumed by the calves in UM 1 and UM 2 (100% and 99.5%

respectively) was reported to have been obtained from within the farm (Table 4.14). In UM 4, about two thirds (65%) of the times during the visits, forage for calves was reported to have been obtained from within the farm, while one third (35%) of the times, it was from both within and outside the farm. The latter was encountered only in UM 4

The methods used for tick control are shown in Table 4.15. There was significant ($p<0.01$) difference in the methods used for tick control across the AEZs. Overall, the most widely reported method was hand-washing, followed by hand spraying; the least reported method was dipping. Upper Midlands 4 recorded the highest use of acaricides; 17%, 12% and 0.2% for hand-washing, hand-spraying and dipping respectively and were significantly ($p<0.01$) different. In UM 1 and UM 2, the use of acaricides was reported 2% and 0.2% of the times respectively. The results on current acaricide use agreed with the results on past acaricide use as reported in retrospect (Table 4.15).

Table 4.16 shows the distribution of *Rhipicephalus appendiculatus* tick classes. Ticks were most abundant in UM 4, few in UM 1 and very few were observed in UM 2. The class most frequently observed was non-engorged adult female ticks (26% in UM 4, 1.7% in UM 1 and 0.2% in UM 2) followed by adult male ticks (24% in UM 4, 1.2% in UM 1 and 0% in UM 2). Engorged adult females and total nymphs were observed in the same frequencies (6.5% and 6.3% in UM 4 respectively, 1.5.% and 0.6% respectively in UM 1 and 0.2% and 0% respectively in UM 2). The class less frequently observed was engorged nymphs (2.0%, 0.2% and 0% in UM 4, UM 1 and UM 2 respectively).

Table 4.11. Distribution of calf numbers within farm, calf management and housing from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Variable		Number of farms (no. of calves)	Percentage of farms
Calf numbers			
1 calf	UM 1	50 (50)	90
	UM 2	38 (38)	86
	UM 4	79 (79)	90
	Total	167 (167)	89
2 calves	UM 1	3 (6)	5
	UM 2	6 (12)	14
	UM 4	8 (16)	9
	Total	17 (34)	9
> 2 calves	UM 1	3 (19)	5
	UM 2	0	0
	UM 4	1 (3)	1
	Total	4 (22)	2
Grazing system[1]			
Upper Midlands 1			
	Zero-grazing	34	44.7
	Open grazing	42	55.3
Upper Midlands 2			
	Zero-grazing	50	100
	Open grazing	0	0
Upper Midlands 4			
	Zero-grazing	50	50.5
	Open grazing	49	49.5
Current calf housing[2]			
Upper Midlands 1			
	Indoors	218	24.0
	Outdoors	195	21.5
	Combination	494	54.5
Upper Midlands 2			
	Indoors	94	15.1
	Outdoors	142	22.8
	Combination	387	62.1
Upper Midlands 4			
	Indoors	127	11.4
	Outdoors	764	68.3
	Combination	227	20.3

[1]refers to grazing system on the farm during the first visit
[2]refers to routine practice where the calf spent the day throughout the study period, and this was significantly different across the AEZs ($p < 0.001$).

Table 4. 12. Distribution of breeds by AEZ for the 225 calves studied in the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Breed[a]	Number of calves	Total calves	Total (%)
Friesian			
Upper Midlands 1	22		
Upper Midlands 2	17	41	18.2
Upper Midlands 4	2		
Guernsey			
Upper Midlands 1	0		
Upper Midlands 2	1	2	0.9
Upper Midlands 4	1		
Ayrshire			
Upper Midlands 1	8		
Upper Midlands 2	3	14	6.2
Upper Midlands 4	3		
Jersey			
Upper Midlands 1	0		
Upper Midlands 2	0	1	0.4
Upper Midlands 4	1		
Zebu			
Upper Midlands 1	0		
Upper Midlands 2	0	8	3.6
Upper Midlands 4	8		
Cross (Exotic*Exotic)			
Upper Midlands 1	46		
Upper Midlands 2	29	146	64.9
Upper Midlands 4	71		
Cross (Exotic*Zebu)			
Upper Midlands 1	0		
Upper Midlands 2	0	13	5.8
Upper Midlands 4	13		

[a]Breed classification through phenotypic identification by the author and based on the first visit of the longitudinal study. Breed of calf was significantly different across the AEZs ($p < 0.001$).

Table 4.13. Distribution of calf feeding management practice by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Category[a]		Number of farm observations	Percentage[b]
Forage			
Upper Midlands 1	Yes	843	92.8
	No	65	7.2
Upper Midlands 2	Yes	578	92.3
	No	48	7.7
Upper Midlands 4	Yes	982	87.6
	No	139	12.4
Concentrate feeds			
Upper Midlands 1	Yes	69	7.6
	No	839	92.4
Upper Midlands 2	Yes	105	16.8
	No	521	83.2
Upper Midlands 4	Yes	217	19.4
	No	904	80.6
Mineral supplements			
Upper Midlands 1	Yes	2	0.2
	No	906	99.8
Upper Midlands 2	Yes	1	0.2
	No	625	99.8
Upper Midlands 4	Yes	167	14.9
	No	85.1	85.1
Legumes			
Upper Midlands 1	Yes	4	0.4
	No	904	99.6
Upper Midlands 2	Yes	0	0
	No	626	100
Upper Midlands 4	Yes	1	0.1
	No	1120	99.9
Milk			
Upper Midlands 1	Yes	559	61.6
	No	349	38.4
Upper Midlands 2	Yes	363	58.0
	No	263	42.0
Upper Midlands 4	Yes	1043	93.0
	No	78	7.0

[a]The frequency refers to the total number of observations over the entire longitudinal study
[b]Percent refers to percent within an AEZ

Table 4.14. Distribution of source of forage by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Category	Number of farm observations	Percentage
Source of forage		
Upper Midlands 1		
Owners farm	843	100
Outside the farm	0	0
Combination of above	0	0
Upper Midlands 2		
Owners farm	571	99.5
Outside the farm	0	0
Combination of above	3	0.5
Upper Midlands 4		
Owners farm	635	64.5
Outside the farm	7	0.7
Combination of above	343	34.8

Table 4.15. Distribution of tick control management by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Category[a]	Number of farm observations	Percentage[b]
Tick control method		
Upper Midlands 1		
No tick control	888	97.8
Dipping	0	0
Spraying	16	1.8
Handwash	4	0.4
Upper Midlands 2		
No tick control	625	99.8
Dipping	0	0
Spraying	1	0.2
Handwash	0	0
Upper Midlands 4		
No tick control	798	71.2
Dipping	2	0.2
Spraying	129	11.5
Handwash	192	17.1
Tick control of calf before?		
Upper Midlands 1		
Yes	14	1.5
No	894	98.5
Upper Midlands 2		
Yes	1	0.2
No	625	99.8
Upper Midlands 4		
Yes	295	26.3
No	826	73.7

Table 4.16. Distribution of *Rhipicephalus appendiculatus* tick by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Tick stage	Calf observations	Percentage
Adult males		
Upper Midlands 1		
Ticks present	11	1.2
No ticks present	897	98.8
Upper Midlands 2		
Ticks present	0	0
No ticks present	626	100
Upper Midlands 4		
Ticks present	264	23.6
No ticks present	857	76.4
Adult females non-engorged		
Upper Midlands 1		
Ticks present	15	1.7
No ticks present	893	98.3
Upper Midlands 2		
Ticks present	1	0.2
No ticks present	625	99.8
Upper Midlands 4		
Ticks present	286	25.5
No ticks present	835	74.5
Adult females engorged		
Upper Midlands 1		
Ticks present	14	1.5
No ticks present	894	98.5
Upper Midlands 2		
Ticks present	1	0.2
No ticks present	625	99.8
Upper Midlands 4		
Ticks present	73	6.5
No ticks present	1048	93.5
Nymphs total		
Upper Midlands 1		
Ticks present	5	0.6
No ticks present	903	99.4
Upper Midlands 2		
Ticks present	0	0
No ticks present	626	100
Upper Midlands 4		
Ticks present	71	6.3
No ticks present	1050	93.7
Nymphs engorged		
Upper Midlands 1		
Ticks present	4	0.4
No ticks present	904	99.6
Upper Midlands 2		
Ticks present	0	0
No ticks present	626	100
Upper Midlands 4		
Ticks present	22	2.0
No ticks present	1099	98.0

4.2.2 Measures of calf health

4.2.2.1 Incidence of calf morbidity and cause-specific morbidity rates

The overall calf morbidity with case definition for ECF (clinical and confirmed by ELISA test) are shown in Table 4.17. East Coast fever stratified on the basis of AEZ and grazing system and the causes of morbidity are shown in Table 4.18.

The overall crude calf morbidity rate in all AEZs during the first year of life for female calves was 20.7%. The detailed visit by visit crude morbidity, ECF-morbidity and the respective survivorship are shown in Figure 4.3 and Appendices 2.1 and 2.3. The crude morbidity rates were significantly ($p<0.001$) different across the AEZs and these were: 15.5% in UM 1, 7.0% in UM 2 and 30.7% in UM 4. East Coast fever was the main cause of female calf morbidity in UM 1 and UM 4 and had an overall cause-specific morbidity rate of 14.1% in all AEZs during the first year of life. In UM 4, 20.8% of all female calves (one fifth of every female calf) were at risk of becoming infected with ECF during the first year of life. In UM 4, this risk of ECF morbidity was significantly ($p<0.05$) different between zero-grazing (6.1%) and open-grazing (14.4%) systems. In UM 1, the risk of becoming sick from ECF was 12.4% during the first year of life for female calves. The ECF morbidity in UM 1 was also significantly ($p<0.05$) different between zero-grazing (1.5%) and open grazing (10.9%) systems. In UM 2, ECF cause-specific morbidity was 2.3%. In addition, it was observed that the ECF cause-specific morbidity rates were significantly different across the AEZs ($p<0.05$).

Of the calves that developed clinical ECF, 35% (8/23) in UM 1, 6% (1/17) in UM 2 and 46% (17/37) in UM 4 were confirmed sero-positive to *T. parva*. Other causes of female calf morbidity were: diarrhoea, 4.6% in UM 4 and 4.1% in UM ; unknown causes, 9.4% in UM 4 and 4.1% in UM 1. Other minor causes of calf morbidity are also shown in

Table 4.18. These included diarrhoea, joint ill, eye infection, insect bites and unknown causes.

4.2.2.2 Incidence of calf mortality and cause-specific mortality rates

The overall calf mortality with case definition for ECF (clinical and confirmed the ELISA test) are shown in Table 4.17. East Coast fever stratified on the basis of AEZ and grazing system and the causes of morbidity are shown in Table 4.19.

The overall crude calf mortality rate in all AEZs during the first year of life for female calves was 7.4%. The detailed results for visit by visit crude mortality and ECF-specific risk rates and the respective survivorship are shown in Figure 4.3 and Appendices 2.2 and 2.4. The crude mortality rates were significantly ($p<0.05$) different across the AEZs and these were: 7.4% in UM 1, 2.3% in UM 2 and 10.5% in UM 4. East Coast fever was the main cause of female calf mortality in UM 1 and UM 4 and had an overall cause-specific mortality rate of 4.5% in all AEZs in female calves during the first year of life. In UM 4, 7.0% of every female calf was at risk of dying from ECF during the first year of life while in UM 1 the risk of death from ECF was 4.5%. No ECF-specific mortality was recorded in UM 2. The differences in ECF cause-specific mortality rates between UM 1 and UM 4 were not statistically ($p>0.05$) different.

Of the calves that died from clinically diagnosed ECF, 13% (3/23) in UM 1 and 16% (6/37) in UM 4 sero-converted to *T. parva*. None in UM 2 sero-converted to *T. parva*. The proportion of calves that died from ECF (from those initially diagnosed with clinical ECF) were, 38% (3/8) in UM 1, 35% (6/17) in UM 4 and none in UM 2. Unknown causes of calf mortality encountered were 6.9% in UM 4 and 4.1% in UM 1 (Table 4.19).

Table 4. 17. Case definition criteria for ECF-morbidity and mortality for the 225 cohort of females calves from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

AEZ	Total cases	Calves showing ECF clinical signs[a]	Calves with ECF signs and +ve* to ELISA test[b]	Calves with ECF signs and –ve** to ELISA test[c]
ECF-morbidity cases				
UM 1	10	8	6	2
UM 2	3	1	1	0
UM 4	25	17	12	5
ECF-mortality cases				
UM 1	5	3	1	2
UM 2	1	0	0	0
UM 4	9	6	1	5

[a]Suspected ECF morbidity cases based on clinical signs defined in Table 3.3.
[b]Confirmed ECF morbidity based on both clinical signs defined in Table 3.3 and a positive ELISA test.
[c]Suspected ECF mortality based on clinical signs defined in Table 3.3 but rapid death occurred before ELISA test was performed to confirm.
*+ve: means positive to ELISA test
**-ve: means negative to ELISA test

Table 4.18. Incidence of crude and cause-specific calf morbidity rates (cause-specific risk rates by AEZ in brackets) from 225 calves studied in the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Disease/Condition	UM 1 (%)	UM 2 (%)	UM 4 (%)	Total Cases	Risk rates
East Coast fever[a]	8 (12.4)[1]	1 (2.3)[2]	17 (20.9)[3]	26	14.1
Zero-grazing	1 (1.5)[1]		5 (6.1)[1]		
Open-grazing	7 (10.9)[2]		12 (14.7)[2]		
East Coast fever[b]	6 (9.3)[1]	1 (2.3)[2]	12 (14.7)[3]	19	10.1
Zero-grazing	1 (1.5)[1]		4 (4.9)[1]		
Open-grazing	5 (7.6)[2]		8 (9.8)[2]		
Diarrhoea/scours	0	1 (2.3)	2 (2.5)	3	1.6
Joint ill	1 (1.6)	0	0	1	0.5
Abscesses	0	0	1 (1.2)	1	0.5
Eye infection	0	0	1 (1.2	1	0.5
Insect bites	1 (1.6)	0	0	1	0.5
Unknown	0	1 (2.3)	4 (4.9)	5	2.7
Total	10	3	25	38	20.7
Risk rates (%)	15.5[1]	7.0[2]	30.7[3]	20.7	

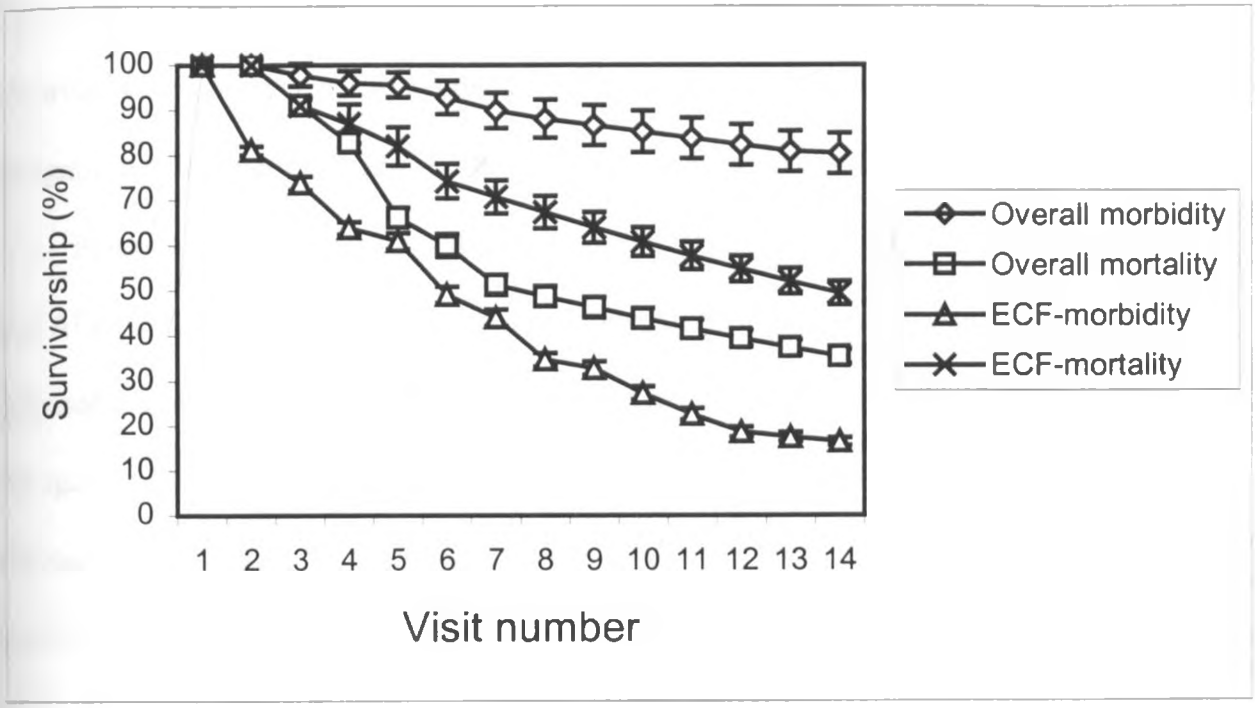
[a]ECF morbidity and risk rates based on clinical diagnosis alone
[b]ECF morbidity and risk rates based on clinical signs and confirmation by ELISA test
[1,2]Risk rate estimates for morbidity across AEZ or between grazing system with different superscripts significantly ($p<0.05$) different.

Table 4.19. Incidence of crude and cause-specific calf mortality rates (cause-specific risk rates by AEZ in brackets) from 225 calves studied in the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Disease/Condition	UM 1 (%)	UM 2 (%)	UM 4 (%)	Total cases	Risk rates
East Coast fever[a]	3 (4.5) [1]	0	6 (7.0)[1]	9	4.5
Zero-grazing	0 (0.0)[1]		1 (1.1)[1]		
Open-grazing	3 (4.5)[2]		5 (5.9)[2]		
East Coast fever[b]	1 (1.5) [1]	0	1 (1.1)[1]	1	0.5
Zero-grazing	0 (0.0)[1]		0 (0.0)		
Open-grazing	1 (1.5)[2]		1 (1.1)		
Joint ill	1 (1.5)	0	0	1	0.4
Insect bites	1 (1.5)	0	0	1	0.4
Unknown	0	1 (2.3)	3 (3.5)	4	2.0
Total	5	1	9	15	7.4
Risk rates (%)	7.4[1]	2.3[2]	10.5[1]	7.4	

[a]ECF mortality and risk rates based on clinical diagnosis alone
[b]ECF mortality and risk rates based on clinical signs and confirmation by ELISA test
[1,2]Risk rate estimates for mortality across AEZ or between grazing system with different superscripts significantly ($p<0.05$) different.

Figure 4.3 Survivorship curves (fitted with 95% confidence limits) for the overall morbidity and mortality and ECF-morbidity and mortality for 225 cohort of female calves studied in the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



4.2.3 Descriptive analysis and factors associated with *T. parva* antibodies

4.2.3.1 Descriptive analysis of antibody titres

The proportion of calves with positive antibody titres to *T. parva* in all AEZs was generally high at the recruitment age due to maternally derived colostral antibodies. The proportion of seropositive calves declined towards the third month of age and started to rise again steadily afterwards as a result of exposure *T. parva* parasite.

