STUDIES ON THE PREVALANCE OF EAST COAST FEVER AMONG CATTLE IN KILOSA DISTRICT

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ABSTRACT

The most important bovine theilerial species in sub-Saharan Africa, *Theileria parva,* causes widespread morbidity and mortality in endemic areas. A study was conducted in Kilosa District, Morogoro Region, to determine sero - prevalence of *Theileria parva,* knowledge, attitude and practices of livestock keepers on East Coast Fever (ECF). The prevalence of ECF in indigenous cattle was determined by measuring serum antibodies to *Theileria parva* using ELISA technique. Three hundred and eighty two (382) serum samples were collected and analysed, whereby 31 (8.1%) tested positive for *T. parva* infection. Knowledge of the farmers on disease constraints, tick species, tick control measures and socio economic characteristics were determined using questionnaire and Focus group discussion. Majority of the respondents (49.1%) had no education, while (47.1%) had primary school education and few (3.8%) had an adult learning education. The major source of household income in the study areas were sales of livestock, livestock products and crop products. Majority of the farmers were able to mention common ticks, but they were not able to identify exactly which tick transmits ECF. Hand spraying was the commonest method for acaricide application, because dips were not in working condition and the tendency of the pastoralists to move from one place to another searching for pasture. Majority reported that they did not know other control measures while few said that they usually treat their animals which fell sick. Most of the farmers declared that they were not aware of ECF immunization and very few were aware of ECF immunization. It is concluded that cattle production in Kilosa District is maintained under a state of endemic instability, as the seroprevalence for *Theileria parva* was less than 70%, suggesting that appropriate tick and theileriosis control strategies are required. Thus, there is a need to develop tick control strategies that can be adopted by farmers in Kilosa District in order to reduce losses due to ECF.

DECLARATION

I, Mary Aloyce Tarimo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

_____ _____

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The above declaration is confirmed by,

_____ _____

Prof. Elikira N. Kimbita Date

(Supervisor)

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DEDICATION

This dissertation is dedicated to my dear son Kelvin, my mom and dad.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABTS	Azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt
ASDP	Agricultural Sector Development Programme
ECF	East Coast Fever
EDI	ELISA Data Interchange
ELISA	Enzyme-Linked Immunosorbent Assay
HRP	Horse Raddish Peroxidase
IFAT	Imminofluorescent Antibody Test
IgG	Immunoglobulin G
ILRI	International Livestock Research Institute
ITM	Infection and Treatment Method
Mabs	Monoclonal antibody
MHC	Major Histocompatibility Complex
PCR	Polymerase Chain Reaction
PIM	Polymorphic Immunodominant Molecule
pp	percentage positivity
PS-MLFD	Permanent Secretary, Ministry of Livestock and Fisheries Development
RFLP	Restriction Fragments Length Polymorphism
RLB	Reverse Line Blot
RNA	Riboxynucleic acid
rRNA	Ribosomal nucleic acid
TBDs	Tick-borne diseases
TVLA	Tanzania Veterinary Laboratory Agency

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

East Coast fever (ECF) is a disease of cattle caused by haemoprotozoan parasite *Theileria parva*, and transmitted by *Rhipicephalus appendiculatus* (brown ear tick). The disease causes high morbidity and mortality in local and exotic cattle (Kivaria *et al.,* 2007) is one of the most important tick-borne diseases which cause cattle production losses in Eastern and Southern Africa through costs of morbidity, mortality and control measures (Mukhebi *et al.,* 1992; Gitau *et al.,* 1994; Perry and Young, 1995; Dolan, 1999, and Kivaria, 2006a). ECF accounts for more than 70% of all cattle death annually in Tanzania (Kivaria *et al.,* 2007). *Theileria parva* is currently distributed within eleven countries in eastern, central and Southern Africa region, where it is a major problem to cattle production (Mukhebi *et al.,* 1992).

Theileria parasites can cause severe, mild and benign theileriosis in domestic and some wild animals. Pathogenic species apart from *T. parva* include *T. annulata, T. hirci, T. lestoquadi, T. ovis*, *T. equi*, and *T. capreoli*. *T. annulata* and *T. parva,* cause death in cattle and buffaloes while *T. taurotragi* and *T. ovis* can cause death in elands and sheep respectively and also infection due to these species can result in abortions and lowered fertility (Jensen *et al*., 2009).

Among all TBDs, East Coast fever (Theileriosis) caused by *T. parva* is economically, the most important tick-borne disease in East and Central Africa (Mbassa *et al*., 2009a). *T. parva* also causes other forms of theileriosis including January disease which occurs mainly in Zimbabwe and Corridor disease which occurs in South Africa. The actual ECF

losses are caused directly by death of animals, whereby the mortality may exceed 90% or indirectly through the costs of control and reduced production capability (Mbassa *et al*., 2009a). In Tanzania it is estimated that the losses due to ticks and tick borne diseases reach US$ 364 million annually (Kivaria, 2006a; Kivaria *et al.*, 2007).

Theileria parva is mainly the parasite of cattle, although other *Theileria* spp such as *T. annulata, T. taurotragi, T. mutans*, *T. velifera* and *T. orientalis* are also known to infect cattle (Morzaria *et al*., 1999a). Cattle undergo severe, mild or subclinical disease whereby immunized, asymptomatic carriers transmit the parasite to ticks and therefore with favorable condition for ticks, such as type of vegetations and cattle management system, cattle maintain the vector and parasite population (Bishop *et al*., 2004).