Figure 4.4 shows the mean antibody titres for calves of different age groups in the three AEZs (with 95% confidence limits). In all the AEZs, the mean antibody titres decreased steadily from birth, to reach their lowest levels at about 94 days old. They then rose again, to reach a maximum titre at the end of the study. The above pattern was reflected in all the estimated statistical measures such as the median, standard deviations, standard errors, range, and 95% confidence limits (CLs) (Appendices 2.5 - 2.7).

Figure 4.5 shows the mean antibody titres (PP values) for UM 1 and UM 4, stratified by grazing system. The mean antibody titres were different across the age groups between zero-grazing and open grazing systems. In UM 1, the mean antibody titres were 17.7 and 29.5 for zero-grazing and open-grazing systems respectively at 9 days old (recruitment age). The mean titre dropped steadily to 8.5 in both grazing systems at 122 days old and rose steadily to 15.3 and 24.5 for zero-grazing and open-grazing respectively at 191 days old. In UM 1, there was significant difference in the antibody titres between zero-grazing and open-grazing systems at 9 days old (T-test, $p<0.1$) only. There were no significant differences in the other visit ages ($p>0.1$). In UM 4, the mean antibody titres were 33.8 and 58.2 for zero-grazing and open-grazing systems respectively at 9 days old. The mean titre dropped steadily to 11.2 and 16.1 for zero-grazing and open-grazing respectively at 108 and 122 days old and rose steadily to 16.3

and 40.0 in zero-grazing and open-grazing respectively at 191 days old. There were significant differences (T-tests), in the antibody titres between zero-grazing and open-grazing systems in the following age groups: 10-50 days old, $p<0.001$; 191 days old, $p<0.01$; 66 and 177 days old, $p<0.05$; 80 and 163 days old, $p<0.1$. No significant ($p<0.1$) differences between antibody titres were found from 94 -150 days old. The results of the descriptive analysis are summarised in Appendices 2.8 and 2.9.

Figure 4.6 shows a scatter graph of antibody profiles by age of calves (in days) for all AEZs with figures 4.7 to 4.9 showing antibody profiles of calves in individual AEZs. Generally, the antibody profiles showed that titres were initially high and declined gradually with age (up to about 100 days old in UM 1 and UM 4, and 80 days old in UM 2). This was followed by a gradual rise in antibody profiles from 100 days old presumably due to sero-conversion to *T. parva*. In UM 2, the peak levels reached about half the peaks observed in UM 1 and UM 4.

The variance components procedure on antibody titres showed that the main variations occurred at the farm and calf levels. About 47% of the total variability was because of visit-to-visit variations in the antibody titres at the individual calf levels. The rest of the variability in antibody titres was due to variations from farm-to-farm (23%), from calf-to-calf (20%) and from AEZ-to-AEZ (10%).

Figure 4.4 Mean antibody titres (percent positivity – PP) by age of calf, stratified by AEZ and fitted with 95% confidence limits, from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

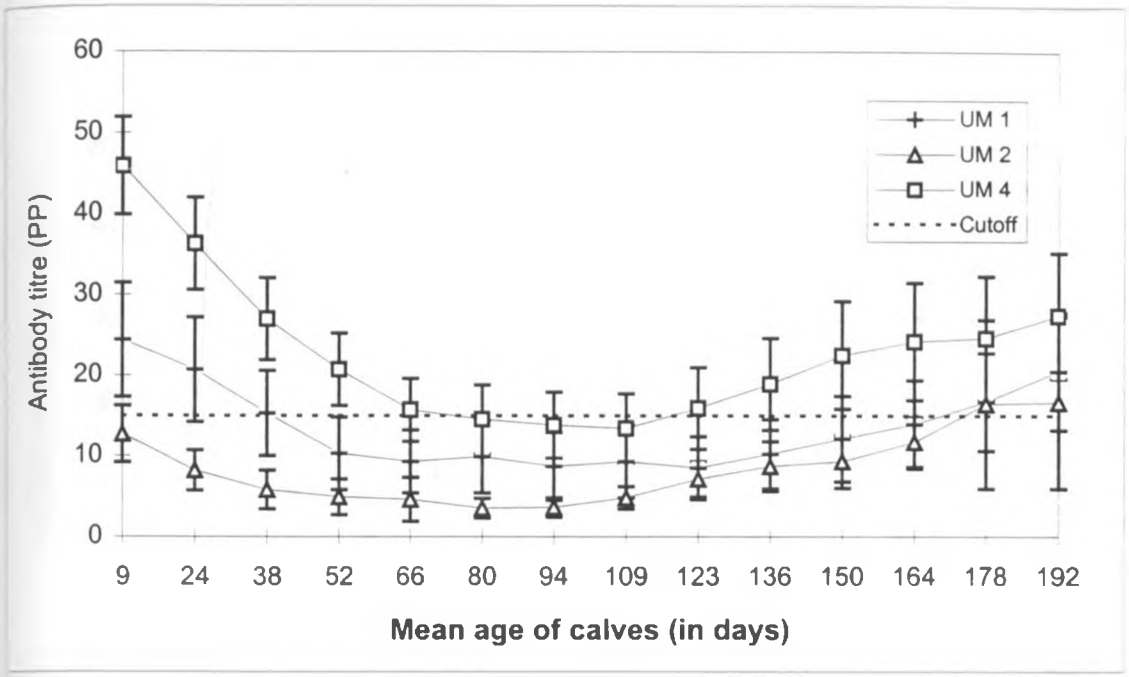


Figure 4.5 Mean antibody titres (percent positivity – PP) by age of calf for UM 1 and UM 4, stratified by grazing system and fitted with 95% confidence limits, from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

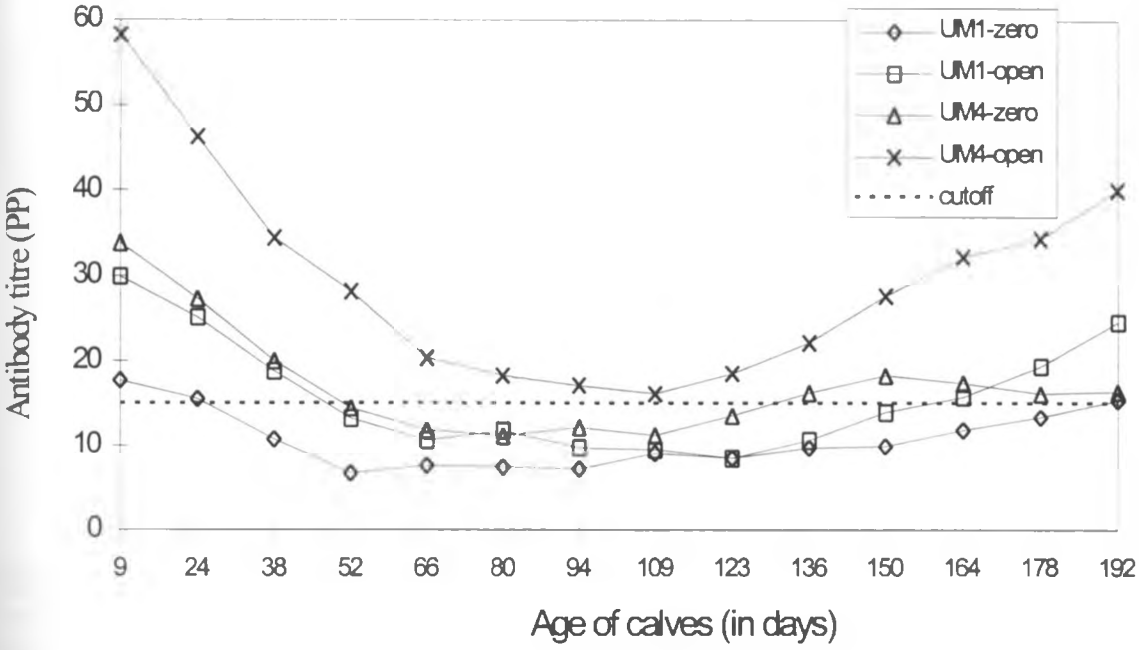


Figure 4.6 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves in all AEZs from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

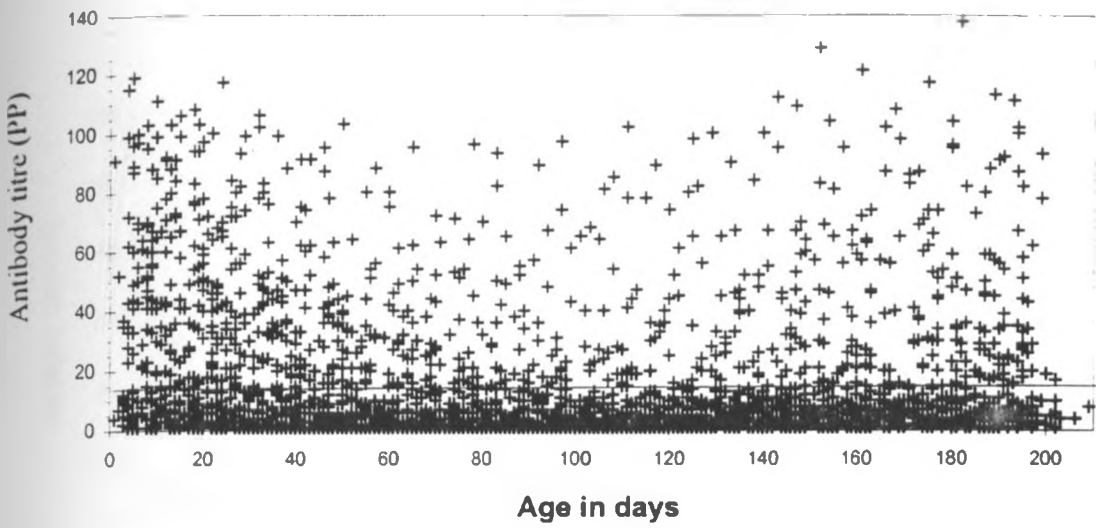


Figure 4.7 Distribution of antibody titres (percent positivity – PP) by calf age (in days) for calves in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

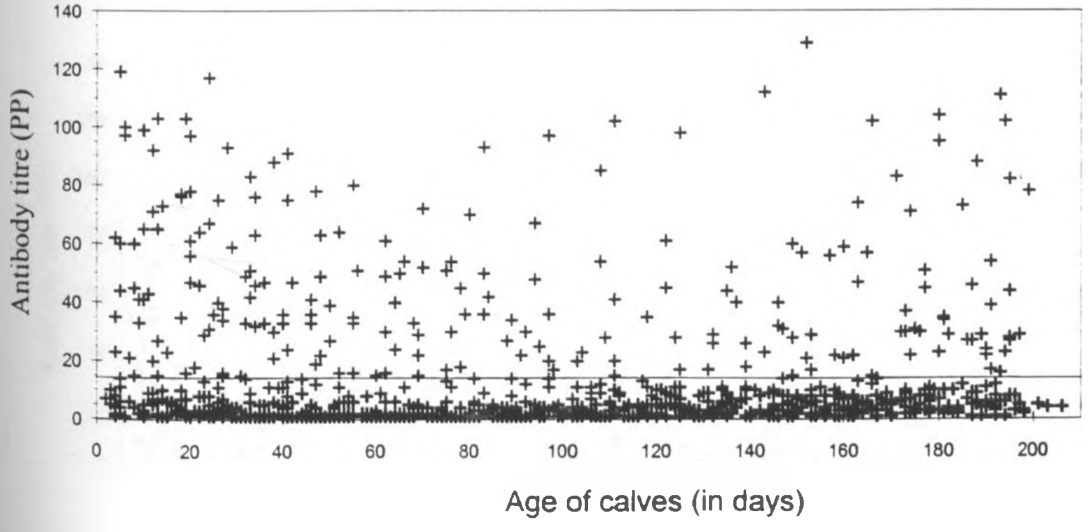


Figure 4.8 Distribution of antibody titres (percent positivity – PP) by calf age (in days) for calves in UM 2 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

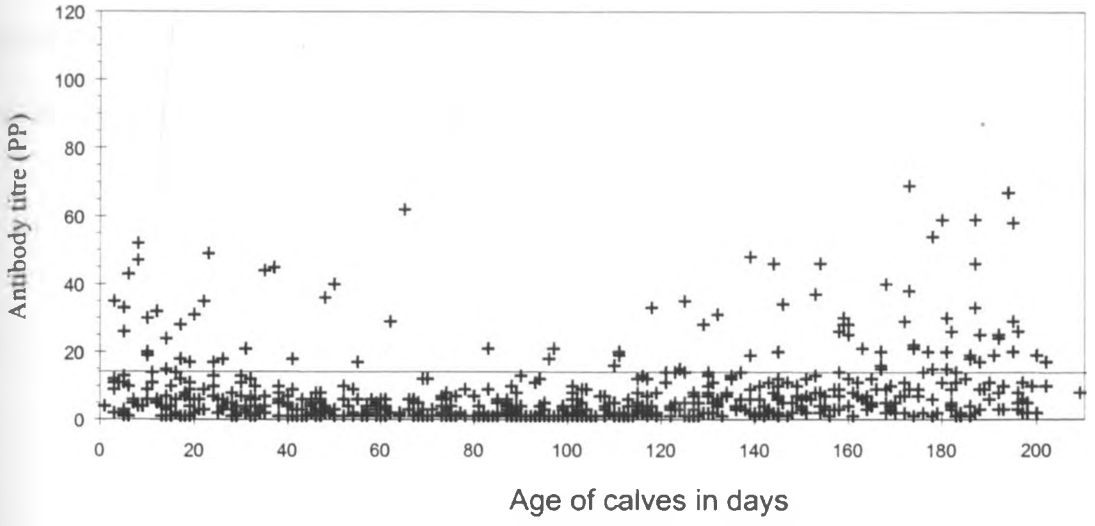
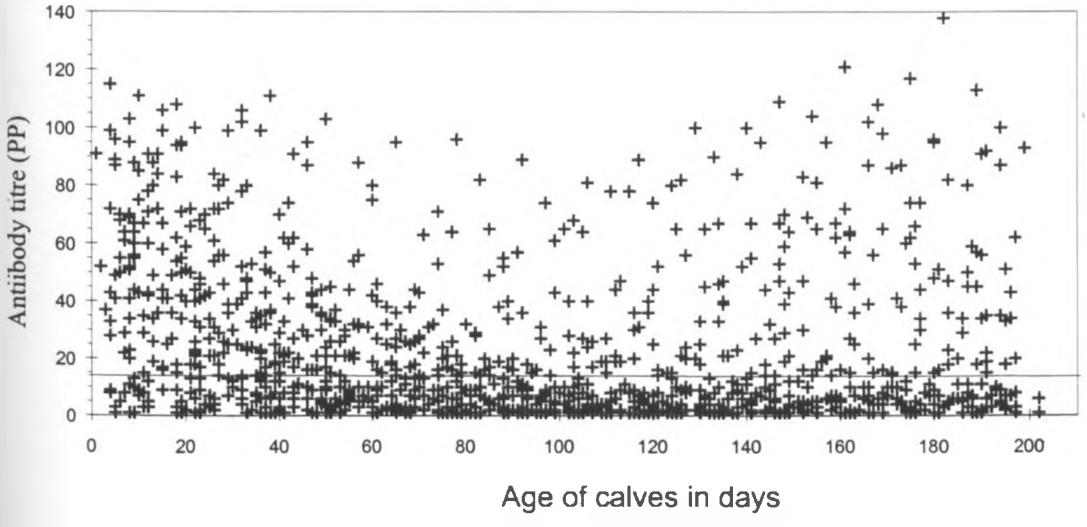


Figure 4.9 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



Figures 4.10 to 4.13 show scatter graphs of antibody profiles by age of calves in UM 1 and UM 4 stratified by grazing system. In general, there were fewer calves raised under zero-grazing management (in both UM 1 and UM 4) that showed the presence of antibodies than those raised under open-grazing management. Following the initial decline of antibodies at 100 days old, higher number of calves in open-grazing system than in zero-grazing showed the presence of antibodies to *T. parva*.

Figure 4.10 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves under zero-grazing in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

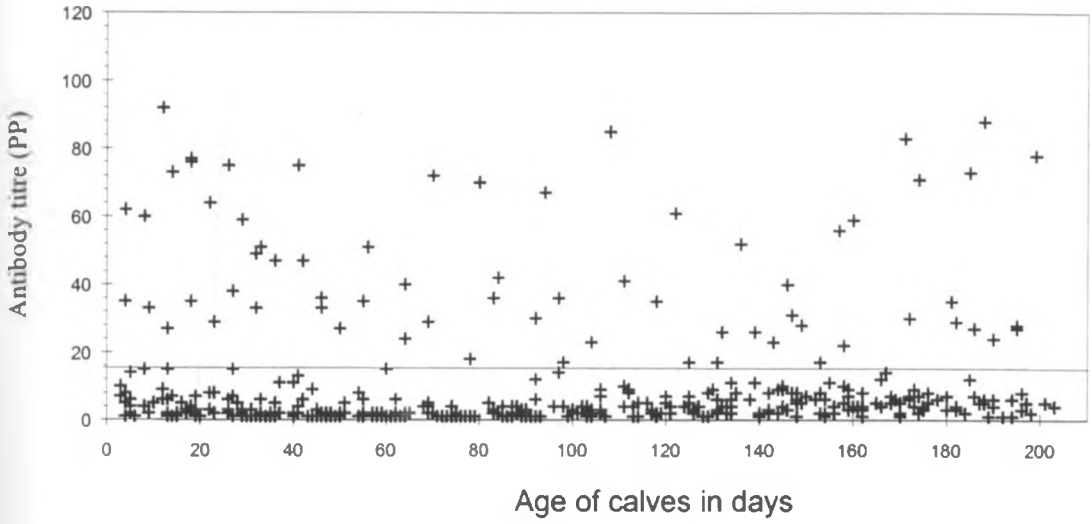


Figure 4.11 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves under open-grazing in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

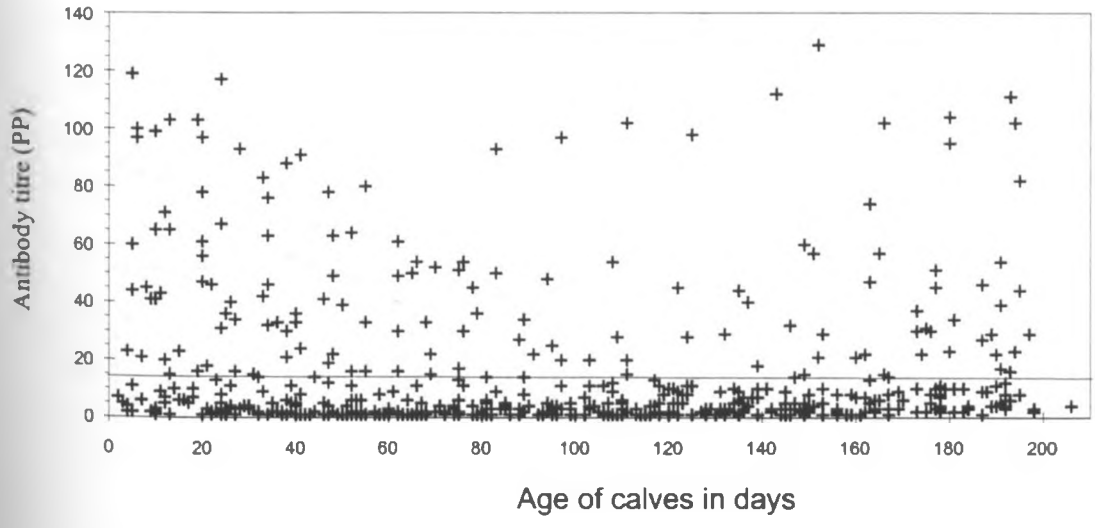


Figure 4 12 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves under zero-grazing in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

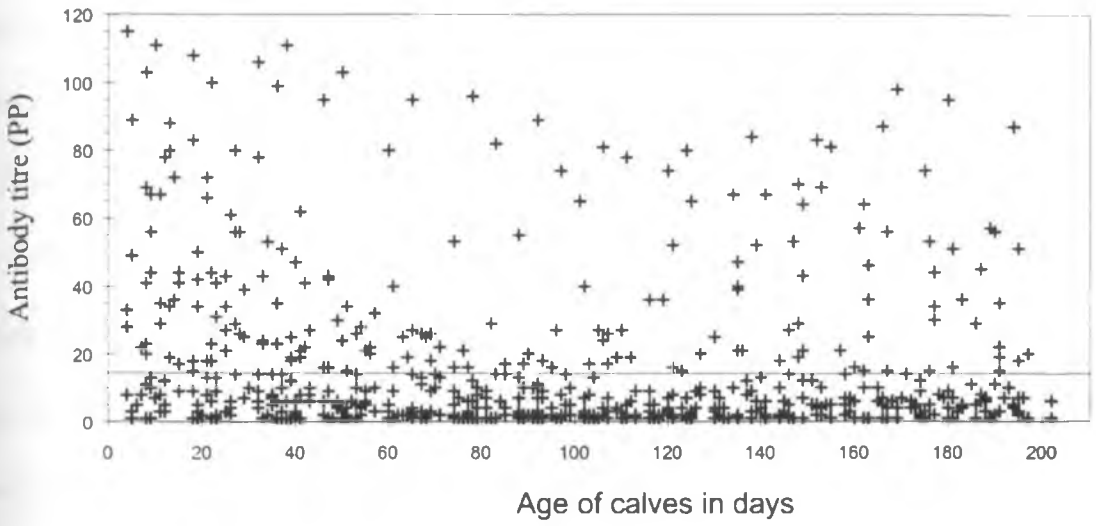
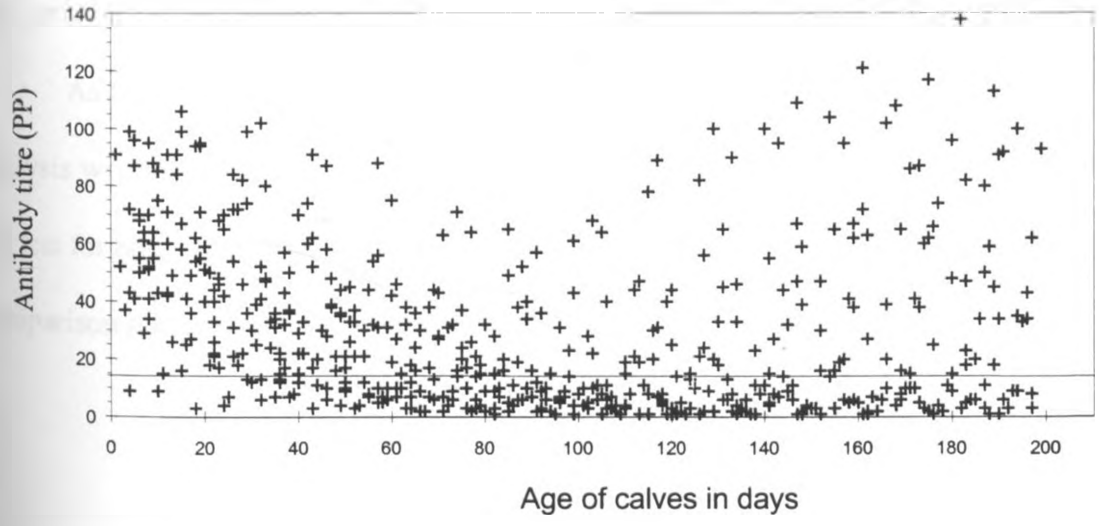


Figure 4.13 Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves under open-grazing in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



4.2.3.2 Estimation of survival times to loss of maternal antibodies and acquisition of antibodies due to natural infections

Figures 4.14 and 4.15 are survival curves showing survival function distribution and survival time to decline of maternal antibodies (from calf birth) and time (in days) tor sero-conversion to *T. parva*. The mean estimated survival times for decline of maternal antibodies to a survival level of 50% in the three AEZs were 161 days for UM 1, 168 days for UM 2 and 138 days for UM 4. The generalised Wilcoxon test showed that the survival times and curves for time to decline of maternal antibodies were homogeneous and were therefore not significantly different across the three AEZs (χ^2=4.1829, df=2 and p=0.1235). The mean estimated survival times for 50% of the calves to survive sero-conversion to *T. parva* in the three AEZs were 120 days for UM 1, 104 days for UM 4 and >200 days for UM 2. The generalised Wilcoxon test showed that the survival times and curves for time to sero-conversion to *T. parva* parasites were not homogeneous and were therefore marginally significantly different across the three AEZs (χ^2=6.1284, df=2 and p=0.0467).

As few clinical ECF cases were recorded in UM 1 and UM 2, the survival data analysis were not performed on ECF data. Although there were more ECF cases in UM 4, the test for homogeneity of survival curves required more than one survival curve for comparison purposes and so survival curves for ECF were not generated.

Figure 4.14 Survival function distribution for time to decline of maternal antibodies to *Theileria parva* by age of calves in days for all calves stratified by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

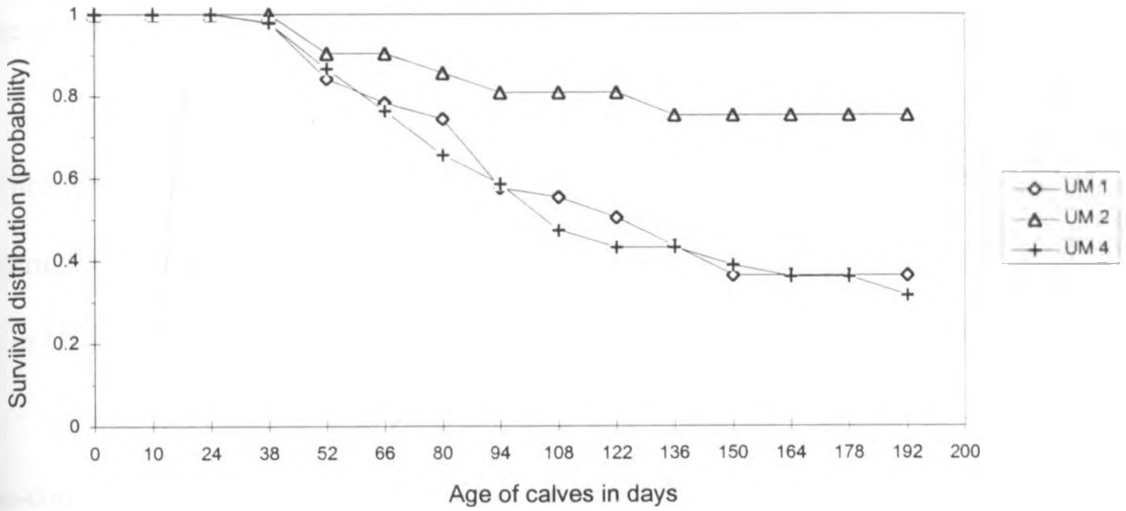


Figure 4.15 Survival function distribution for time to sero-conversion to *Theileria parva* by age of calves in days for all calves stratified by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

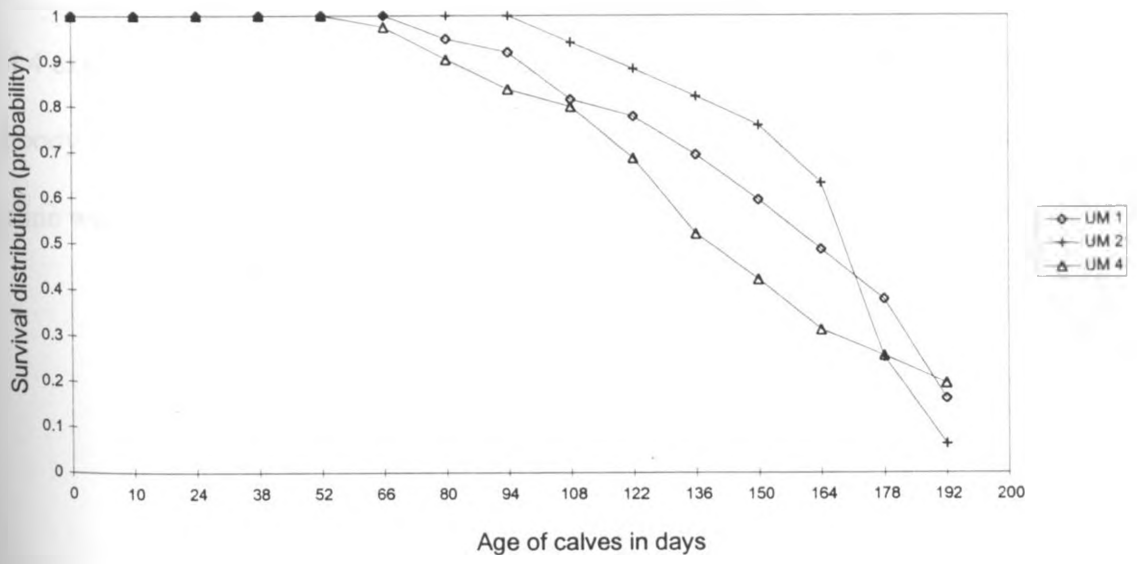


Table 4.20 shows the sero-conversion risk rates to *T. parva* and stratified by grazing management in UM 1 and UM 4. The risk rates were higher in open-grazing management than in the zero-grazing management in UM 1 and UM 4. These differences were however not significant ($p>0.05$) within AEZ.

Appendices 2.10 to 2.13 show scatter graphs of antibody profiles for calves that did not sero-convert to *T. parva* in all AEZs combined and stratified by each AEZ. The maternally-derived antibodies declined to levels below the cut-off by the age of 100 days old in UM 1, 60 days old in UM 2 and 160 days old in UM 4.

Appendices 2.14 to 2.20 show scatter graphs of antibody profiles for calves that sero-converted to *T. parva* in all AEZs combined and for each AEZ and grazing management. The peak titres for maternally-derived antibodies for calves that sero-converted in UM 2 (peak PP of 50) were about half that found in UM 1 and UM 4 (peak PP of 110). In UM 2, sero-conversion started after the age of 100 days while in UM 1 and UM 4 it occurred at all ages. Generally, calves on zero-grazing management in UM 1 and UM 4 experienced a decline in maternally-derived antibodies below cut off levels before antibody titres rose again due to *T. parva* exposure. In open-grazing management, no such decline was observed and calves appeared to be exposed to *T. parva* continually.

Table 4.20. Sero-conversion risk rates for calves to *T. parva* by AEZ from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Grazing system	Number sero-converted	Total Number	Risk rate
UM 1			
Zero-grazing	8	34	12.4
Open-grazing	15	42	23.3
Total	23	76	35.7
UM 2			
Zero-grazing	17	50	39.5
UM 4			
Zero-grazing	16	50	19.6
Open-grazing	21	49	25.8
Total	37	99	45.4

4.2.3.8 Multi-level modelling for factors associated with *T. parva* antibody titres

The results of the Mixed Model Procedure for *T. parva* antibody titres are described below. First, the results from the uni-variate models are described followed by those from the multi-variate models. These are classified by visit and calf level factors.

The fixed-effects factors that were significantly associated with *T. parva* antibody titres in the uni-variate models are shown in Table 4.21. Calves raised outdoors were associated with higher antibody titres than those raised indoors, while younger calves were weakly associated with higher antibody titres than older ones. Calves with higher mean daily weight gains were associated with low antibody titres than those with lower mean weight gains, while calves that had acaricides applied on them or had history of acaricide application were associated with lower antibody titres than those with no acaricide application. The presence of male, female (non-engorged and engorged) and nymphs (total) *R. appendiculatus* classes were associated with higher antibody titres in calves.

Calves raised in UM 4 were associated with higher antibody titres than those raised in the other two AEZs, while calves raised in zero-grazing were associated with lower antibody titres than those raised in open grazing. Zebu and their crosses were associated with higher antibody titres than Taurine breeds, while calves whose dams had higher antibody titres were also associated with higher antibody titres. Farm, the random-effect factor was not associated with *T. parva* antibodies.

The fixed-effect factors significantly associated with antibody titres in the multi-variate mixed models are shown on Table 4.22. Calves that were raised in UM 4 were associated with higher antibody titres than those raised in the other two AEZs while calves raised in zero-grazing were associated with lower antibody titres than those raised

in open-grazing system. Calves that received forage from within the farm, were associated with higher antibody titres than those that received forage from outside the farm while calves whose dams had higher antibody titres were also associated with higher antibody titres. Calves that were reported to have suffered from ECF or received acaricide treatment or recorded higher mean daily weight gains were associated with lower antibody titres, while those housed indoors were associated with higher antibody titres. The presence of male and female (non-engorged) *R. appendiculatus* tick classes were associated with higher antibody titres in calves while the presence of female (engorged) *R. appendiculatus* tick class was associated with lower antibody titres in calves. The calf level factors were: grazing management and antibody titres of the dam. The details on factors significantly associated antibody titres stratified by AEZ in the multi-variate model are shown on Appendix 2.21. Farm, the random-effect factor was not associated with *T. parva* antibodies.

Table 4. 21. Factors significantly associated with *T. parva* antibody titres in the uni-variate mixed models in all AEZs from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Parameter estimate (b)	Standard error (Se (b))	P-value
Visit level factors			
Housing (indoors=1; outdoors=0)	7.89	1.11	0.0001
Age of calf (in days - absolute values)	-0.04	0.01	0.0001
Daily weight gains (absolute values)	-8.08	1.89	0.0001
Acaricide application (any method=1; none=0)	3.25	1.26	0.010
Past acaricide use (any method=1; none=0)	2.99	1.31	0.022
Males[b] (yes=1; none=0)	12.51	1.33	0.0001
Females (non-engorged)[c] (yes=1; none=0)	10.93	1.30	0.0001
Females (engorged)[d] (yes=1; none=0)	4.68	2.17	0.031
Nymphs (total)[e] (yes=1; none=0)	8.85	2.39	0.0001
Calf level factors			
AEZ (UM 4=1; others=0)	3.87	0.45	0.0001
Grazing (Zero=1; open=0)	-11.41	1.25	0.0001
Calf breed (Zebu=1; Taurine=0)	13.93	1.88	0.0001
Dam antibodies (absolute values)	0.45	0.02	0.0001

[a]Random-effects variables in all the uni-variate mixed model not significant (p>0.05)
[b]Male *R. appendiculatus* tick
[c]Female *R. appendiculatus* tick (non-engorged)
[d]Female *R. appendiculatus* tick (engorged)
[e]Nymphs *R. appendiculatus* tick (total)

Table 4. 22. Factors significantly associated with *T. parva* antibody titres in the multivariate mixed models in all AEZs from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Parameter estimate (b)	Standard error (Se(b))	P-value
Visit level factors			
Housing (indoors=1; outdoors=0)	2.36	1.22	0.053
Source of forage (owners farm=1; outside=0)	3.08	1.18	0.009
Daily weight gains (absolute values)	-6.74	1.81	0.0001
East Coast fever (yes=1; no=0)	-14.26	3.89	0.0001
Past acaricide application (any method=1; none=0)	-5.44	3.01	0.071
Males[b] (yes=1; none=0)	3.94	1.74	0.023
Females (non-engorged)[c] (yes=1; none=0)	3.67	1.66	0.027
Females (engorged)[d] (yes=1; none=0)	-5.22	2.27	0.022
Calf level factors			
AEZ (UM 4=1; others=0)	3.13	2.59	0.001
Grazing (Zero=1; open=0)	-4.33	1.47	0.003
Dam antibodies (absolute values)	0.38	0.03	0.0001

[a]Random-effects variables in the mixed model not significant ($p>0.05$)
[b]Male *R. appendiculatus* tick
[c]Female *R. appendiculatus* tick (non-engorged)
[d]Female *R. appendiculatus* tick (engorged)

4.2.3.9 Multi-level models for risk factors associated with sero-conversion to *T. parva* and ECF

The risk factors associated with sero-conversion to *T. parva* from the multi-level Glimmix model are shown in Table 4.23 and are classified by visit and calf level factors. Zebu breeds and their crosses with exotic breeds were associated with lower risks of sero-conversion to *T. parva* than the exotic breeds. Calves whose dam antibodies were high and positive were significantly associated with higher risk of sero-conversion to *T. parva* as were older calves than the young ones. Calves that were reported to have been washed with acaricides to control ticks were associated with higher risks of sero-conversion while calf morbidity was significantly ($p<0.05$) associated with higher risk of seroconversion. Calves that acquired ECF were associated with significantly ($p<0.05$) lower sero-conversion risk rates that those which did not sero-convert. The presence of nymphs (total) and nymph (engorged) of *R. appendiculatus* on calves was associated with significantly lower and higher risk of sero-conversion to *T. parva* respectively. An analysis using multi-variate logistic regression showed that the risk factors associated with sero-conversion to *T. parva* were not different from those that were obtained from Glimmix model.

The results for the risk factors associated with ECF in both multiple logistic regression and Glimmix models were not obtained as model fitness was poor for the former and there was no convergence in the model for the latter.