The distribution of *T. parva* correlates well with the distribution of their vectors. Under field conditions transmission of East Coast fever is mainly by vector tick *Rhipicephalus appendiculatus* (brown ear tick)**,** which is restricted to eastern, central and southern Africa (Lawrence, 1991). Tick population and distribution are not the only factors that determine the *T. parva* epidemiology in a region. Cattle type, tick control methods and cattle management system also bring different levels of interaction between hosts and vectors (Bazarusanga *et al*., 2007). The occurrence and importance of tick-borne diseases depends on the interactions which involve the causative organisms (the parasite), the tick vectors (invertebrate), the vertebrate hosts and the environment (Norval *et al*., 1992). *Theileria* parasites are vector specific, for example *T. mutans, T. velifera* and *T. orientalis* are transmitted by ticks of the genus *Amblyomma,* whereas *T. parva* and *T. taurotragi,* are transmitted predominantly by *R. appendiculatus.*

Theileria taurotragi can also be transmitted by *R. pulchellus* (Morzaria, 1999a). Infection with *Theileria parva* sporozoites starts by infecting T or B cells then differentiation into macroschizonts in bovine lymphoid cells causes the massive destruction of lymphocytes in thymus, spleen and lymph nodes (Mbassa *et al*., 1994, Perry and Randolph, 1999). Some of macroschizonts differentiate into merozoites which invade erythrocytes and differentiate into piroplasms, which are infective to ticks. The main clinical signs of ECF appear during the schizont stage of the parasite and host death can occur before the stage of piroplasm (Yamada *et al*., 2009). The main clinical sign are high fever, swollen lymph nodes, hypoxia, anaemia, haemorrhages along the digestive tract, interstitial pneumonia and pulmonary oedema causing dyspnoea (Tindih *et al*., 2010 and Mbassa *et al*., 1994). Death can occur within three weeks of infection in susceptible untreated cattle, and animals that survive infection either naturally or following treatment are solidly immune to that disease (Patel *et al*., 2011).

ECF is controlled mainly by acaricides and chemotherapy, although these methods of control have become less reliable, acceptable and sustainable for some reasons. These include the high cost of acaricides and chemotherapeutic drugs which are paid in foreign currency, poor maintenance of dips or spray races, water shortages, acaricide resistance, illegal cattle movements and contamination of the environment or food with toxic residues and availability of alternative tick hosts (Mukhebi *et al*., 1992). Treatment of ECF using drugs such as parvaquone, buparvaquone and halofuginone lactate show effectiveness but they are very expensive.

Infection and treatment method (ITM) is another method of control of ECF through immunization, whereby animals are injected with *T. parva* sporozoites from infected *Rhipicephalus appendiculatus* ticks and simultaneously treated with 30% oxytetracycline

(Di Giulio *et al*., 1997; Mbassa *et al*., 1998a; Mbassa *et al*., 1998b). Immunization of cattle against East Coast fever by infection and treatment method (ITM) offers the prospect of a less costly and more effective control of the disease without continued reliance on the expensive acaricides (Kivaria *et al*., 2007).

However, ITM is facing some drawbacks concerning not only its economic implication, as one has to continue using acaricide against other tick-borne diseases (Kivaria *et al*., 2007). The immunity status following ECF immunization is strain/stock specific (Radley, 1978). The resulting immune response coupled with low levels and continuous natural challenge protects the animal for the rest of its life. Widely used immunizing stock is the 'Muguga cocktail' which is composed of *T. parva* Muguga, Kiambu 5 and Serengeti-transformed stocks (Radley *et al.*, 1975). The same species of *Theileria* also may differ in many aspects such as genetic makeup, immunological state, pathogenecity and even evolutionally (Geysen *et al*., 1999; Bishop *et al.*, 2001, Oura *et al.*, 2003).

1.2 Problem Statement and Justification

Tick and tick borne diseases are serious constraints affecting cattle production in Tanzania. Theileriosis, babesiosis, anaplasmosis and cowdriosis are most prevalent and cause the greatest impact on cattle industry in Tanzania. East coast fevers (ECF) kill millions of cattle every year and devastates the livelihood of those who depend on livestock for their survival (Swai *et al*., 2007 and Kivaria, 2006a). It is estimated that 80% of the 20 million herd of cattle are at risk each year in Tanzania alone, and other direct economic losses are estimated to be US$248 million, including an estimated mortality of 0.92 million animal (Kivaria, 2006a). ECF is known to occur in Tanzania but little information is available concerning its present status (distribution, prevalence, and economic importance) in the livestock sector (Kambarage, 1985; Lynen *et al*., 1999).

Early investigations were not production system-specific and did not target biological, management and social economic parameters to establish presence and magnitude due to tick-borne diseases (Pegram and Chizyuka, 1987). Ecological and climatic variations induce changes in tick population dynamics which result in different epidemiological situations of theileriosis in the endemic regions (Fandamu *et al*., 2005). Collection of epidemiological data on theileriosis is a first prerequisite on the roadmap to develop a control strategy.

East Coast fever can be prevented by either controlling the vector (ticks) that transmit the pathogens, treating infected animals by chemotherapy and also through immunization by ITM. However prevention is most commonly achieved through the control of the vectors and for many years the main control for ECF and other tick borne diseases (TBDs) has been effected through dipping of animals in plunge dips/spray race or hand spray using acaricides. This control method generally is not sustainable due to selection of acaricide resistant ticks and availability of alternative tick hosts (Mukhebi *et al.,* 1992). Also financial constraints of the livestock keepers, mean that acaricides are too expensive for the average agro-pastoralist and pastoralist (Mugisha *et al*., 2005). Because of high price of acaricide, livestock keepers use inappropriate rate of acaricide than that recommended by the manufacturer (Okello-Onen and Rutagwenda 1998). Control of tick-borne diseases in East Africa has proved difficult largely because of lack of epidemiological information and also control strategies commonly applied are not integrated in the production system. As a result, in most cases control efforts have not been corresponding to the magnitude of the disease problem (Norval *et al*., 1992).