Table 4. 23. Risk factors associated with sero-conversion to *T. parva* from the multi-level Glimmix model in all AEZs from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Parameter estimate (b)	Standard error (Se(b))	p-value
Visit level factors			
Breed of calf (Zebu*=1; Taurine=0)	-1.43	0.81	0.081
Age of calf (in days - absolute values)	0.02	0.01	0.000
Acaricide application (any method=1; none=0)	1.20	0.54	0.027
Calf sickness (yes=1; no=0)	1.44	0.62	0.023
East Coast fever (yes=1; no=0)	-4.07	1.99	0.042
Nymphs[b] (engorged) (yes=1; none=0)	-5.18	1.92	0.008
Nymphs[c] (total) (yes=1; none=0)	2.91	1.47	0.050
Calf level factors			
Dam antibodies (absolute values)	0.02	0.01	0.034

[a]Random-effects variable not significant in the model ($p<0.05$)
[b]Nymph *R. appendiculatus* tick (engorged)
[c]Nymph *R. appendiculatus* tick (total)
*refers to Zebu and their crosses with exotic

4.2.4 Mean daily weight gains and association with ECF, *T. parva* and other factors

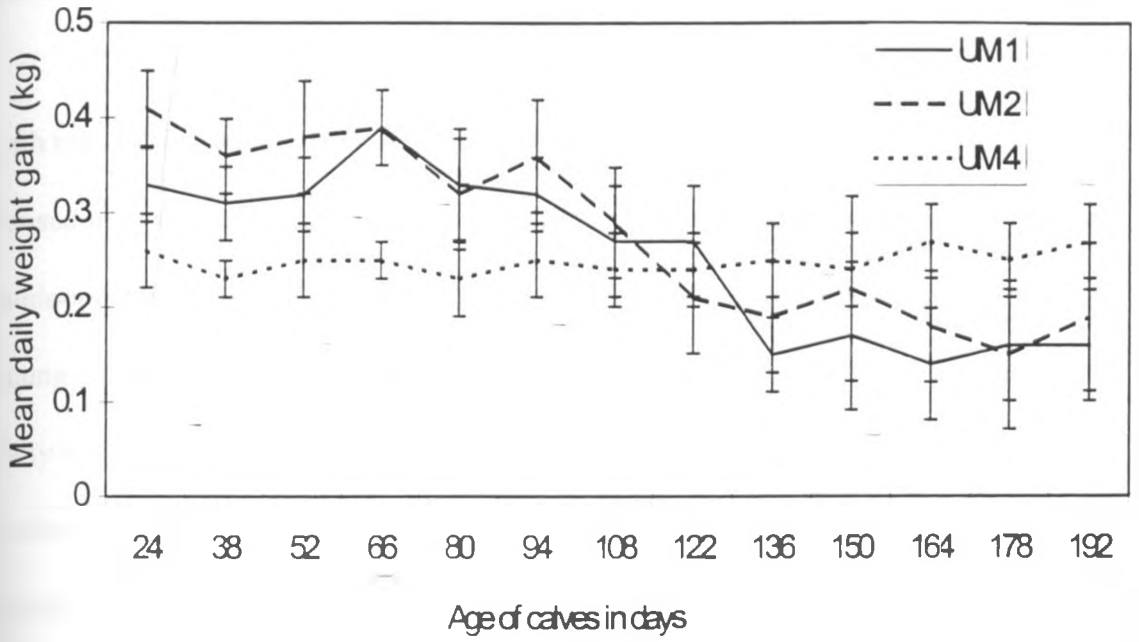
4.2.4.1 Descriptive analysis on mean daily weight gains

Figure 4.16 shows the mean daily weight gain curves by age of calf fitted with confidence limits (95%). The overall mean daily weight gains for all calves in the study were: 0.25 kgs, 0.29 kgs and 0.25 kgs in UM 1, UM 2 and UM 4 respectively. The scatter plots for the distribution of the mean daily weight gains for each AEZ are shown in Appendices 3.4 - 3.6. Figure 4.16 further shows that in UM 1, the mean daily weight gains dropped from a high initial mean of 0.39 kgs at 52 days old to as low as 0.14 kgs at 150 days old. In UM 2, the mean daily weight gain dropped from a mean of 0.41 kgs at 10 days old to 0.15 kgs at 166 days old. In UM 4, the generally low mean daily weight gains of 0.23 - 0.25 kgs were maintained throughout age groups. For calves that experienced morbidity events (any morbidity or ECF-morbidity), there was an initial drop in mean daily weight gains and about 60% of these calves never compensated for the weight losses in the visits that followed immediately after while the rest 40% compensated for these weight losses in the next visits. The detailed results of the descriptive analyses on mean daily weight gains for each AEZ are shown in Appendices 3.1 - 3.3.

The variance components procedure on mean daily weight gains for all calf ages showed the main variations at the calf and farm levels. The greatest proportion of variability (87% of total variability) for all calves was attributed to visit-to-visit variations in the mean daily weight gains of the calves. The rest of the variability was due to variations from farm-to-farm (9% of total variability) and from calf-to-calf (4% of total variability). There was insignificant variability from AEZ-to-AEZ. For calves less than 3 months old, all calf and farm levels showed some variations. The greatest proportion of variability in this group of calves (61% of total variability) was because of visit-to-visit

variation in the mean daily weight gains of the calves. The rest of the variability was due to variation from AEZ-to-AEZ (12% of total variability), farm-to-farm (10% of total variability) and from calf-to-calf (16% of total variability). For calves more than 3 months old, the major source of variability was within calf measurements. The greatest proportion of variability in this group of calves (91% of total variability) was because of visit-to-visit variation in the mean daily weight gains of calves. The rest of the variability was due to farm-to-farm (4% of total variability) and from calf-to-calf (4% of total variability). There was insignificant variability from AEZ-to-AEZ.

Figure 4.16 Mean daily weight gains curves by AEZ and age of calves with 95% confidence limits fitted for all from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).



4.2.4.2 Multi-level models for factors associated with mean daily weight gains

The results of the Mixed Model Procedure for calf daily weight gains are described below. First, the results from the uni-variate models are described followed by those from the multi-variate models. These are classified by visit and calf level factors.

The explanatory factors (fixed-effects factors) that were significantly associated with mean daily weight gains in the uni-variate models are shown on Table 4.24. Calves housed indoors were associated with higher mean daily weight gains than those housed outdoors, while older calves were associated with lower mean daily weight gains than young calves. Calves that received concentrate feeds were associated with higher mean daily weight gains than those that did not receive, while calves that either had higher antibody titres or experienced any morbidity were associated with lower mean daily weight gains than those with lower antibody titres or those that had no experience of morbidity. The presence of male, female (non-engorged) and nymphs (total) *R. appendiculatus* classes were associated with lower mean daily weight gains in calves. Calves raised under zero-grazing management were associated with lower mean daily weight gains than those raised under open-grazing management. Zebu and their crosses were associated with lower mean daily weight gains than Taurine breeds. Calves born by those dams with higher *T. parva* antibody titres were associated with lower mean daily weight gains than calves whose dams had lower antibody titres. Farm, the random-effect factor was not significantly associated with *T. parva* antibodies.

The factors significantly associated with mean daily weight gains in the multi-variate mixed models are shown on Table 4.25. Older calves were associated with lower mean daily weight gains than young calves, while calves with higher *T. parva* antibody titres were associated with lower mean daily weight gains than those with lower *T. parva*

antibody titres. Calves that received concentrate feed supplements were associated with higher mean daily weight gains than those not receiving, while Zebu and their crosses were associated with lower mean weight gains than the Taurine breeds. Calves that experienced any morbidity were associated with lower mean daily weight gains at the time of morbidity than non-affected calves. Calves that experienced ECF-morbidity were associated with higher mean daily weight gains at the time of morbidity than non-affected calves. The presence of female (non-engorged) *R. appendiculatus* on calves was associated with lower mean daily weight gains in calves. Farm, the random-effect factor was not associated with *T. parva* antibodies. The details on explanatory factors significantly associated with mean daily weight gains stratified by AEZ in the multi-variate model are shown on Appendix 3.7.

Table 4.24. Factors significantly associated with mean daily weight gains in the uni-variate mixed models in all AEZs from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Parameter estimate (b)	Standard error (Se(b))	P-value
Visit level factors			
Housing (indoors=1; outdoors=0)	0.03	0.01	0.043
Age of calf (in days - absolute values)	-0.00	0.00	0.0001
Concentrate feeds (yes=1; no=0)	0.03	0.01	0.015
Calf antibodies (absolute values)	-0.00	0.00	0.0001
Calf sickness (yes=1; no=0)	-0.10	0.02	0.0001
Males[b] (yes=1; none=0)	-0.04	0.01	0.004
Females (non-engorged)[c] (yes=1; none=0)	-0.02	0.01	0.079
Nymphs (total)[d] (yes=1; none=0)	-0.07	0.02	0.004
Calf level factors			
Grazing (open=1; zero=0)	0.03	0.01	0.005
Calf breed (Zebu=1; Taurine=0)	-0.09	0.02	0.0001
Dam antibodies (absolute values)	-0.00	0.00	0.090

[a]Random-effects variables in all the uni-variate mixed model not significant (p>0.05)
[b]Male *R. appendiculatus* tick
[c]Female *R. appendiculatus* tick (non-engorged)
[d]Nymphs *R. appendiculatus* tick (total)

Table 4.25. Factors significantly associated with mean daily weight gains in the multi-variate mixed models in all AEZs from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Parameter estimate	Standard error	P-value
Visit level factors			
Age of calf (in days - absolute values)	-0.00	0.00	0.0001
Calf antibodies (absolute values)	-0.00	0.00	0.001
Concentrate feeds (yes=1; no=0)	0.06	0.01	0.0001
Calf sickness (yes=1; no=0)	-0.13	0.03	0.0001
East Coast fever (yes=1; no=0)	0.08	0.04	0.072
Females (non-engorged)[b] (yes=1; none=0)	0.04	0.02	0.035
Calf level factors			
Calf breed (Zebu=1; Taurine=0)	-0.08	0.02	0.0001

[a]Random-effects variables in all the uni-variate mixed model not significant ($p>0.05$)
[b]Female *R. appendiculatus* tick (non-engorged)

CHAPTER 5

DISCUSSION

5.1 THE CROSS SECTIONAL STUDY

5.1.1 Components of study design and description of epidemiologic states

This study provided a preliminary assessment of serum antibody prevalence to *T. parva, T. mutans* and *B. bigemina* in Murang'a District in animals other calves less than six months of age. The latter were not sampled so as to minimise the possibility of detecting passively-derived colostral antibodies, which in *T. parva* infection are considered to decline to undetectable levels by 2-4 months of age (Burridge and Kimber, 1973). Serum antibody prevalence values assist in determining the presence and degree of endemic stability and instability TBD parasites (Perry, 1997). Endemic stability is more likely to exist where the prevalence of serum antibodies to the infection is high (>70%), while the antibody prevalence is usually low (<30%) in an endemic instability state (Norval *et al.*, 1992; Deem *et al.*, 1993; Perry and Young, 1995; Perry, 1997). There was large variability in serum antibody prevalence to the three TBDs across the AEZs suggesting existence of different epidemiological status for these diseases in the district.

An endemically stable state appeared to be present in UM 4 for *T. parva*, based on serum antibody prevalence over 70%. However, the validity of such a state would only be confirmed if other estimates such as ECF-fatality rate are known or established. Similar results have been obtained from past studies in Kenya conducted in Trans Mara District, (Moll *et al.*, 1986), the Lake Victoria Basin (Morzaria *et al.*, 1988a), and Kilifi District (Deem *et al.*, 1993). Antibody prevalence in other AEZs were lower, suggesting the

existence of endemically unstable states. For *T. mutans*, all AEZs showed antibody prevalence below 40% and this was suggestive of unstable states. *Babesia bigemina* antibody profiles showed a different pattern, with two AEZs (LH 1 and UM 1) showing intermediate prevalence (approximately 50%) while the other three showed low prevalence, suggesting instability. An interesting observation was that while an endemically stable state for *T. parva* appeared to occur in the lower altitude zones, a more or less similar situation was observed in higher altitude zones for *B. bigemina*. This was possibly explained by the tick dynamics across the AEZs. In lower AEZs, high numbers of *R. appendiculatus* ticks were present, while high numbers of *Boophilus* species were present in the higher AEZs.

5.1.2 Risk factors associated with prevalence to *T. parva*, *T. mutans* and *B. bigemina*

Serum antibody prevalence was associated with the three main factors considered, that is grazing management, breed and AEZ. Grazing management and AEZ were considered more important determinants of serum antibody prevalence than breed in the study area since antibody prevalence was not different among breeds within an AEZ. Three AEZs (UM 1, 2 and 3) were mainly characterised by an intensive grazing system in which virtually all animals were kept under a zero-grazing (strict confinement) management system. In zero-grazing, calves are not exposed to open pastures and have minimal contact with adult animals. It was likely that calves raised on pastures were frequently exposed to infected ticks thus developing circulating antibodies.

The association between serum antibody prevalence and the breed of calf may be explained by the distribution of these breeds. There was a high correlation between breed, AEZ and grazing system, with Zebu and their crosses kept under conditions of higher tick exposure (UM 4 and unrestricted grazing). However, after controlling for these two factors

(AEZ and grazing management), breed was not associated with differences in prevalence. The UM 4 has drier area than the other four AEZs and Zebu cattle and their crosses are predominant here, especially where open grazing system is practised. Zebu breeds from endemic theileriosis areas are known to be less susceptible to the effects of *T. parva* infections thus they likely may have a survival advantage in this zone over Taurine breeds and their crosses (Moll *et al.*, 1984, 1986; Perry *et al.*, 1992).

The difference in prevalence between male and female calves for *Babesia bigemina* was possibly explained by the differences in their management. Female calves are generally housed and well cared for due to the value attached to them, whereas the males are generally grazed freely due to lower value attached to them, thus increasing the risk of their exposure to ticks. Older calves were associated with higher prevalence as older calves tend to be released outside the housing area and allowed access to pastures.

Tick control was negatively associated with serum antibody prevalence. This may be due to the fact that tick control is generally poor throughout the district, or the fact that reporting was inaccurate. Tick control in highland areas is less commonly practised than was previously the case (either inadequately or ineffectively applied). Also, as it is still unlawful to move cattle with ticks, some respondents may have been reluctant to report minimal acaricide use. Similar observations were made in the coastal area of Kenya (Maloo *et al.*, 1994). This also shows the limitation of a cross-sectional study in accurately reporting the actual routine tick control since some farmers may report that they use acaricides when they actually do not for fear of prosecution.

This study shows that different epidemiological states may exist in Murang'a district for tick-borne diseases and these may result in different production losses. The important factor defining these states was the agro-ecological zone, which was associated with grazing

management and breed of calves. However, it is difficult to understand clearly the factors associated with acquisition of infection from a cross-sectional study of serum antibody prevalence and the potential outcome from these infections. Data on disease incidence and production loss can only be obtained from a longitudinal study. Despite this shortfall, the results from the cross-sectional study provided baseline information for the design of the longitudinal study.

5.2 THE LONGITUDINAL STUDY

5.2.1 Farmers response, farm demographics and calf breeds

The results from the longitudinal study provided more detailed assessment on the epidemiology of *T. parva* infections, in particular ECF, and the risk factors associated with it in Murang'a District. The farmers willingness to participate in the study was high even though the calves were bled at short intervals. The two weekly visits and sampling were meant to capture any changes in general farm management and to help detect when young calves were first exposed to *T. parva* infection, and their reaction following infection. The response rate of over 90% compared very well with that obtained from a previous study in the neighbouring Kiambu District where over 90% voluntary participatory rate was achieved (Gitau *et al.*, 1994a).

The study purposively recruited females calves are different from male calves as more attention is given to female calves which are less likely to be culled early in life (Gitau *et al.*, 1994b,c). Widespread upgrading of cattle breeds has taken place in smallholder dairy farms in Murang'a District as less than 10% of calf breeds were phenotypically identified as of indigenous (Zebu) breeds or crosses of Zebu with exotic

breeds. This observation agreed with the results from the neighbouring Kiambu District where no indigenous cattle were found in smallholder dairy farms (Gitau *et al.*, 1994a). The most popular cattle breed was the Friesian, an indication that after Kenya attained independence, upgrading of dairy cattle was aimed at improving milk production in the district (Mbogoh, 1984a).

5.2.2 Calf grazing management and feeding practices

Although the study purposively targeted farms that practised open and zero-grazing management systems, the results indicated that farmers practised the two grazing management systems in some AEZs depending on land availability. Given the small land size available and the high human population density in Murang'a District, the intensive grazing management system has been widely adopted. Most dairy cattle were raised under zero-grazing or strict confinement. This intensive grazing system was supported by the fact that fodder for calves was exclusively obtained from within these smallholder farms in nearly all the farms in UM 1 and UM 2 and over two thirds of the farms in UM 4. This showed that cattle in these smallholder farms were hardly moved out of the farm in search of fodder, in agreement with earlier observations in Kiambu and Murang'a districts (Gitau *et al.*, 1994a; Methu *et al.*, 1996).

The study further showed that feeding of commercial concentrates to calves in all AEZs was not common as was with mineral supplementation. The reason behind this was the high cost of commercial concentrate feeds which meant that many smallholder farmers could not afford especially those without non-farm employment or other income (van Schaik *et al.*, 1996).

5.2.3 Tick control methods and tick numbers

In agreement with the results from the cross-sectional study, the majority of farmers did not practice tick control on their animals. However, there was higher use of acaricides in UM 4 which was the zone with highest risks of *T. parva* infections and ECF The reason why the use of acaricides was not common was likely because they were expensive and few farmers could afford to buy them regularly given other domestic financial needs. Another reason was that communal dips where farmers used to dip their animals without cost or at a highly subsidised rates were no longer functional. This was attributed to the discontinuation of free or subsidised acaricides and maintenance of dips by the government following the economic reforms that introduced cost sharing measures in Kenya. However, it was observed that farmers from the areas at high risk to *T. parva* infection, ECF or those who previously experienced cattle losses from ECF or other tick-borne diseases, were willing to spend money on acaricides rather than risk further losses.

The total number of *R. appendiculatus* ticks counted on the calves was associated with AEZ, grazing management, antibody titres and ECF-specific morbidity and mortality rates. The fact that about 94% of the tick counts were made in UM 4 showed that UM 4 was a high tick challenge area. This observation was in agreement with the high antibody titres estimated in this zone (especially in open grazing management) even though ECF-morbidity and ECF-mortality were not correspondingly high indicating possibility of an endemically stable state. There was further association between the presence of ticks or tick numbers and the reported acaricide application in UM 4. Few ticks or none were recorded from calves that were washed with acaricides, an indication that tick control by acaricide application was effective in preventing ticks on calves.

5.2.4 Measures of calf health

The overall crude calf morbidity of 20.7% estimated in the cohort of female calves in this study in all AEZs was moderately high. There was a strong association between calf morbidity, AEZ and grazing management system. The high overall calf morbidity in UM 4 was explained by the high ECF-morbidity estimated in this AEZ and agreed with the findings from the cross-sectional study that classified UM 4 as a high risk zone to *T. parva* infection. A similar observation was made in UM 1 where high calf morbidity was due to high ECF-morbidity rate. Calf morbidity due to ECF in UM 1 and UM 4 was two thirds and one third in UM 2.