Previous reports on the prevalence of *Theileria* spp. pathogens (mostly *T. parva* and *T. taurotragi*) in indigenous cattle in Tanzania were mostly based on findings in Giemsa

stained blood and/or brain smears (Mbassa *et al*., 1994). Kilosa District has a large number of pastoralists from different places of Tanzania, who own big herds of cattle. The cattle management system used is communal grazing and exposure of cattle to tick challenge is big and this plays a big role in livestock production drawback. Most livestock keepers use acaricide as major tick control method which is not sustainable due to high price and other setbacks. Therefore proper epidemiological studies, disease prevalence and control strategies should be integrated in the production system to match the disease problem.

1.3 Objectives

1.3.1 Main objective

To determine the prevalence of *T. parva* infection and impact of immunization in cattle in Kilosa District.

1.3.2 Specific objective

(1) To determine prevalence of *T. parva* infections in Kilosa District.

(2) To determine awareness of ECF among farmers in Kilosa District.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Theileria* Species

Theileria species cause severe, mild and benign theileriosis in some domestic and some wild animals. There are about seventeen *Theileria* species but the most highly pathogenic ones are *T. parva, T. annulata, T. hirci, T. lestoquadi, T. ovis* and *T. capreoli*. Hard ticks of the genera *Rhipicephalus, Hyalomma, Amblyomma, Dermacentor*, *Boophilus* and *Haemaphysalis* are known to be the vectors of *Theileria* species. Life cycle of *Theileria* species involves sexually reproduction and finally developments to infective stage occur in the salivary glands of tick vector.

Theileria parva and *Theileria annulata* are responsible for theileriosis in most endemic areas and are the most pathogenic and economically important among all *Theileria* species (Mukhebi *et al*., 1992). *Theileria annulata* can infect both cattle and buffaloes (El-Deeb and Younis 2009). *Theileria parva*, the causative agent of ECF, Corridor disease and January disease, occurs in Eastern, Central and South Africa whereas *T. annulata* occurs around the Mediterranean basin, in the Middle East and in Southern Asia (Norval *et al*., 1992). Buffalo (*Syncerus caffer*) is known to be the carrier of multiple *Theileria* species (Sibeko, 2009).

There are several methods used to determine different species of *Theileria*. These include schizont, merozoite and piroplasm morphology, host immunological responses, monoclonal antibodies, biochemical, genome, chromosome and specific conserved molecular markers, infectivity in arthropod vectors and mammalian hosts, and specificity to vectors and mammal hosts. Different molecular markers and methods have been used

to differentiate and to detect different species of *Theileria* (Bazarusanga *et al*., 2007). Coexistence of Theileria spp is possible as was reported by Bazarusanga *et al.* (2007) who detect four spp (*T. parva T. mutans T. taurotragi* and *T. velifera*) in Rwanda by RFLP-PCR analysis of 18S rRNA gene using enzyme digestion assay.

2.2 *Theileria parva* Parasite

Theileria parva (Theiler, 1904) is a protozoan parasite, transmitted by the tick vector *Rhipicephalus appendiculatus*. It parasitise T and B cells of cattle and some wild animals such as Cape Buffalo, (*Syncerus caffer*) causing classical East Coast fever to cattle (Norval *et al*.,1991, Perry *et al.,*1991). Wildlife animals such as Cape buffalo (*Syncerus caffer*) are considered to be an important reservoir of various tick-borne haemoparasites of veterinary importance and which are pathogenic to cattle including *T. parva* (Uilenberg *et al.,* 1982). *Theileria parva* parasite is most important tick- borne parasite of cattle in East and Central Africa also is the most pathogenic and economically significant (Norval *et al*., 1992).

Theileria parva belong to Kingdom: Protista, Subkingdom: Protozoa, Phylum: Apicomplexa, Class: Sporozea, Subclass: Piroplasmia (piroform, round, rod-shaped parasites), Order: Piroplasmida, Family: Theileriidae, Genus: *Theileria* and Species: *Theileria parva* (Levine *et al.,* 1980).

Trinomial system of classification of the three forms of *T. parva* was proposed, *T. parva parva* for parasites causing classical ECF, *T. parva lawrencei* for parasites causing Corridor disease and *T. parva bovis* for those parasites causing January disease (Uilenberg, 1976 and Lawrence, 1979). However recent system of classifying *T .parva,* classify this parasite according to their host of origin, as cattle-derived or buffalo-derived

(Norval *et al*., 1992). It was therefore recommended that *T. parva* parasites which cause ECF and January disease to be classified as cattle-derived because transmission occur from cattle to cattle by ticks and those which cause corridor disease to be classified as buffalo-derived because transmission occurs from buffalo to cattle through infected ticks (Norval *et al.*,1992 and Oura *et al*., 2011).

2.3 Vectors of *Theileria parva*

The three-host ticks, *Rhipicephalus appendiculatus* are the chief transmitters of ECF to cattle (Odongo *et al*., 2009). *Rhipicephalus appendiculatus* occurs over large areas in Kenya, Uganda, Rwanda, Burundi, Tanzania, Zambia, Malawi, Zimbabwe, Swaziland and South Africa (Norval *et al.,* 1992). Tick population dynamics is a main factor affecting the efficiency in transmission of tick-borne diseases. The concept of endemic stability is an important hypothesis that has been developed during years of observations on ECF and other TBDs in the field (Norval *et al.,* 1992). Climatic conditions, vegetation and host availability are factors known to determine the distribution of the vector, which in turn determines the distribution of the parasite itself (Lawrence, 1991). These vector ticks are very numerous in tropical areas, particularly East Africa, whereby the problem is attributed to communal pastoral grazing of livestock and sharing of pastures between domestic and wild animals.

2.4 Hosts of *Theileria parva*

The hosts of *T.parva* include, *Bos indicus*, *Bos taurus*, African buffalo (*Syncerus caffer*) waterbuck (*Kobus deffassa*) and Egyptian buffalo (*Bubalus bubalis*) (Mbassa *et al.,* 1998b, Uilenberg *et al.,* 1982). The African buffalo (*Syncerus caffer*) are natural reservoir host of *T. parva* parasite. Wherever the suitable tick species of *R. appendiculatus* and *R. zambeziensis* are present cattle may become infected but the presence of that parasite in

this animal does not mean disease (Sibeko *et al*., 2011). The studies conducted in livestock-wildlife overlap areas reported *T. parva* 100% infection in growing calves and high prevalence in adults mainly of the buffalo-derived type indicating a broad sharing of parasites between cattle and buffaloes (Mbassa *et al*., 1998b and Sibeko *et al.,* 2011).

2.5 Economic Implications of *Theileria parva*

Theileria parva is currently distributed within fourteen countries in Eastern, Central and Southern Africa where it is a major constraint to cattle production. In the affected 14 countries where the disease is found, about one million cattle per year die, with a further 28 million of the 47 million cattle in the region being at risk of contracting the disease (Patel *et al*., 2011). *Theileria parva* by far is the most pathogenic and economically significant *Theileria* specie in Eastern, Central and Southern Africa (Norval *et al*., 1992). East Coast fever, the disease caused by this parasite causes high morbidity and mortality, and is considered as the important restriction to the improvement of the livestock industry in Africa (Yamada *et al*., 2009 and Kivaria *et al*., 2007).

The clinical prevalence of ECF in calves in traditional cattle herds in Tanzania in cool months of the year (May-July) is close to 100% (Mbassa *et al*., 2008) and mortality rate can reach 100% if there is no treatment. *Theileria* parasites can infect new born animals in their early life causing big losses if no treatment is provided (Bazarusanga *et al*., 2007 and Mbassa *et al*., 2009a). Apart from death, farmers face a lot of loses such as impaired weight gain, weak calves, low grade meat, decreased milk production, and enhanced costs of veterinary services (drugs, laboratory diagnosis, surveillance, vaccinations, administration, training, prophylaxis, dipping and others) (Mukhebi *et al.,*1992). It is estimated that losses of more than US$300 million per year occur in East Central and

South Africa regions, where losses of US$ 168 million occur in Eastern Africa alone (Mukhebi *et al.,* 1992).

2.6 Life Cycle of *Theileria parva*

The life cycle of protozoan *T. parva* starts in the tick vector *(Rhipicephallus appendiculatus*) which feed on infected cattle as larva or nymph picking the piroplasms in the red blood cells. When ingested by a feeding tick, piroplasms give rise to gametes, which undergo syngamy in the gut to form diploid zygotes which invade epithelial cells of the tick gut to develop into motile kinete and then migrate to the tick salivary glands (Katzer *et al*., 2006). The tick transmits the disease when feeding as nymph/adult on a new host after kinete develop into sporozoites, released in the tick saliva and enters the animal (Lawrence *et al*., 1994a).

The occurrence of the disease is determined by the density of infected animals, the numbers of ticks infesting the host, and also on the distribution of the vector which depends on the climatic conditions. Depending on the relative suitability of climate for tick survival, epidemiological states ranging from epidemic to stable or unstable endemic situation (Bazarusanga *et al*., 2007).

Ticks with infection inoculate infective sporozoites three days after attaching to its host and within short time sporozoites invade host lymphocytes. Invasion and entry of the parasite into the cell is accompanied by transformation of the infected cell to a state of uncontrolled proliferation (Dobbelaere *et al*., 2000). Schizonts in infected cells undergo further differentiation to merozoites, and as the cell ruptures they invade erythrocytes and develop into piroplasms, the infective stage for ticks (Norval *et al.,* 1988).

2.7 Diseases Caused by *T. parva* Infection

Three major disease syndromes caused by T. parva in cattle are East Coast fever, Corridor disease and January disease. East Coast fever and January disease results from cattle-cattle transmission while corridor disease is buffalo-to-cattle transmission.

2.7.1 East coast fever

Among the three disease syndromes East Coast fever is a fatal disease of cattle and is caused by the cattle derived strains. The severity of the disease differs depending on cattle breed, exotic cattle being more prone to infection, than zebu cattle (Di Giulio *et al*., 2009). Also the severity of the disease may vary depending on factors such as the virulence of the parasite strain, sporozoite infection rates in ticks and previous exposure to the parasite. Indigenous cattle in East Coast fever-endemic areas are observed to experience mild disease or subclinical infection, while newly introduced indigenous or exotic cattle usually develop severe disease (Lynen *et al*., 1999).

Under experimental conditions incubation period may ranges from 8 to 12 days, while under field conditions incubation period may extend up to three weeks after attachment of infected ticks depending on environmental conditions and other challenges (Fandamu, 2005). ECF is characterized by high schizont parasitosis and piroplasms parasitaemia. Initially the disease syndrome is characterized by elevated body temperature (40-42°C) and swollen lymph nodes (Matovelo *et al*., 2002). The schizont is the pathogenic stage of *T. parva* infection. It initially causes a lymphoproliferative, and later a lymphodestructive disease. The infected animal shows enlarged lymph nodes, fever, a gradually increasing respiratory rate, dyspnoea and/or diarrhoea. If untreated anorexia develops, loss of condition follows and nervous signs may be observed (Mbassa *et al*., 2006).

A nervous syndrome called ‘turning sickness’ can be observed in *T. parva* endemic areas, and is suspected to be associated with the presence of aggregated schizont-infected lymphocytes, causing thrombosis and ischaemic necrosis throughout the brain (Mbassa *et al*., 2006). Death usually occurs within 30 days after infection in susceptible cattle. Mortality in fully susceptible cattle can be nearly 100% (Mbassa *et al*., 2008).