These observations showed that ECF was the major cause of calf morbidity in UM 1 and UM 4 and not in UM 2. The high association between calf morbidity and ECF-morbidity in UM 1 and UM 4 was also associated with grazing practices. In these two AEZs, over two thirds of calf morbidity was recorded in the open-grazing system. In UM 2, where calf morbidity and ECF-morbidity rates were low and weakly associated, zero-grazing system (restricted grazing) was the only grazing management in place. These observations showed that the high calf morbidity in UM 1 and UM 4 was due to the high ECF incidence and open grazing practice which increased the exposure of calves to *T. parva* parasites, thus increasing the ECF incidence. An interesting finding from this study was that the ECF incidence across the AEZs and between grazing systems differed more than the incidence of sero-conversion to *T. parva*. It appeared that either the dose of exposure to *T. parva* parasite or tick infection rates were lower in higher altitude zones than in lower altitude zones. Since the risk of ECF was higher and appeared more important in UM 4 and under open grazing management, efforts for the control of this disease should be directed here.

The crude calf mortality of 7.4% in the cohort of female calves estimated in this study was generally low and was lower than the 13% among female calves in smallholder farms in Kiambu District in Kenya (Gitau *et al.*, 1994b). Calf mortality was associated with AEZ, calf morbidity, ECF and grazing management. Calf mortality rates estimated in UM 1 and UM 4 were more than four times that estimated in UM 2. The ECF-mortality rates estimated in UM 1 and UM 4 were moderately high for a single cause-specific mortality, given that none was reported in UM 2. This could have been explained by the differences in the grazing management across the AEZs where in UM 2, only zero-grazing management was in place, whereas both zero and open grazing management were practised in UM 1 and UM 4. About two thirds of all calf morbidity ended up as ECF-mortality and over three quarters of the ECF-mortality was recorded in open-grazing system. In all AEZs, about 60% of all calf mortality was due to ECF indicating that ECF was the main cause of calf mortalities among the cohort of female calves studied in smallholder dairy farms in Murang'a District. Further to the above observation, about one third of all calves that became clinically sick from ECF died later in in these two AEZs. This again shows the importance of implementing ECF control measures in some AEZs and especially in open grazing managed farms.

Although the incidence of crude calf morbidity and mortality were easily estimated from this study, it was difficult in some instances to make the cause-specific diagnosis. This problem was encountered more often while attempting to confirm ECF mortality since calves that died acutely from clinically diagnosed ECF had not developed adequate antibody titres to *T. parva*. Although lymph node biopsy smears would have assisted in confirming diagnosis in such cases, accurate timing was often difficult and also presented logistical problems in such kind of a study where intensive follow-up and visits

were made. In addition to the above, UM 2 provided a unique problem of very low ECF-morbidity and no ECF-mortality. In an area such as UM 2, a bigger sample size of calves would be required to make better estimates on the potential losses from both the ECF-morbidity and mortality. Other specific causes of acute deaths were not easily verified.

5.2.5 Profiles of antibody titres to *T. parva* parasite

During the first few weeks of life, positive antibody titres to *T. parva* observed in the calves were attributed to the presence of maternally-derived antibodies in the colostrum from the dam. The maternally-derived antibodies however declined rapidly to negative levels by the end of the third month of life. This was consistent with observations in earlier experimental studies (Burridge and Kimber, 1973; Mining *et al.*, 1997). In the subsequent months, consistent rise in antibody titres were attributed to the exposure to *T. parva*.

The proportion of variability in *T. parva* antibodies in the individual-calf antibody titres from visit to visit (which contributed to 50% of total variability) indicated that antibody titres were quite variable at the calf level. This indicated that decline in maternally-derived antibodies and rise in antibody titres following exposure to *T. parva* were both rapidly occurring processes. The farm-to-farm and calf-to-calf variations in antibody titres indicated that calf management practices at the farm and calf levels played important roles in the exposure of calves to ticks. Any future ECF and tick control strategies should thus take into considerations farms and calves at greatest risk and more efforts should be directed there.

There was a strong association between AEZ, grazing management, calves showing maternally-derived antibodies and those showing antibodies due to *T. parva*

exposure at six months of age. The prevalence of antibody titres for calves at birth in UM 4, the high risk AEZ, was more than twice that found in the other two low risk AEZs (UM 1 and UM 2) and this correlated well with antibody prevalence in the dams. During ages 2-3 months, when the maternally-derived antibodies were expected to decline significantly, more than a quarter of the calves in UM 4 had positive antibody titres while in UM 1 and UM 2, the number was less than one tenth. The high *T. parva* prevalence during the last visit in UM 4 agreed with the results from the cross-sectional study that estimated the highest mean prevalence to *T. parva* antibodies in this zone. At the age of six months, the proportion of calves showing positive antibody titres in UM 4 was 45% while in UM 1 and UM 2 it was 38% and 39% respectively, closely agreeing with the results from the cross-sectional study which had estimated antibody prevalence at 27% and 29% in UM 1 and UM 2 respectively. But in UM 4, the estimate was lower than that estimated from the cross-sectional study and this was probably due to high ECF-mortality (lowering the proportion of positive calves) and the zero-grazed farms added from the neighbouring UM 3. Although age at last visit in the longitudinal study was about 6 months compared to 6-18 months old calves in the cross-sectional study, the comparably high prevalence still placed UM 4 as the high risk zone. This suggested that calves were exposed to a more continuous challenge by ticks in UM 4 and this was likely to result to an endemically stable state than would be in the other two AEZs.

After stratifying all calves by grazing management system, the antibody prevalence at birth and six months of age were twice in open-grazing farms compared to zero-grazing farms irrespective of the AEZ. The near 100% antibody prevalence among calves in an open grazing system in UM 4 equalled the dam antibody prevalence showing that calves ingested colostrum. The latter suggested that (based on antibody prevalence),

an endemically stable state existed for *T. parva* under the open grazing management in UM 4. The generally higher antibody prevalence rates recorded in open grazing than zero grazing management systems in all AEZs suggested that the former was likely to be associated with endemic stable state than the latter. The above results agree with observations from other studies that showed a strong association between *T. parva* exposure, mean antibody titres and grazing management (Moll *et al.*, 1986; Young, 1981 ; Maloo, *et al.*, 1994). Irrespective of the AEZ, open grazing management increased the risk of tick challenge to calves and thus infection with *T. parva* parasites while zero grazing management reduced this risk.

5.2.6 Survival times to decline of maternal antibodies and seroconversion to *T. parva*

The survival times to the decline of the maternally-derived *T. parva* colostral antibodies in calves were the same across the three AEZs. The decline of maternally-derived antibodies was not associted with AEZ and grazing system, and this could be explained as a biological process that depends on ingestion of antibody-rich colostrum by the calf. On the contrary, the survival times to seroconversion to *T. parva* were not the same across the AEZs. These differences were explained by the different tick challanges across the AEZs with higher tick challanges in low altitude AEZ than high altitude AEZs. The above observations suggested that endemic stable states were likely to develop faster in UM 4 than in UM 1 and UM 2 and therefore presented different requirements for the control of theileriosis.

5.2.7 Risk factors associated with *T. parva* infections and antibody titres

Both farm and calf level factors were associated with the risk of *T. parva* infections and *T. parva* antibody titres. This study showed that calves that were exposed to open pastures or relied on fodder feeds imported from outside the farm stood a higher risk of being infected with *T. parva* parasite. This was attributed to higher tick challenge in open-grazed farms. The above observation showed that even within the same geographical zone, the risk of *T. parva* infections was not uniform but varied from farm-to-farm depending on the grazing management system in place. It would therefore be misleading and inadequate to classify *T. parva* infections by AEZ alone in situations where different grazing systems are practised within a geographical zone. However, though sero-conversion to *T. parva* showed the extent of the spread of *T. parva* parasite, it was a less important measure of the impact of theileriosis on productivity across AEZs and farms than ECF-morbidity and mortality were. The reason was that the higher proportion of calves that became exposed to *T. parva* did not develop ECF and these were not quantified as losses.

The application of acaricides for tick control is aimed at preventing ticks from attaching to cattle or to kill the ones attached and this breaks the transmission cycle of *T. parva* parasites from an infected to a susceptible host. This study showed that *T. parva* infection was higher (from multi-variate model) even when acaricides were reported to be in use indicating the application of acaricides was ineffective even when administered by farmers privately. The reasons could have been that farmers either were applying inadequate dosage levels of acaricides or only used them when they had tick problems, the latter being the most likely reason. The apparently lower risk of *T. parva* infections among Zebu and their crosses were probably explained by calf number more than the

actual risks per se. There were more exotic calves exposed in terms of numbers than Zebu and their crosses from the study. The age-related risk of *T. parva* exposure followed the naturally expected patterns where the risks increased with the ages of the calves. This study showed that more calves continued to acquire *T. parva* infections with age which may lead to the development of an endemic stable state.

The major cause of calf morbidity in this study was ECF. The results showed that calves suffering from any clinical illness were not necessarily those associated with high risk of sero-conversion to *T. parva* but instead stood a higher risk that the illness was ECF. The reason behind this observation was that sero-conversion to *T. parva* did not result in the development of clinical ECF in the majority of the calves. The latter is the theory behind endemic stability and has been observed in other smallholder dairy farming areas in Kenya (O'Callaghan *et al.*, 1994). This may have meant that in UM 4, where the majority of calves developed antibodies to *T. parva* and yet there were fewer reports on ECF morbidity and mortality than expected, an endemic stable state may have developed.

The presence of *R. appendiculatus* ticks showed mixed relationship with the risk of *T. parva* infection. For some calves, the number of ticks observed on them were not directly related to threshold of antibodies to *T. parva*. Some calves with very many tick did not show a dramatic rise in antibody titres. This observation did not appear to be biologically true. The reasons may have been the difficulties of quantifying the absolute number of ticks that attached on a calf, given that the various tick stages attach and drop on a host and secondly, tick counts were made once every two weeks and not on a daily basis. This was especially true in UM 1 and UM 2 where the tick populations were very low, (in UM 2 only two adult *R. appendiculatus* ticks were recorded over the one-and-a-half-year period), yet the proportion of calves sero-converting was 34%. This meant that

various tick stages attached and dropped from the host before they were observed during the farm visits. The results thus suggest that reliance on tick counts may be a poor indicator of the risk to *T. parva* infection in smallholder dairy farms.

It was apparent from this study that the number of risk factors associated with *T. parva* infections were diverse and may have exerted different influences in different geographical zones. Given the above, the ECF control strategy should take different priorities in different farming systems and geographical zones. The highest priority for ECF control through tick control or immunisation against ECF should be given to open grazing management and in UM 4, the areas where the potential scale of ECF problem was high. Based on the findings from this study, it is also recommended that studies on the impact of *T. parva* infections on smallholder dairy productivity should estimate ECF morbidity and mortality rates *as T. parva* exposure alone is a poor indicator of productivity loss.

5.2.8 Factors associated with calf mean daily weight gains

The study showed that the large proportion of variability in the calf mean daily weight gains were from visit to visit variations within the individual calf indicating that individual calf weight measures were highly variable. The various data analyses showed that mean daily weight gains in calves were mainly associated with visit level factors. Factors affecting calf growth therefore had the greatest influence at the individual calf level as observed in another study (Gitau *et al.*, 1994c). The farm-to-farm and calf-to-calf variations in mean daily weight gains were major sources of variations (Gitau *et al.* 1994c). These results indicated that management practices at the farm level did not play a major role in influencing calf growth in smallholder daily farms. Improvement on calf

growth in Murang'a District should therefore target the individual calf level management practices.

The inverse relationship between age of calf and mean daily weight gains was probably related to nutritional factors. Young calves up to three months depended on milk as the main diet (the weaning age). After three months of age, milk was replaced gradually by forage feeds with commercial concentrate feed supplements for some calves (over 80% did not receive). This resulted in lower mean daily weight gains as the forage feed alone could not meet and maintain the dietary requirements for the fast growing heifer calves. The age-related mean daily weight gains was more evident in UM 2 where the highest overall mean daily weight gains were recorded early in life but later declined by over 50% after 3 months of age and did not recover by 6 months of age. The observed inverse relationship between mean daily weight gains and age of calf suggest that improvements on calf growth should be directed at calf nutrition among other factors.

The inverse relationship between mean daily weight gains and *T. parva* antibody titres may have meant that even though clinical ECF was not observed, sub-clinical ECF may have occurred and this could have been responsible for the decline in calf growth.

The presence of disease in a dairy production enterprise is known to impair productivity (Radostitis and Blood, 1985). The results from this study supported the above observation as it was shown that calves that suffered any morbidity were associated with low mean daily weight gains and recorded poor growth. However, this was not true with calves with ECF-morbidity. In contrast, in a study in Trans Mara District in Kenya, Moll *et al.*, (1986) reported that theileriosis was the major cause of poor calf growth. Although recovery in mean daily weight gains were recorded for some calves in Murang'a District after morbidity events, most were not able to immediately compensate

for the weight loss after recovery and this was due to the severity of morbidity and the poor nutritional availability.

Tick infestation is associated with direct loss in productivity through blood sucking or indirectly through transmission of tick-borne infections (Norval *et al.*, 1992). In this study, the loss in productivity associated with tick infestations was hypothesised to be indirect and related to theileriosis transmitted by *R. appendiculatus*. But since the rising *T. parva* antibody titres were associated with low mean daily weight gains, it was likely that the presence of *R. appendiculatus* were associated with an indirect loss in productivity through the transmission of *T. parva* infection. *Rhipicephalus appendiculatus* tick numbers were low and thus the blood sucking effect may not have been responsible for poor calf growth.

CHAPTER 6

CONCLUSIONS

Studies on the epidemiology of tick-borne infections and in particular theileriosis in smallholder dairy farms in Kenya and in other areas where the diseases have a great impact on productivity are lacking. The purpose of this chapter is to highlight the important findings and conclusions that arose from the study and to suggest areas of future research and possible control measures where the risk from these diseases is highest.

Most data that describe the status, extent and impact of tick-borne diseases on cattle in Kenya and other regions are based on passively-derived data or retrospective studies. Although such data are useful indicators of the existence of tick-borne diseases in certain areas, they cannot be extrapolated to explain the disease status in the population. There is therefore a need to improve on the quality of data available on tick-borne diseases by carrying out appropriate studies such as prospective observational studies. Data obtained from prospective studies can be used to estimate population parameters on tick-borne diseases and help to identify the specific constraints on health and productivity associated tick-borne diseases.

The available land among the smallholder dairy farms in Murang'a District is small and is used for dairying, and growing of both food and cash crops. Given the small land size and the competitive land use for various farming enterprises, appropriate intensification is needed to maximise productivity on all these enterprises. This calls for improvement and efficient management of the dairy enterprise to enable the farmers to optimise production performances.

Data gathering was mainly facilitated by the close visit intervals and sampling and farmers willingness to participate. The short visit intervals enabled farmers to recall and monitor closely the management and outcome events. In addition to close monitoring, the farmers were very cooperative in the initial response to participate in the study and afterwards allowing their calves to participate in the study even with short bleeding interval. The close monitoring interval and the remarkable participation by the farmers made data gathering easy and thus helped to improve on the precision on the estimates on calf productivity made. As farmers willingness to participate in the study was very encouraging, it is my hope that many researchers will take this advantage and conduct many on-farm observational studies. Such studies will help to improve animal health and productivity data bases on the smallholder dairy farms which are currently inadequate or are unreliable.

The serum antibody prevalence rates estimated on tick-borne diseases from the cross-sectional study varied significantly across AEZs and between grazing system. These two stratifying factors, that is AEZ and grazing system, were most important in explaining the variation in the prevalence rates estimated since all the other factors were distributed within them. However, this variation was more marked for *T. parva* parasite than for *T. mutans* and *B. bigemina*. The antibody prevalence results provided a reliable baseline for explaining the distribution of tick-borne infections in Murang'a District and enabled the identification of potential endemic states to the tick-borne parasites. The main limitation of the cross-sectional study was the inability to make estimates on calf productivity parameters such as the incidence of morbidity, mortality and ECF and calf growth measures such as mean daily weights gains. This limitation was overcome by carrying out a longitudinal study that comprised the second phase of the study.

The longitudinal study estimated female calf morbidity and mortality rates at 20.7% and 7.4% respectively. Calf morbidity estimated from this study was moderately high while calf mortality was considered to be low. This study showed that the major cause of female calf morbidity and mortality was ECF since about one third of the female calves born became sick (over 80% from ECF) and about one tenth died (about 60% from ECF). The high ECF morbidity and mortality were mainly associated with AEZ and grazing management. Since the risk of ECF was high and more important in UM 4 and under open grazing management, farmers in these areas should continue with efforts of controlling the disease through tick control by acaricide application. Any efforts on ECF control including immunisation should be directed in these areas as it is here that the animals are at the greatest risk of the disease. East Coast fever immunisation appears to be the choice for future ECF control given that the treatment of ECF is expensive and use of communal-based dip tanks has virtually collapsed in Kenya. Since it was established that most morbidity and mortality in the smallholder dairy farms in Murang'a were from ECF, then immunisation against ECF will provide a cost-effective ECF control option to be adopted by the farmers.

Although all AEZs were associated with moderately high and similar sero-conversion rates to *T. parva* parasite, ECF morbidity and mortality rates were different across the AEZs. Sero-conversion showed that *T. parva* parasite was present in all AEZs. The differences in ECF morbidity and mortality rates across the AEZs could only be explained by the different risks of exposure to *T. parva* parasite which were associated with varying tick populations across the AEZs and the different grazing systems adopted within an AEZ. As mentioned earlier, ECF morbidity and mortality rates were better and more reliable in estimating calf productivity losses than *T. parva* exposure alone. Some

crucial points arising from these observations are: i) studies on the epidemiology to *T. parva* need to be stratified by grazing management as a generalisation may be misleading, ii) tick and ECF control would require different approaches between the grazing management systems, and iii) the high priority ECF control AEZ based on the results from this study is UM 4 where currently, the tick challenge and parasite exposure are high.

Another important aspect that deserves serious attention is calf growth. The mean daily weight gains from this study indicated that calf growth was poor. The mean daily weight gain of 0.25 kgs was below the recommended daily weight gains of 0.50 - 0.73 kgs from the developed countries or the 0.4 - 0.5 kgs recommended target for smallholder dairy farms in central highlands of Kenya. Since calf morbidity and ECF were associated with calf growth, efforts need to be directed towards improved calf performance. The latter could be achieved through extension services which are aimed at preventing or reducing the overall calf and ECF-morbidity. However, since calf nutrition was also associated with calf growth, the extension services should also incorporate proper feeding management in particular supplementing the growing heifers with commercial feed concentrates.

Finally this study has contributed towards an understanding of the current status and the epidemiology of theileriosis in smallholder dairy farms in Murang'a District in central highlands of Kenya.