In dead animals the postmortem reveals haemorrhages in mucous membranes, heart, subcutaneous, pulmonary oedema, froth in lungs, trachea and nostrils, (Mbassa *et al*., 2006). Some of the infected animals may recover, however the recovered animals may remain emaciated and unproductive for months.

2.7.2 Corridor disease

Corridor disease is an acute, usually fatal disease of cattle resembling ECF (Uilenberg, 1999). It is caused by infection with *T. parva* strains from African buffaloes, one of the wild ruminant species that is a carrier of the causative organism (Sibeko, 2009). Corridor disease occurs in Southern and East Africa especially in areas where there is contact between cattle and infected buffalo. The main vectors for corridor disease are *R. appendiculatus, R.zambeziensis* and *R. duttoni* (Blouin and Stoltsz, 1989). The disease was diagnosed in a corridor land between Hluhluwe and Umfolozi Game Reserve in South Africa, hence the name Corridor disease (Neitz *et al*., 1955). Transmission of the disease occurs in cattle sharing the same grazing area with infected buffalo in the presence of the tick vector. The pathogenesis and pathology of Corridor disease are similar to those of ECF. Clinical features exhibited are also the same as ECF except that the course is usually shorter, death occurring only three to four days after the onset of the first clinical sign (Lawrence *et al*., 1994a). Corridor disease is generally regarded as self limiting as cattle usually die in the acute stage before the parasite develops into

erythrocytic piroplasm stage which is the one picked up by the feeding tick (Uilenberg, 1999, Oura *et al.*, 2011). Among the important diseases transmitted from buffalo to cattle, Corridor disease is currently the second after foot-and-mouth disease in South Africa (Sibeko, 2009).

2.7.3 January disease

January disease is the type of theileriosis which is found in Zimbabwe where the disease adheres to the strict seasonality which is between December and March, coinciding with the seasonal activity of adult R. *appendiculatus*. It is an acute, fatal disease caused by the cattle-derived *T. parva* parasite formally known as *T. parva bovis*. January disease exhibits the same clinical features as ECF. The pathogenesis and pathology of the disease are also very similar to those of ECF (Lawrence *et al.*, 1994b).

2.7.4 Carrier state

A carrier state of *T. parva* is the persistence of a tick-transmissible infection over prolonged period of time among host animals, both cattle and buffalo after surviving *T. parva* infection (Young *et al*., 1986). Number of *T. parva* strains inducing carrier state is not well known but it is considered to be high. In endemic areas (Kenya) the carrier state approach 100% (Young and Leitch., 1981). Differences in the frequency of detectable carrier state, which did not appear to correlate with time was reported by Geysen (2000) and argues that the parasite densities seems to fluctuate and can fall below the detectable level during sampling time. Most *T. parva* stocks produce a carrier state in recovered cattle, and studies using DNA markers for parasite strains have shown that *T. parva* carrier animals are a source of parasites which can be transmitted naturally by ticks in the field. Immunized and disease recovered animals may develop low *T. parva* parasitaemia which leads to carrier state (Mbassa *et al*., 2009a; Bishop *et al*., 2002; Oura *et al*.,

2007).Vaccine components can remain in the body of immunized cattle for up to 4 years, developing to carrier state of theileriosis which can be transmitted to field ticks and then to susceptible cattle (Geysen *et al*., 1999; Bishop *et al*., 2002; Oura *et al*., 2007). Carrier animals do not suffer or show clinical disease, and yet may be a source of infection (Di Giulio *et al*., 2009).

2.8 Immunity to *Theileria parva* Infection

Animals that recover from ECF are immune to subsequent challenge with the same strains but may be susceptible to some heterologous strains. Most recovered or immunized animals remain carriers of the infection. The immune response to these parasites is complicated. The most relevant antibody responses are those directed against sporozoite surface antigens (Musoke *et al*., 1982). Antibodies against sporozoites appear to recognise a wide range of *T. parva* isolates and are correlated with some protection (Musoke *et al.*, 1984). Although the molecular basis of the strain specificity is not clearly established, there is evidence suggesting that it may be due to diversity in the antigens presented by the class I major histocompatibility complex (MHC) molecules on the surface of infected cells (MacHugh *et al*., 2009). Infected animals can recover from ECF either naturally or after treatment with tetracyclines or antitheilerial drugs and are subsequently able to resist challenge with the homologous strain of parasite (Di Giulio *et al*., 2009). The serum from immune cattle contains antibodies against all stages of the *T. parva* parasite (Burridge and Kimber, 1972).

The role of antibodies in the neutralization of sporozoites of *Theileria parva* was investigated, and the results show that serum obtained from cattle recovered from East Coast fever (ECF) and a rabbit immunized with sporozoites was capable of neutralizing the parasites (Musoke *et al*., 1984). Humoral antibodies may play a role in resistance to

reinfection with *T. parva*. This mechanism for acquired resistance is proposed based upon the established biological properties of bovine IgG2 immunoglobulins (Musoke *et al*., 1984). Immune response in *T. parva* infection is strain/stock specificity, such that some cattle immunized with one stock are susceptible to challenge with heterologous stocks (Patel *et al*., 2011).

2.9 Diagnosis of *Theileria parva* Infections

For routine diagnosis, conventional methods are used, whereas serological and molecular methods are utilized for research purposes and epidemiological studies. Conventional methods involve microscopic examination of Giemsa stained thin/thick blood films for detection of piroplasms and lymph node biopsy smears for detection of schizonts. The mostly used serology tests are Indirect Immunofluorescent Atibody Test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA). Several molecular biology techniques have been employed as well. These include; conventional PCR assay, PCR-based hybridization assay, PCR-based RFLP assays, Real time PCR assays and Loop-mediated isothermal amplification (LAMP) assay.