CHAPTER 7

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LIST OF APPENDICES

Appendix 1.1

TICK-BORNE DISEASE PROJECT - MURANG'A DISTRICT

FARM QUESTIONNAIRE - CROSS-SECTIONAL STUDY

1. Date (day/month/year):__/__/

2. Investigator Administering Survey:

3. Agro-ecological zone:
   1) LH 1
   2) UM 1
   3) UM 2
   4) UM 3
   5) UM 4

4. Division
   1) Kangema
   2) Kiharu
   3) Kigumo
   4) Kandara
   5) Gatanga
   6) Makuyu

5. Sub-location/Farm ID: _____ owner's name _____

6. What farm related activities are present in the farm?

	acreage	% of total
1) Dairy farming	_____	_____
2) Coffee	_____	_____
3) Tea	_____	_____
4) Horticultural products	_____	_____
5) Subsistence crops	_____	_____
6) other. Specify:	_____	_____

7. Give a breakdown of the number of cattle present on your farm by breed.

	calves < 6mths	calves 6-18mths	adult > 18mths
1) Friesian	_____	_____	_____
2) Guernsey	_____	_____	_____
3) Ayrshire	_____	_____	_____
4) Jersey	_____	_____	_____
5) Zebu	_____	_____	_____
6) Cross (exo*exo)_____		_____	_____
7) Cross (exo*local____		_____	_____
8) Other (specify)_____		_____	_____

8. Is the farm owner employed or has another business outside the farm?
   0) No
   1) Part time

2) Full time

9. Who looks after the animals?
   1) Owner
   2) Wife
   3) Children
   4) Employee

10. Do you control ticks on your animals? If so, by what method?
   0) no tick control
   1) dipping
   2) spraying
   3) combination of spraying/dipping.
   4) `hand dressing'
   5) other. Specify:

11. What time of the year do you control for ticks?
   1) Regularly
   2) when tick burden is high
   3) varies with season
   4) other. Specify:

12. Does the frequency of tick control vary with season or tick burden?
   0) No
   1) Yes

13. Where do your animals spend during the day?
   1) grazing (private paddocks)
   2) grazing (communal pastures)
   3) zero grazing (complete confinement)
   4) semi-zero grazing (sometimes confined)
   5) other. Specify:

14. How do your animals get access to forage?
   1) grazing entirely on pastures
   2) cut/purchased and transported to animals
   3) combination of the above

15. **If under grazing**, size of grazing area _____acres.

16. What is the source of the forage for the animals?
   1) grown within the farm
   2) from neighbourhood
   3) `imported' from far (owner's other farms or bought)
   4) others specify _____

17. What type of housing/shelter is available for the animals?
   1) closed (wooden walls with a roof)
   2) closed (concrete walls with a roof),

3) open (no walls but with a roof)
4) open (no walls no roofs)
5) other. Specify:

18. Where do your cattle have access to water during the wet season?
1) water provided in housing area
2) water available on pasture/grazing
3) water available in stream/river away from housing area
4) other. Specify:

19. Where do your cattle have access to water during the dry season?
1) water provided in housing area
2) water available on pasture/grazing
3) water available in stream/river away from housing area
4) other. Specify:

20. If animals have access to water outside housing area, how long do they travel?
1) < 0.5 Km
2) > 0.5 - 1 Km
3) > 1 Km - 2 Km
4) > 2 Km - 3 Km
5) > 3 Km

21. Does the source of water vary between wet and dry season?
0) No
1) Yes

Appendix 1.2

TICK-BORNE DISEASE PROJECT - MURANG'A DISTRICT

CALF QUESTIONNAIRE - CROSS-SECTIONAL STUDY

To be filled out for **EACH** calf between six and 18 months old.

1. Date (day/month/year):__/__/

2. Investigator Administering Survey: _____

3a. Agro-ecological zone:
1) LH 1
2) UM 1
3) UM 2
4) UM 3
5) UM 4

3b. Division
1) Kangema
2) Kiharu
3) Kigumo
4) Kandara
5) Gatanga
6) Makuyu

4. Location/Sub-loc./Farm ID: _____ owner's name _____

5. Calf ID - (Name or Number):

6. Breed:
1) Friesian
2) Guernsey
3) Ayrshire
4) Jersey
5) Zebu
6) Cross (exo*exo)
7) Cross (exo*loc)
8) Other: specify

7. Breed of Dam:
1) Friesian
2) Guernsey
3) Ayrshire
4) Jersey
5) Zebu
6) Cross (exo*exo)
7) Cross (exo*loc)
8) Not Known
9) Others: specify

8. Breed of Sire:
1) Friesian
2) Guernsey
3) Ayrshire
4) Jersey
5) Zebu
6) Cross (exo*exo)
7) Cross (exo*loc)
8) Not known
9) Others: specify

9. Date of Birth (d/m/y):__/__/__, if unavailable, approx. age ____months old

10. Sex:
0) male
1) female

11. What are you presently feeding this calf?
1) forage
2) grain/concentrate
3) mineral supplement
4) legumes. Specify:
5) other. Specify:

12. What is the source of the forage feed?
1) owners farm
2) brought from outside the farm
3) combination of 1 & 2

13. Has this calf experienced any "sickness" since birth?
0) No
1) Yes

14. Has this calf received any vaccinations since birth?
0) no vaccines
1) vaccinated.

15. Name the vaccines Product Date(s)

16. Has this calf been "dewormed" since birth?
0) not dewormed
1) dewormed.

17. Has any prophylactic medication been administered to this calf since birth?
0) no prophylactic medication
1) prophylactic medication administered

18. When was this calf last treated for ticks?
0) do not treat for ticks
1) calf not yet treated for ticks

2) < 7 days
3) 8 days - 2 weeks
4) > 2 - 4 weeks
5) > 1 month

Has this calf ever been diagnosed as having, or treated for any of the following tick-borne diseases? Give details as indicated.

19. Theileriosis ("Ngai"/East Coast Fever)
0) No
1) Yes. Give details:

Date: _____ Severity:_____ Response:
_____ _____

20. Anaplasmosis ("Ndigana"/Gall-sickness)
0) No
1) Yes. Give details:

Date: _____ Severity:_____ Response:
_____ _____

21. Babesiosis ("Guthuguma Thakame"/Red Water)
0) No
1) Yes. Give details:

Date: _____ Severity:_____ Response:
_____ _____

Appendix 1.3
TICK-BORNE DISEASE PROJECT - MURANG'A DISTRICT - CALF FORM

CALF QUESTIONNAIRE - LONGITUDINAL STUDY

To be filled out for **ALL FEMALE CALVES** from birth until **six months of age**.

1. Date (day/month/year):__/__/

2. Investigator Administering Survey:

3. Agro-ecological zone: 1) UM 1
2) UM 2
3) UM 4

4. Farm ID: _____

5. Owners name: _____

6. Visit number: _____

7. Grazing system: 1) Zero-grazing
2) Open-grazing

8. Calf ID - (Name or Number): _____

9. Breed: 1) Friesian
2) Guernsey
3) Ayrshire
4) Jersey
5) Zebu
6) Cross (exo*exo)
7) Cross (exo*loc)
8) Other: specify

10. Date of Birth (d/m/y):__/__/__, (or approx. age ____months old).

11. Where is this calf currently kept at the moment?
1) Indoors
2) Outdoors
3) Combination of 1 & 2

12. What are you presently feeding this calf?
1) forage
2) grain/concentrate
3) mineral supplement
4) legumes. Specify:

5) other. Specify:

14. What is the source of the forage feed?
    1) owners farm
    2) brought from outside the farm
    3) combination of 1 & 2

15. What method of tick treatment do you apply on this calf?
    0) None
    1) Dipping
    2) Spraying
    3) Handwash
    4) Combination of 1, 2 & 3

16. Was this calf treated for ticks since the last visit?
    0) No
    1) Yes

17. Has this calf experienced any "sickness" since birth or last visit?
    0) No
    1) Yes

18. If **CURRENTLY SICK**, specify details on vital parameters

Temp_____Resp._____MM_____LNs_____

19. If the calf is withdrawn from the study, state the reason
    1) Died
    2) Withdrawn
    3) Sold
    4) Others. Specify_____

20. Weight: _____ kg (to the nearest 0.5)

Tick Form - Fill in details as specified

Rhipecephalus appendiculatus		Number
Adults	Males	_____
	Females	_____
	Engorged	_____
Nymphs	Total	_____
	Engorged	_____

Rhipecephalus evertsi

Males	_____
Females	_____
Engorged	_____

Boophilus decoloratus

Females engorged	_____

Amblyoma variegatum

Males	_____
Females	_____
Engorged	_____

Appendix 2.1. Follow-up life table showing overall morbidity survivorship for the 225 cohort of females calves from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Visit Number	Initial at risk	Number of cases	Number of withdrawals	Risk rate	Survival rate	Survivorship to beginning of interval	Standard error
1	225	2	0	0.00	100.00	100.00	0.00
2	225	5	4	2.22	97.78	100.00	1.00
3	223	4	2	1.81	98.19	97.78	1.30
4	219	1	9	0.47	99.53	96.01	1.40
5	210	6	8	2.91	97.09	95.57	1.43
6	202	6	10	3.05	96.95	92.79	1.87
7	192	4	6	2.11	97.89	89.96	2.00
8	186	3	5	1.63	98.37	88.06	2.14
9	181	3	6	1.60	98.40	86.62	2.26
10	177	3	7	1.73	98.27	85.24	2.37
11	170	0	4	0.00	98.27	83.76	2.32
12	166	0	3	0.00	98.27	82.31	2.29
13	163	1	5	0.62	99.38	80.89	2.30
14	158	0	2	0.00	99.38	80.39	2.30

Appendix 2.2. Follow-up life table showing overall mortality survivorship for the 225 cohort of females calves from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Visit Number	Initial at risk	Number of cases	Number of withdrawals	Risk rate	Survival rate	Survivorship to beginning of interval	Standard error
1	225	0	0	0.00	100.00	100.00	0.00
2	225	2	1	0.89	91.10	100.00	0.60
3	224	2	2	0.90	91.00	91.10	1.89
4	222	4	5	2.01	79.90	82.90	1.14
5	217	2	6	0.94	90.60	66.24	1.01
6	210	3	7	1.45	85.50	60.01	1.28
7	205	1	5	0.49	95.10	51.31	1.13
8	201	1	4	0.50	95.00	48.80	1.10
9	196	1	5	0.52	94.80	46.36	1.10
10	191	0	7	0.00	94.80	43.95	1.10
11	184	0	4	0.00	94.80	41.66	1.00
12	180	0	3	0.00	94.80	39.49	0.90
13	177	0	5	0.00	94.80	37.44	0.87
14	172	0	2	0.00	94.80	35.49	0.82

Appendix 2.3. Follow-up life table showing ECF-morbidity survivorship for the 225 cohort of females calves from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Visit Number	Initial at risk	Number of cases	Number of withdrawals	Risk rate	Survival rate	Survivorship to beginning of interval	Standard error
1	225	2	0	0.89	91.10	100.00	0.00
2	225	2	4	0.90	91.00	81.08	0.50
3	223	3	2	1.35	86.50	73.78	0.76
4	219	1	9	0.47	95.53	63.82	0.72
5	210	4	8	1.94	80.60	60.97	0.91
6	202	2	10	1.01	98.80	49.14	0.90
7	192	4	6	2.12	78.80	44.13	0.94
8	186	1	5	0.54	94.60	34.77	0.76
9	181	3	6	1.69	83.10	32.89	0.79
10	177	3	7	1.73	82.70	27.34	0.76
11	170	0	4	0.00	82.70	22.61	0.63
12	166	1	3	0.61	93.90	18.70	0.54
13	163	0	5	0.00	93.90	17.56	0.50
14	158	0	2	0.00	93.90	16.49	0.47

Appendix 2.4. Follow-up life table showing ECF-mortality survivorship for the 225 cohort of females calves from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Visit Number	Initial at risk	Number of cases	Number of withdrawals	Risk rate	Survival rate	Survivorship to beginning of interval	Standard error
1	225	0	0	0.00	100.00	100.00	0.00
2	225	2	1	0.89	91.10	100.00	0.63
3	224	1	2	0.45	95.50	91.10	0.71
4	222	2	5	1.00	90.00	87.00	2.20
5	217	2	6	0.94	90.60	81.99	2.20
6	210	1	7	0.48	95.20	74.28	1.98
7	205	0	5	0.00	95.20	70.72	1.88
8	201	1	4	0.50	95.00	67.32	1.83
9	196	0	5	0.00	95.00	63.96	1.74
10	191	0	7	0.00	95.00	60.76	1.65
11	184	0	4	0.00	95.00	57.72	1.57
12	180	0	3	0.00	95.00	54.83	1.49
13	177	0	5	0.00	95.00	52.09	1.42
14	172	0	2	0.00	95.00	49.49	1.35

Appendix 2.5. Mean antibody titres for calves in Upper Midlands 1 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Standard Deviation	Standard Error	Range	Confidence Limits (95%)
9	76	24.4	8	31.1	3.6	1 - 119	17.3, 31.5
24	76	20.7	6	28.7	3.3	1 - 117	14.2, 27.2
38	74	15.3	4	23.3	2.7	1 - 91	10.0, 20.6
52	70	10.4	2	18.9	2.3	1 - 80	5.9, 14.9
66	70	9.3	2	16.6	2.0	1 - 72	5.4, 13.2
80	66	9.9	2	18.7	2.3	1 - 93	5.4, 14.4
94	64	8.7	3	16.4	2.1	1 - 97	4.4, 12.8
109	63	9.3	3	18.1	2.3	1 - 102	4.8, 13.8
123	62	8.5	4	15.7	2.0	1 - 98	4.6, 12.4
136	61	10.2	4	17.0	2.2	1 - 112	5.9, 14.5
150	58	12.1	5	20.8	2.7	1 - 129	6.8, 17.4
164	57	13.9	6	21.1	2.8	1 - 102	8.4, 19.4
178	56	16.7	7	23.2	3.1	1 - 104	10.6, 22.8
192	53	20.5	8	26.8	3.7	1 - 111	13.2, 27.8

Appendix 2.6. Mean antibody titres for calves in Upper Midlands 2 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Standard Deviation	Standard Error	Range	Confidence Limits (95%)
10	50	12.7	9	12.8	1.8	1 - 52	9.2, 16.2
24	50	8.2	6	9.5	1.3	1 - 49	5.7, 10.7
39	50	5.8	4	8.7	1.2	1 - 45	3.5, 8.2
53	49	4.9	3	7.6	1.1	1 - 40	2.7, 7.1
67	49	4.6	2	4.6	1.4	1 - 62	1.9, 7.3
81	48	3.5	2	4.3	0.6	1 - 21	2.3, 4.7
96	48	3.6	2	4.2	0.6	1 - 21	2.4, 4.8
109	45	4.8	4	4.4	0.7	1 - 20	3.4, 6.2
124	44	7.1	5	7.5	1.1	1 - 35	4.9, 9.3
138	42	8.7	5	10.1	1.6	1 - 48	5.6, 11.8
151	40	9.3	7	10.6	1.7	1 - 46	6.0, 12.6
166	38	11.7	9	9.6	1.6	1 - 40	8.6, 14.8
180	37	16.4	11	16.8	2.8	1 - 69 1	0.9, 21.9
192	36	16.6	10	17.1	2.9	1 - 67 1	0.9, 22.3

Appendix 2.7. Mean antibody titres for calves in Upper Midlands 4 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Standard Deviation	Standard Error	Range	Confidence Limits (95%)
10	99	45.9	43	31.1	3.1	1 - 115	40.0, 52.0
24	96	36.3	31	28.2	2.9	1 - 108	30.6, 42.0
38	93	27.0	20	25.2	2.6	1 - 106	21.9, 32.1
52	89	20.7	12	22.0	2.3	1 - 103	16.2, 25.2
66	86	15.7	10	18.6	2.0	1 - 95	11.8, 19.6
80	81	14.5	7	19.5	2.2	1 - 96	10.2, 18.8
94	79	13.8	6	18.8	2.1	1 - 89	9.7, 17.9
108	77	13.4	6	19.0	2.2	1 - 81	9.1, 17.7
122	73	15.9	6	22.4	2.6	1 - 100	10.8, 21.0
136	71	18.9	8	24.4	2.9	1 - 95	13.2, 24.6
150	70	22.5	9	28.1	3.4	1 - 109	15.8, 29.2
163	69	24.2	7	30.9	3.7	1 - 121	16.9, 31.5
177	68	24.6	10	30.3	3.7	1 - 117	16.5, 31.9
191	66	27.4	11	32.4	4.0	1 - 138	19.6, 35.2

Appendix 2.8. Mean antibody titres for calves in Upper Midlands 1 by age (in days) and grazing system from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Grazing System[a]	Mean[b]	Median	Standard Deviation	Standard Error	Range
9	34	1	17.7	6.5	25.0	4.3	1 - 92
	42	2	29.9	10.5	34.6	5.3	1 - 119
24	34	1	15.5	3.5	23.2	4.0	1 - 77
	42	2	25.1	9	32.2	5.0	1 - 117
38	32	1	10.7	2	18.4	3.2	1 - 75
	42	2	18.8	5	26.1	4.0	1 - 91
52	30	1	6.7	2	12.7	2.3	1 - 51
	40	2	13.2	2	22.2	3.5	1 - 80
66	30	1	7.6	2	15.3	2.8	1 - 72
	40	2	10.6	2	17.5	2.7	1 - 61
80	28	1	7.4	1	15.9	3.0	1 - 70
	38	2	11.7	3	20.6	3.3	1 - 93
94	26	1	7.2	2	13.9	2.7	1 - 67
	38	2	9.7	3	18.1	3.0	1 - 97
109	26	1	9.1	3.5	17.7	3.4	1 - 85
	37	2	9.5	3	18.7	3.1	1 - 102
123	26	1	8.5	4	13.7	2.7	1 - 61
	36	2	8.5	3.5	17.2	2.9	1 - 98
136	26	1	9.7	6	11.3	2.2	1 - 52
	35	2	10.6	3	20.4	3.4	1 - 112
150	26	1	9.9	6	12.7	2.5	1 - 56
	32	2	13.9	4.5	25.7	4.5	1 - 129
164	26	1	11.8	5	18.3	3.5	1 - 83
	31	2	15.7	7	23.3	4.1	1 - 102
178	24	1	13.3	5	20.4	4.1	1 - 73
	32	2	19.3	10	25.2	4.5	1 - 104
192	23	1	15.3	6	23.1	4.8	1 - 88
	30	2	24.5	11.5	29.1	5.3	1 - 111

[a]Zero-grazing = 1; Open grazing = 2.
[a]T-test shows significant differences in antibody titres between zero and open grazing only at 9 days old ($P < 0.1$).