2.9.1 Conventional methods

The common field diagnosis for theileriosis is based on clinical signs of the disease and microscopic examination of blood and lymph node smears for the presence of piroplasms and schizonts respectively. This is a method of choice for early and rapid diagnosis and treatment of the disease. These blood films are fixed in methanol and stained in 10% Giemsa stain for 30 minutes. Since piroplasms can be detected in clinically normal carrier animals, these should not be used to confirm the positive case during diagnosis unless the schizonts are seen (Norval *et al.,* 1992).

At these stages the parasites can be differentiated from other blood parasites by morphological appearance and staining properties, but the disadvantage of that method is that, *T. parva* schizonts and piroplasms are difficult to differentiate from those of other *Theileria* parasites (Sibeko, 2009, Morzaria *et al*., 1999b). In dead animals, impression smears from cut lymph nodes or other lymphoid organs like spleen can be prepared, fixed, stained with Giemsa and examined under microscopy. Normally, piroplasms appear 5-8 days following the detection of schizonts, and their detection can be effected through thin blood film preparations.

2.9.2 Serological methods

Serological tests are reliable methods for detection of low grade or previous infections where measurement of antibody levels of a cattle herd is used for assessing the response to natural infection, and also to vaccination for the purpose of disease control (Thrusfield, 2000). Serological methods such, as the indirect fluorescent antibody test (IFAT), and enzyme linked immunosorbent assay (ELISA) tests are available for the detection and qantification of antibodies to tick borne parasites. The most widely used serological assay for detection of *T. parva* antibodies is indirect fluorescent antibody test (IFAT) although it has cross-reaction with other *Theileria* species (Burridge and Kimber, 1972, Goddeeris *et al*., 1982). Indirect ELISA has been used as highly specific and sensitive test for detection of *Theileria* spp. (*T. parva* and *T. mutans*) infection antibodies (Katende *et al*., 1998).

The Polymorphic Immunodominant Molecule (PIM) based ELISA is highly sensitive and specific and is used for the screening of large number of bovine sera antibodies against *T. parva* in epidemiological studies. The principle behind the ELISA technique is based on PIM-base antigen expressed as recombinant fusion protein glutathione S-tranferase

(Katende *et al.,* 1998). To detect *T. parva* antibodies both schizonts and piroplasms can be used, although the schizonts antigen is preferred as it confers a long duration of a serological response. Evidence of *Theileria parva* infection is assessed by increased antibody levels as measured in an indirect ELISA test by the percent positivity (pp) of serum samples relative to a strong positive reference serum (Katende *et al*., 1998).

Although ELISA is more sensitive (>99% sensitivity) and specific (94%-98% specificity) than IFAT, it has the same problem as IFAT since an animal may remain positive while it has already cleared the parasites (Bishop *et al*., 1992 and Sibeko, 2009). Other serological tests for diagnosis of theileriosis include coagulation test, capillary tube agglutination, indirect hemagglutination assay, complement fixation, and immunodiffusion test. Assessment of stable and unstable epidemiological states have been based on the prevalence results of serological diagnostic tests in extensive field survey (Norval *et al.,* 1992; Perry *et al.,* 1996).

2.9.3 Molecular techniques

Molecular tools can be used to differentiate *Theileria* specie. The tests have proved to be highly sensitive and specific for detecting parasite DNA in blood. These molecular techniques range from the classical single polymerase chain reaction (PCR) to more advanced techniques based on the use of DNA probes (Collins *et al*., 2002). Early molecular detection techniques involved the use of probes to detect repetitive regions in parasite genomic deoxyribonucleic acid (DNA). With PCR it is not always possible to detect mixed infections, but reverse line blot (RLB) hybridisation assay which target the 18S ribosomal ribonucleic acid (rRNA) gene has been developed for identification and differentiation of distinct piroplasm species present in the same sample and therefore can detect subclinical infections (Gubbels *et al*., 1999).

2.10 Control and Treatment of Theileriosis

Control of ECF is feasible but it requires a good plan and any tick control measures must consider other local tick-borne diseases in that particular area (Kivaria *et al.,* 2006b). Different methods have been employed to control East Coast Fever. Control of the vector by using acaricides was one of the methods used for a long time although it faces some problems. Immunization of cattle using infection-and-treatment method is practical and is gaining acceptance in some areas. It involves simultaneous injection of sporozoite stabilate of the appropriate strain(s) of *Theileria* derived from infected ticks and a single dose of long-acting oxytetracycline (Lynen *et al.,* 1999). Theilericidal compound are highly effective when applied in the early stages of clinical disease but is less effective in the advanced stages in which there is extensive destruction of lymphoid and hematopoietic tissues (Sibeko, 2009). Separation of grazing areas to avoid interaction between infected buffalo and cattle can also minimize the rate of infection.

2.10.1 Vector control (tick control)

Acaricides application for tick control have been used for a long time, although tick control practices are not always fully effective due to a number of factors including development of acaricide resistance, the high cost of acaricides, poor management of tick control, and illegal cattle movement in many areas (George *et al*., 2004). The selection of acaricide resistant ticks and availability of alternative tick hosts has also provided marked drawbacks to the sustainability of this control method (Mukhebi *et al*., 1992).

Control of theileriosis and other tick-borne diseases will continue to rely on application of acaricides in many endemic areas through dipping or hand spraying. Hand picking and killing of ticks, biological tick control, and keeping tick resistance breeds are also control measures but not largely practiced in the field (Norval *et al*., 1992). By using the principal

of endemic stability it is possible to dip animals once in two weeks instead of the recommended four times with good results and therefore reduction of the costs of dipping (Mbassa *et al*., 2009b). Dipping become very expensive, and inconsistent due to lack of facilities such as finances for rehabilitation of dip tanks, provisions of acaricides and water (Kivaria, 2006b; Eisler *et al.,* 2003; Mugisha *et al.,* 2005).

2.10.2 Immunization against ECF

Naturally acquired immunity in infected animals has led to the development of Infection and Treatment Method of immunization (ITM), using live *T.parva* sporozoites (Radley *et al*., 1975). Immunization of cattle against theileriosis by the infection and treatment method (ITM) (Radley, 1978, 1981), offers the prospect of a less costly and more effective control of the disease without continued reliance on expensive acaricides. The vaccination regime involve inoculation of cattle with sporozoites of the original stabilate, at the same time treating with long-acting formulation of oxytetracycline (Di Giulio *et al*., 2009).

Benefits for immunization process against ECF is increasing of the survival rate of calves in which the mortality rate decreases down to 2% annually among the pastoralists (Lynen *et al.,* 2006). Also with vaccination, tick resistance to acaricides has been reduced with reduced frequency of using acaricides and lower tick controll costs by up to 50% (Lynen *et al.,* 1999). A shortcoming of the infection and treatment immunisation procedure which has limited its practical application is that immunization with one stock of the parasite does not provide protection against all other stocks of *Theileria* parasite (Radley *et al.,*1975, Irvin and Mwamachi, 1983).Vaccine production involves different processes such as passage of the parasite through ticks and cattle, which may result in recombination which affects stabilates composition, therefore extensive infectivity

testing and titration in cattle, to determine the safety and efficacy of the immunizing dose is very important (Di Giulio *et al*., 2009).

Protection engendered by this method of immunisation is ‘strain’ specific; as a result attempts have been made to identify single stocks, or combinations of several parasite stocks that provide broad immunological protection (Bishop *et al*., 2001). Combination of several *T. parva* stocks in the vaccine to induce a broadly protective was planned to be implemented in Eastern Africa where there is heterogeneity in the parasite populations (Oura *et al*., 2005; Odongo *et al*., 2006, Radley *et al*., 1975).

The most widely used ITM vaccine stabilate in Eastern Africa region is trivalent vaccine (‘Muguga Cocktail’), which includes parasites from three stocks: Kiambu 5, Muguga and Serengeti-transformed. This trivalent vaccine has been used to immunize cattle in Malawi, Tanzania, Uganda and Zambia (Musisi *et al.,* 1992). Theileriosis cannot completely be isolated from other tick-borne diseases and immunization against ECF should be considered as only part of an integrated control of the whole package and it has to be cost-effective and sustainable. Risk from other tick-borne diseases such as anaplasmosis and babesiosis limits adoption of reduced acaricide tick control practices following ITM, hence farmers do not see significant reduction in cost after adoption of the method (Kivaria *et al*., 2007).

2.10.3 Treatment of theileriosis

Chemotherapeutic agents such as parvaquone, buparvaquone and halofuginone are available to treat *T. parva* infections. Development of these theilericidal compound, parvaquone and, subsequently, its derivative buparvaquone ensure the survival of cattle with clinical *T.parva* or *T. annulata* infection. Treatments with these agents do not

completely eradicate theilerial infections leading to the development of carrier states in their hosts. These compounds are highly effective when applied in the early stages of clinical disease but they are less effective in the advanced stages in which there is extensive destruction of lymphoid and hematopoietic tissues (Sibeko, 2009). Each of these drugs has been introduced to the market within the last 20 years (Norval *et al.*, 1992). Apart from theilericidal chemotherapeutic available for controlling the disease, scarce resources among the farmers, poor diagnosis and untimely administration of drugs remains as significant constraint.

2.11 Molecular Characterization of *T. parva* Stock

Three *Theileria parva* stocks Muguga, Kiambu 5 and Serengeti-transformed have been used for live vaccination against East Coast fever in cattle in eastern, central and southern Africa. *Theileria parva* antigen genes for polymorphic immunodominant molecule (PIM), piroplasm proteins (p104), p150 and sporozoite surface protein (p67), small and large ribosomal subunit RNA gene have been extensively studied through sequencing them as a means of detecting discriminatory differences among different stocks of *T. pava* isolates (Nene *et al*., 1999; Geysen *et al.*, 2004; Sibeko *et al.*, 2010; Sibeko *et al*., 2011). Under field condition two or more *Theileria* parasites can be similar antigenically and genetically but vary greatly with another *Theileria* parasite from the same field (Irvin and Mwamachi, 1983; Conrad *et al.*, 1987 and Allsopp, 1989). Differentiation of *T. parva* parasites can be done traditionally based on numbers of schizonts and piroplasms present in infected animal and epidemiology of the disease they cause (Norval *et al.*, 1992).

PIM molecule has been extensively characterized and is utilized in recombinant form for diagnosis purpose (Katende *et al*., 1998, Geysen *et al*., 2004). Semi-nested PCR-RFLP have been used in different areas to characterize *Theileria parva* field isolate basing on

p104, p105 and PIM surface protein antigenic genes (Geysen *et al*., 1999; Bishop *et al*., 2001; De Deken *et al*., 2007; Sibeko *et al*., 2011). Semi-nested PCR-RFLP assays exploiting variable region of the parasite antigen genes have been used to recognize the distinctions between buffalo-and cattle-derived *T. parva*. PIM and p104 RFLP profiles from buffalo-derived *T. parva* stocks shows more polymorphism than those from cattle-derived stock (Geysen *et al*., 1999). Mini- and micro-satellite markers are considered to be a powerful tool in discriminating differences between and within the population since they allow simple analysis of variation in the copy of repeat present in loci (Sibeko, 2009).