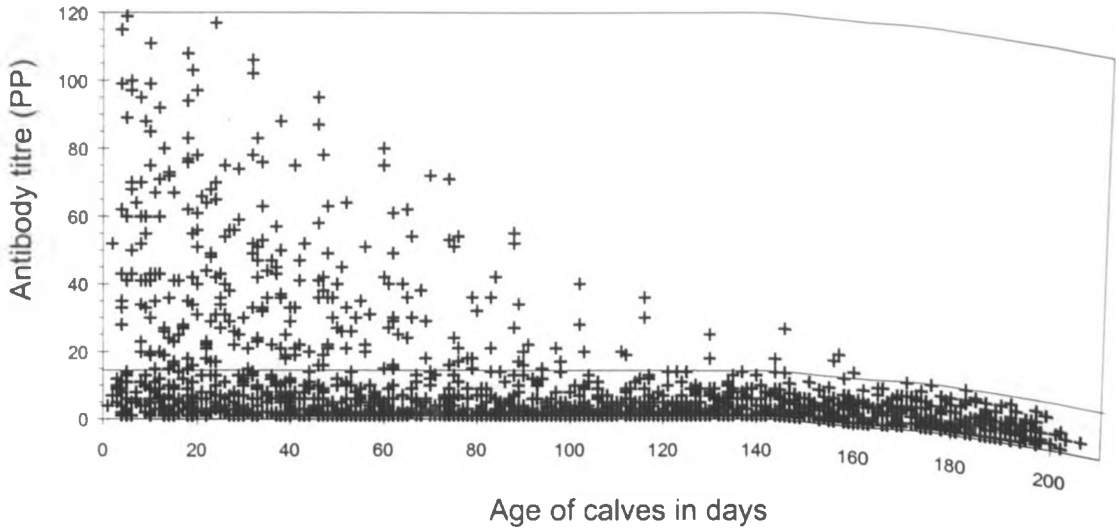
Appendix 2.9. Mean antibody levels for calves in Upper Midlands 4 by age (in days) and grazing system from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Grazing System[a]	Mean[b]	Median	Standard Deviation	Standard Error	Range
10	50	1	33.8	22.5	32.0	4.5	1 - 115
	49	2	58.2	58	24.9	3.5	9 - 106
24	50	1	27.3	18	27.3	3.9	1 - 108
	46	2	46.2	45	26.0	3.8	3 - 99
38	48	1	20.0	10	25.1	3.6	1 - 106
	45	2	34.4	31	23.4	3.4	3 - 102
52	48	1	14.4	6	21.1	3.1	1 - 103
	41	2	28.1	26	20.8	3.3	3 - 88
66	46	1	11.8	3	18.8	2.8	1 - 95
	40	2	20.3	15.5	17.4	2.7	2 - 75
80	43	1	11.1	4	20.0	3.0	1 - 96
	38	2	18.2	12.5	18.5	3.0	1 - 71
94	44	1	12.1	3.5	20.1	3.0	1 - 89
	35	2	17.1	9	17.1	2.9	1 - 61
108	42	1	11.2	3	18.1	2.7	1 - 81
	35	2	16.1	8	20.0	3.4	1 - 78
122	38	1	13.5	4.5	21.1	3.4	1 - 80
	35	2	18.5	8	24.0	4.0	1 - 100
136	38	1	16.2	6.5	21.6	3.5	1 - 84
	33	2	22.1	9	27.2	4.7	1 - 95
150	38	1	18.2	5.5	24.6	4.0	1 - 83
	32	2	27.6	14	31.4	5.5	1 - 109
163	37	1	17.3	6	24.9	4.1	1 - 98
	32	2	32.2	17.5	35.5	6.3	1 - 121
177	36	1	16.1	6.5	22.2	3.7	1 - 95
	32	2	34.3	16.5	35.4	6.2	1 - 117
191	35	1	16.3	7	20.3	3.4	1 - 87
	31	2	40.0	34	38.7	6.9	1 - 138

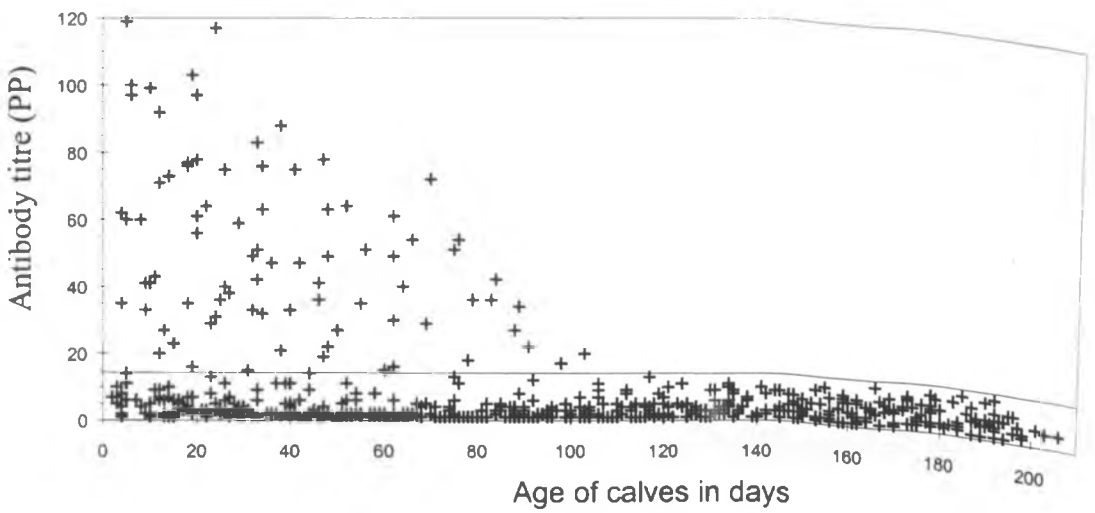
[a]Zero-grazing = 1; Open grazing = 2

[a]T-test shows significant differences in antibody titres as follows: 10-52 days, $p<0.001$; 191 days, $p<0.01$; visits 66 & 177 days, $p<0.05$; 80 & 163 days, $p<0.1$. No significant differences for days 90-150.

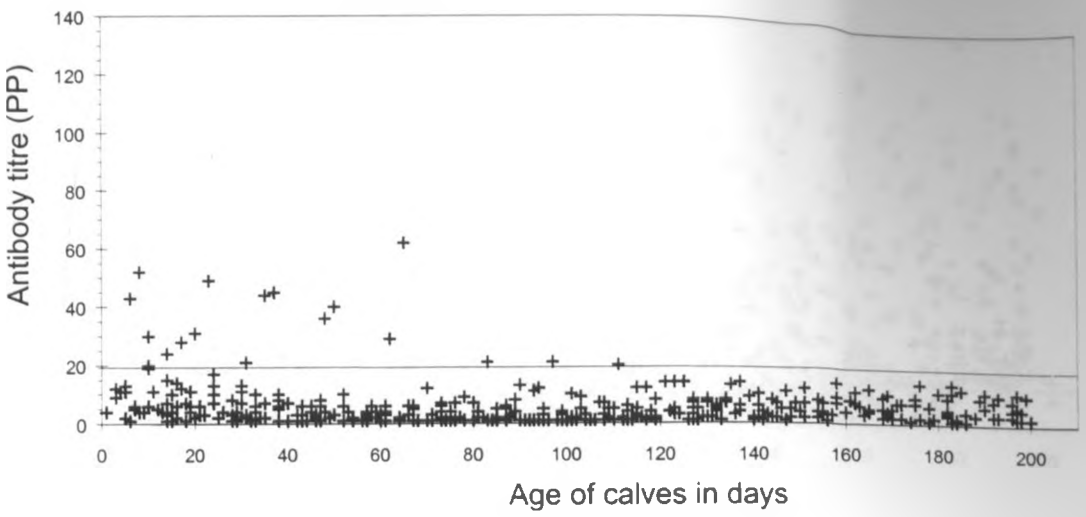
Appendix 2.10. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that did not sero-convert to *T. parva* in all AEZs from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



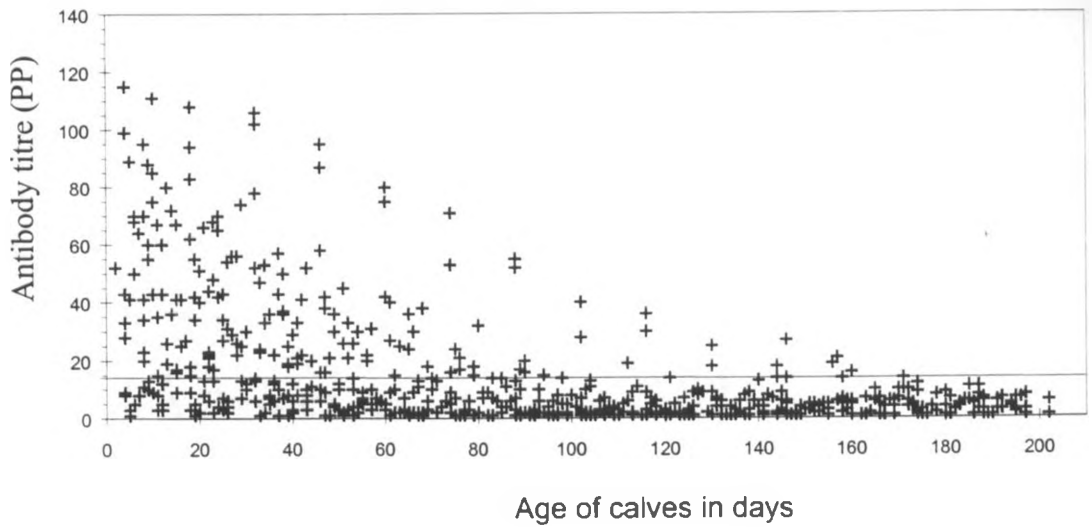
Appendix 2.11. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that did not sero-convert to *T. parva* in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



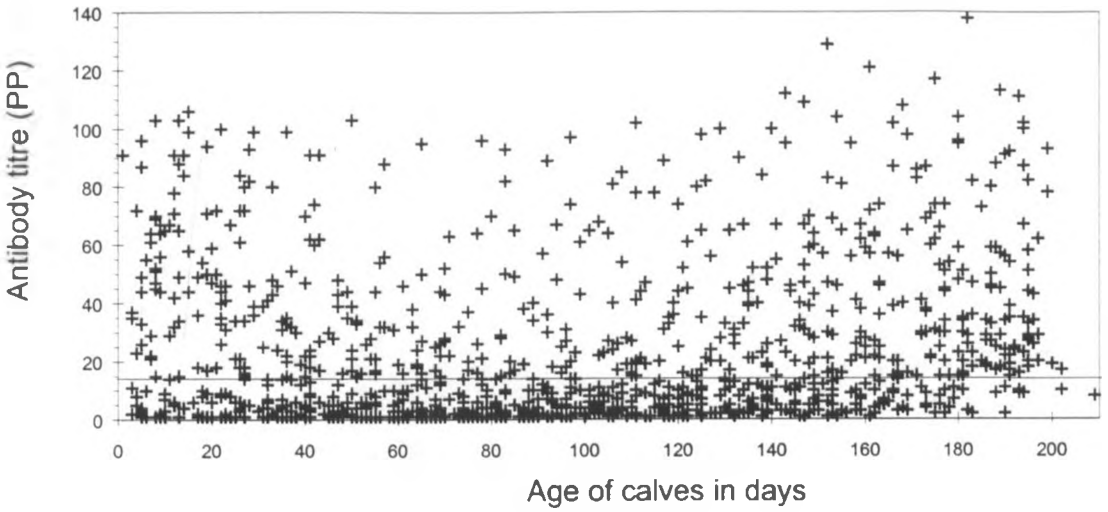
Appendix 2.12. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that did not sero-convert to *T. parva* in UM 2 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



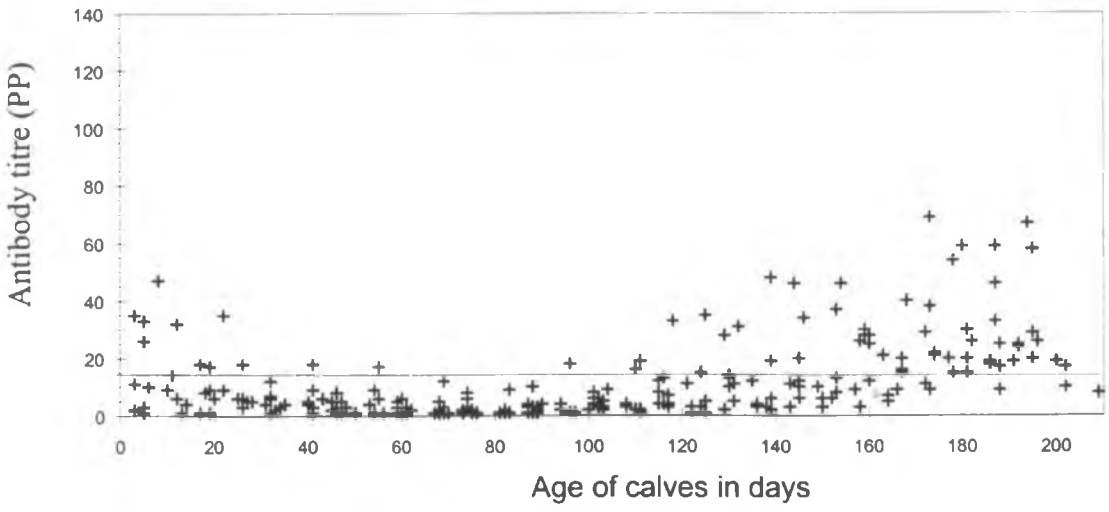
Appendix 2.13. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that did not sero-convert to *T. parva* in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



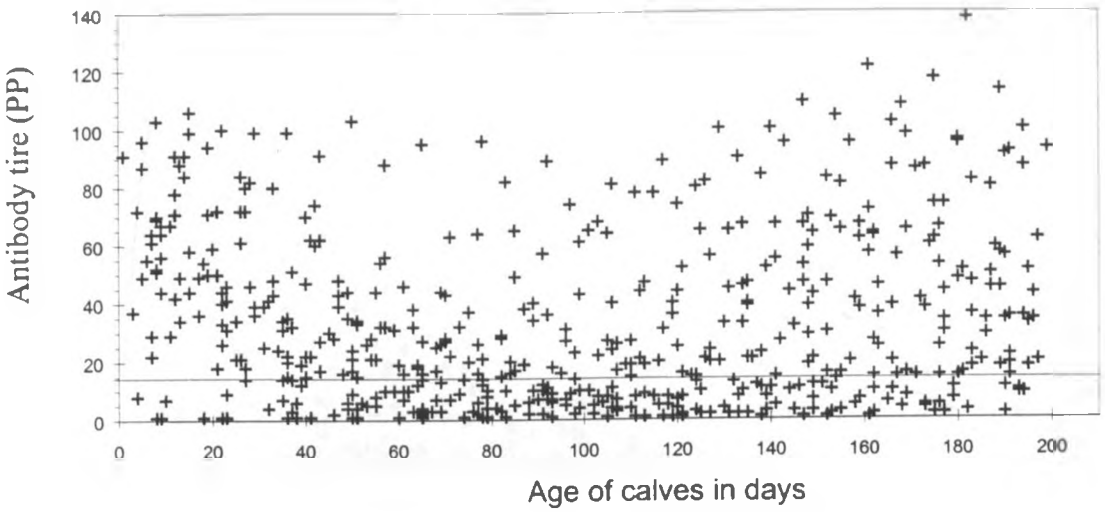
Appendix 2.14. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



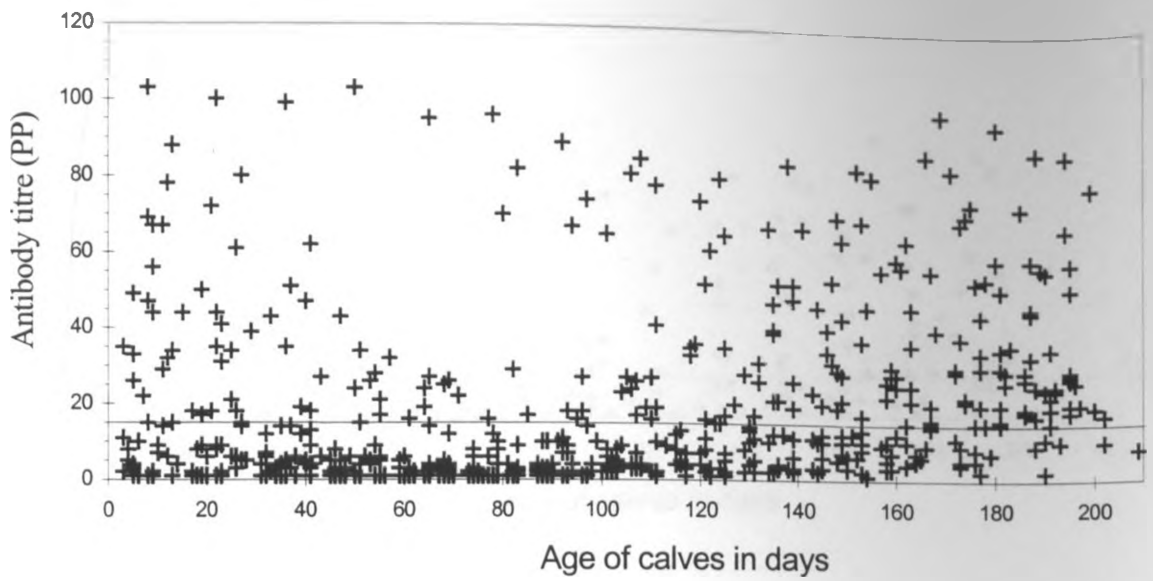
Appendix 2.15. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* in UM 2 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



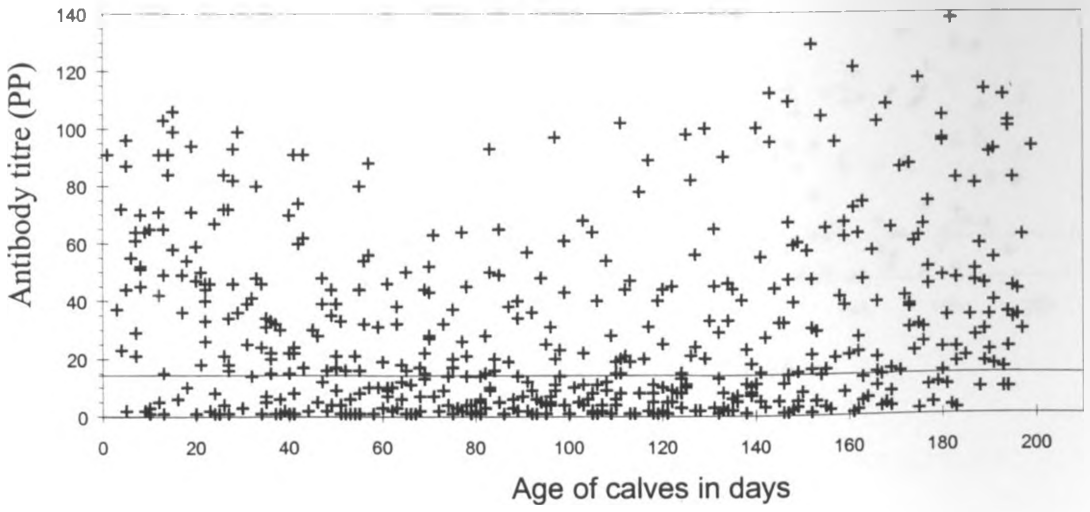
Appendix 2.16. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



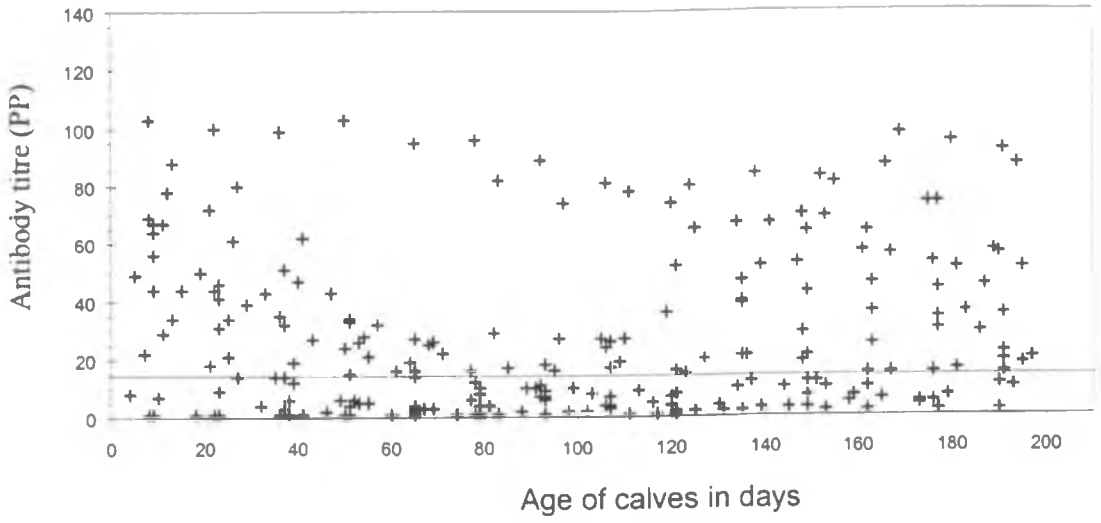
Appendix 2.17. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* under zero-grazing in UM 1 from the longitudinal study in, Kenya Murang'a District (March 1995 - August 1996).



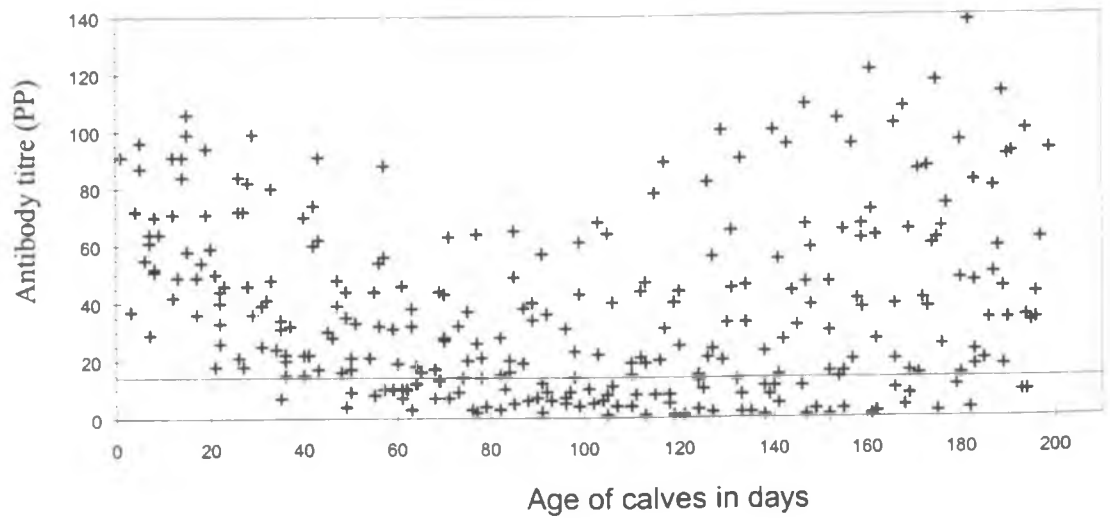
Appendix 2.18. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* under open-grazing in UM 1 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 2.19. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* under zero-grazing in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 2.20. Distribution of antibody titres (percent positivity – PP) by age of calves (in days) for calves that sero-converted to *T. parva* under open-grazing in UM 4 from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 2.21. Factors significantly associated with *T. parva* antibody titres in the multi-variate mixed models stratified by AEZ from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Estimate	Std error	P-value
Upper Midlands 1			
Visit level factors			
Dam antibodies	0.473	0.058	0.0001
Calf level factors			
Calf housing	-2.215	0.945	0.0194
Age of calf	0.018	0.011	0.0962
Daily weight gains	-9.243	2.825	0.0011
Calf sickness	-10.632	5.319	0.0460
Upper Midlands 2			
Visit level factors			
Source of forage	2.832	1.158	0.0145
Daily weight gains	-7.941	1.826	0.0001
Calf level factors			
Dam antibodies	0.482	0.023	0.0001
East Coast fever	-13.250	3.872	0.0006
Upper Midlands 4			
Visit Level factors			
Source of forage	2.928	1.531	0.0562
East Coast fever	-14.441	5.583	0.0093
Age of calf	-0.028	0.011	0.0130
Acaricide application	-6.421	3.770	0.0889
Males[b]	4.319	1.964	0.0281
Females[c] (engorged)	-5.330	2.740	0.0521
Calf level factors			
Dam antibodies	0.416	0.066	0.0001

[a]Random-effects variables not significant in all the uni-variate mixed model ($p<0.05$)
[b]Male *R. appendiculatus* tick
[c]Female *R. appendiculatus* tick

Appendix 3.1. Mean daily weight gains for all calves in Upper Midlands 1 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Mode	Std Dev	Std Error	Range	Percentiles 75%	25%
9	75	0.33	0.33	0.36	0.19	0.02	-0.11 - 0.86	0.43	0.21
24	74	0.31	0.30	0.18	0.17	0.02	-0.07 - 0.86	0.43	0.18
38	71	0.32	0.32	0.29	0.14	0.02	-0.07 - 0.64	0.42	0.21
52	70	0.39	0.29	0.29	0.19	0.02	-0.21 - 0.68	0.40	0.18
66	66	0.33	0.30	0.29	0.21	0.03	-0.13 - 1.06	0.50	0.19
80	64	0.32	0.28	0.14	0.17	0.02	0.08 - 0.93	0.45	0.21
94	63	0.27	0.29	0.14	0.24	0.03	-0.50 - 0.82	0.43	0.14
109	62	0.27	0.27	0.29	0.22	0.03	-0.14 - 0.85	0.43	0.14
123	61	0.15	0.14	0.14	0.19	0.02	-0.40 - 0.57	0.29	0.07
136	58	0.17	0.14	0.00	0.27	0.04	-0.50 - 1.17	0.29	0.00
150	57	0.14	0.14	0.14	0.22	0.03	-0.50 - 0.64	0.25	0.06
164	56	0.16	0.14	0.14	0.19	0.03	-0.43 - 0.71	0.25	0.07
178	54	0.16	0.14	0.21	0.20	0.03	-0.29 - 0.64	0.25	0.07
All	831	0.25	0.25	0.14	0.21	0.01	-0.50 - 1.18	0.37	0.14

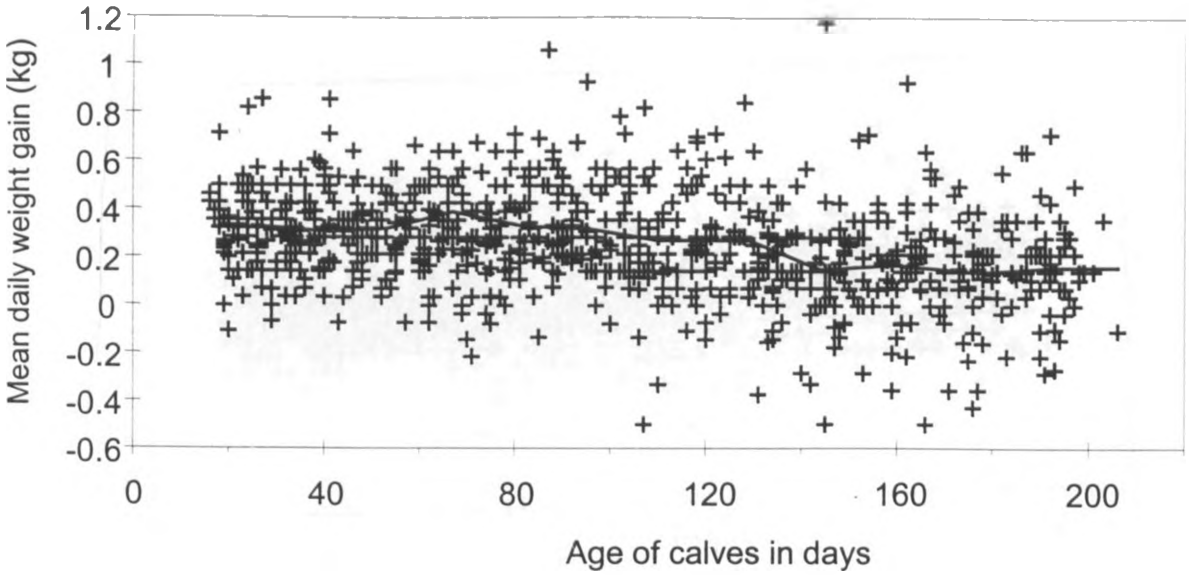
Appendix 3.2. Mean daily weight gains for all calves in Upper Midlands 2 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Mode	Std Dev	Std Error	Range	Percentiles 75%	25%
10	50	0.41	0.43	0.43	0.16	0.02	0.07 - 0.79	0.50	0.32
24	50	0.36	0.36	0.29	0.16	0.02	0.04 - 0.79	0.46	0.29
39	49	0.38	0.36	0.36	0.20	0.03	-0.14 - 0.96	0.46	0.31
53	49	0.39	0.40	0.40	0.17	0.02	0.00 - 1.00	0.50	0.29
67	48	0.32	0.29	0.21	0.18	0.03	0.04 - 0.89	0.43	0.19
81	48	0.36	0.31	0.29	0.18	0.03	0.07 - 0.79	0.50	0.21
96	45	0.29	0.25	0.14	0.21	0.03	-0.18 - 0.86	0.43	0.14
109	44	0.21	0.20	0.14	0.21	0.03	-0.36 - 0.68	0.33	0.07
124	42	0.19	0.15	0.14	0.22	0.03	-0.43 - 0.71	0.29	0.11
138	40	0.22	0.14	0.14	0.29	0.05	-0.29 - 1.18	0.34	0.02
151	38	0.18	0.20	0.14	0.16	0.03	-0.14 - 0.50	0.29	0.14
166	37	0.15	0.14	0.21	0.26	0.04	-0.43 - 0.77	0.29	0.04
178	36	0.19	0.20	0.14	0.23	0.04	-0.36 - 0.57	0.36	0.07
All	576	0.29	0.29	0.29	0.21	0.01	-0.42 - 1.18	0.43	0.14

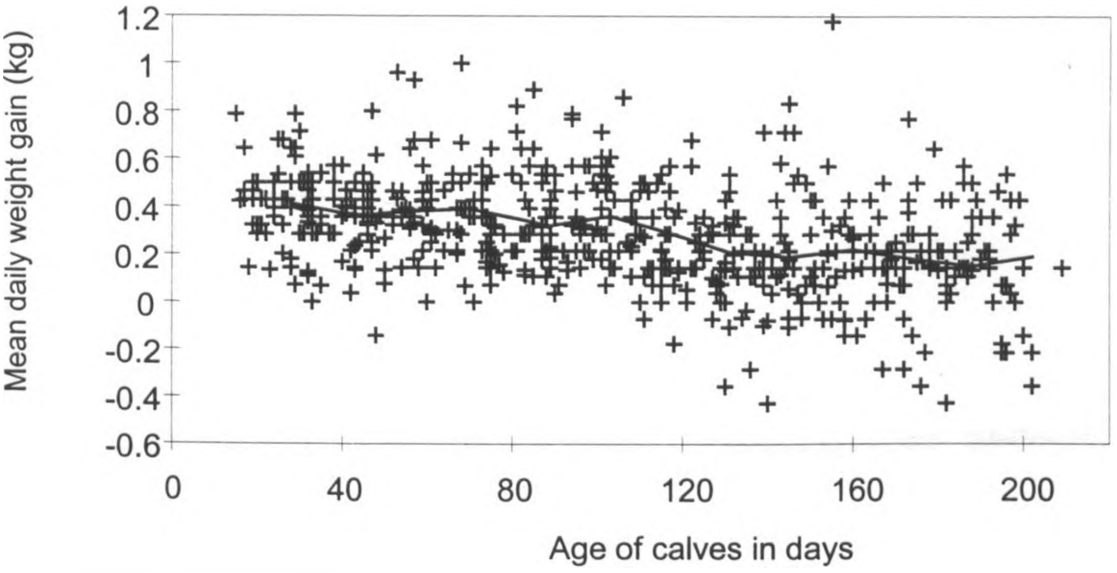
Appendix 3.3. Mean daily weight gains for all calves in Upper Midlands 4 by age (in days) from the longitudinal study in Murang'a District, Kenya (March 1995 - August 1996).

Age	N	Mean	Median	Mode	Std Dev	Std Error	Range	Percentiles 75%	25%
10	96	0.26	0.21	0.14	0.15	0.02	0.00 - 0.71	0.36	0.14
24	93	0.23	0.21	0.21	0.12	0.01	-0.07 - 0.50	0.29	0.14
38	89	0.25	0.21	0.14	0.15	0.02	0.07 - 0.79	0.36	0.14
52	86	0.25	0.21	0.14	0.13	0.01	0.00 - 0.64	0.36	0.14
66	82	0.23	0.21	0.14	0.16	0.02	-0.29 - 0.64	0.29	0.14
80	79	0.25	0.21	0.21	0.16	0.02	0.00 - 0.87	0.32	0.14
94	76	0.24	0.21	0.14	0.17	0.02	-0.11 - 0.86	0.29	0.14
108	74	0.24	0.21	0.21	0.18	0.02	-0.29 - 0.86	0.36	0.14
122	71	0.25	0.21	0.21	0.19	0.02	-0.14 - 0.71	0.36	0.14
136	70	0.24	0.21	0.14	0.15	0.02	0.00 - 0.57	0.36	0.14
150	69	0.27	0.21	0.14	0.20	0.02	-0.14 - 0.93	0.36	0.14
163	68	0.25	0.23	0.00	0.18	0.02	-0.21 - 0.71	0.42	0.10
177	66	0.27	0.23	0.21	0.18	0.02	-0.14 - 0.71	0.43	0.14
All	1019	0.25	0.21	0.14	0.16	0.01	-0.29 - 0.93	0.21	0.14

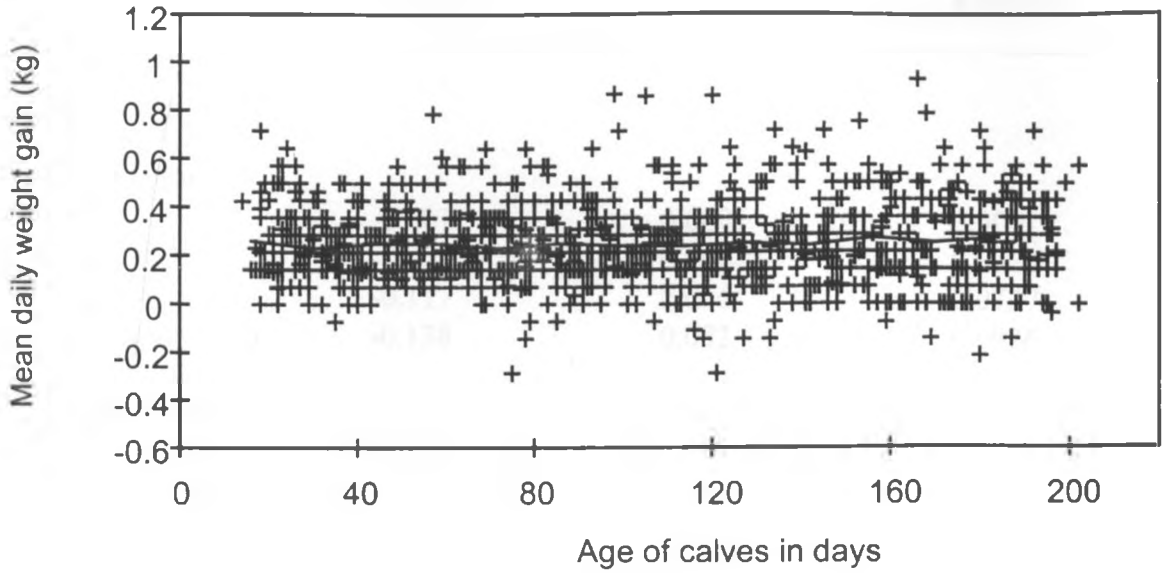
Appendix 3.4. Scatter graph of mean daily weight gains for all calves in UM 1 by age of calf with a fitted mean daily weight gain curve from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 3.5. Scatter graph of mean daily weight gains for all calves in UM 2 by age of calf with a fitted mean daily weight gain curve from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 3.6. Scatter graph of mean daily weight gains for all calves in UM 4 by age of calf with a fitted mean daily weight gain curve from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).



Appendix 3.7. Factors significantly associated with mean daily weight gains in the multi-variate mixed models by AEZ from the longitudinal study in smallholder dairy farms in Murang'a District, Kenya (March 1995 - August 1996).

Variable[a]	Estimate	Std error	P-value
Upper Midlands 1			
Visit level factors			
Age of calf	-0.001	0.0001	0.0001
Calf antibodies	-0.001	0.0003	0.0025
Calf sickness	-0.117	0.066	0.0737
Acaricide application	-0.138	0.082	0.0926
Upper Midlands 2			
Visit level factors			
Age of calf	-0.002	0.0001	0.0001
Concentrate feeds	0.127	0.023	0.0001
Calf sickness	-0.198	0.099	0.0467
ECF	0.360	0.216	0.0963
Upper Midlands 4			
Visit Level factors			
Calf housing	-0.024	0.099	0.0146
Concentrate feeds	0.037	0.014	0.0063
Calf sickness	-0.119	0.026	0.0001
Females[b] (engorged)	0.010	0.005	0.0481
Calf level factors			
Calf breed	-0.065	0.024	0.0072

[a]Random-effects variables not significant in all the uni-variate mixed model ($p<0.05$)
[b]Female *R. appendiculatus* tick