DNA probes can be used to distinguish selected stocks of *T. parva* by hybridization to DNA either from intraerythrocytic piroplasms taken from infected cattle, or from isolates of schizont-infected bovine lymphoblastoid cells that are maintained continuously in vitro (Conrad *et al*., 1987).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Sites

The study was carried out in Kilosa District, Eastern Tanzania between October 2012 and April 2013. The district is subdivided into three zones, the Northern zone (Gairo), Central zone (Kilosa) and Southern zone (Mikumi). Agriculture and cattle rearing are the major economic activities in Kilosa District and main source of income for the population. The majority of the people in Kilosa practice cattle rearing and livestock products such as milk, meat, hides and skins and ghee provide household income. Indigenous cattle kept by the agro-pastoralists and pastoralists comprise over 95% of the cattle herd in the district. The farming system in the study areas could be described as agro-pastoralist and communal grazing system is the main cattle management system in Kilosa District.

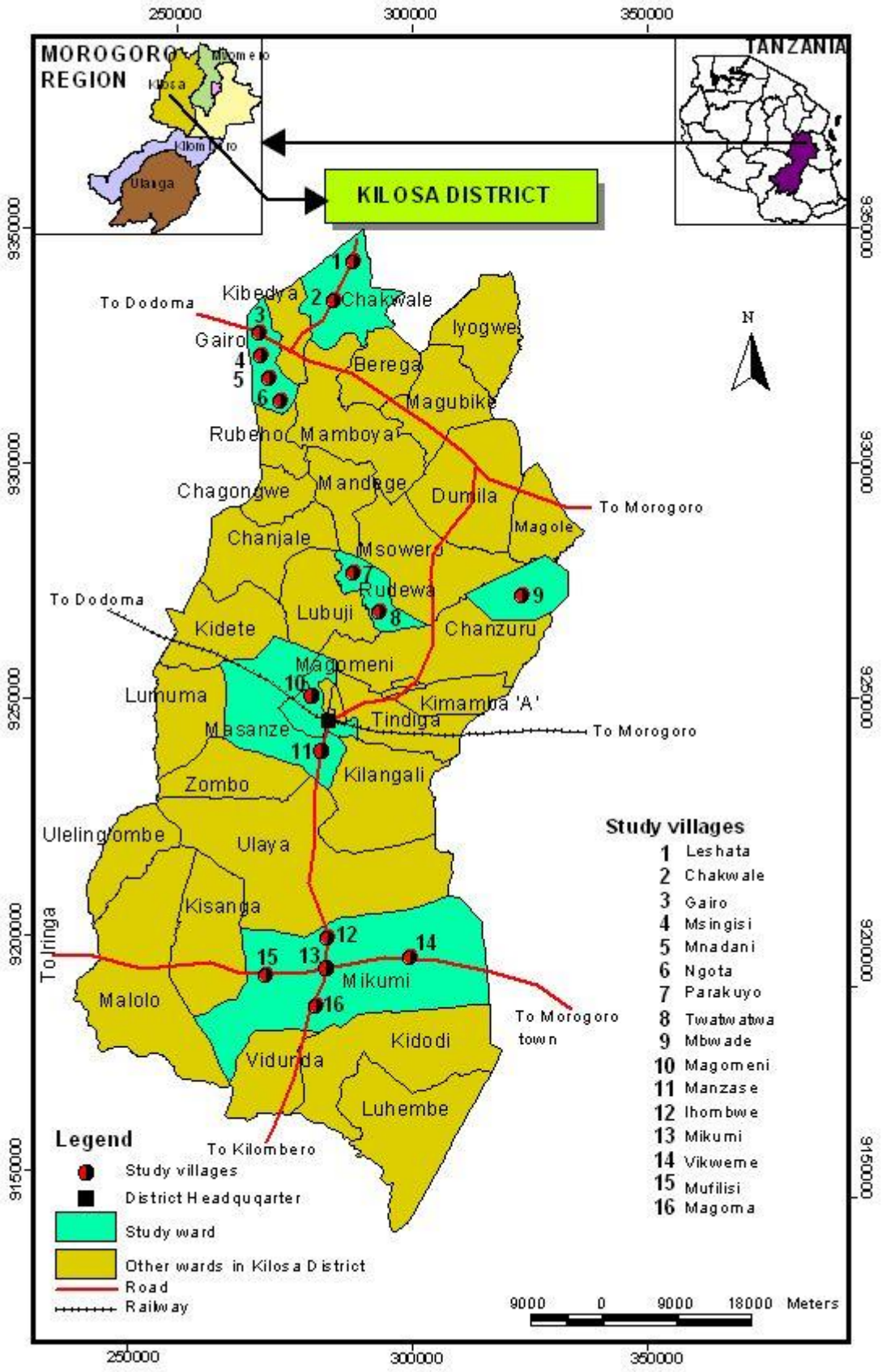

Figure 1: Map of Kilosa district showing where the samples collected

3.2 Sampling Procedure

In this study a multistage sampling technique was employed and the sampling frame were district, ward, village, and finally a household. Purposive samplings were used to pick the villages with large numbers of cattle. A total of eight wards and sixteen villages were surveyed and within a village the list of households keeping cattle was used as a sampling frame from which respondents were selected randomly. The sample sizes were determined using the following formula (Martin *et al.*, 1987).

$$n = \frac{z^2 p(1-p)}{d^2} \quad \text{.....(1)}$$

Where:-

Z = Confidence level (confidence interval CI 95%)

P = Estimated prevalence (50%)

(1-P) = The probability of having no disease

d = Precision level (allowable error 0.05%)

n = Sample size

Level of confidence will be 95%

Precision 0.05 (5%)

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} = 384.16$$

approximately 384 cattle

Simple random selection of households was adopted to select at least three household in each village, giving a total of 48 households in that district. The heads of the households were the main respondents; however, other members of the household attended the interview in the absence of them.

Table 1: Names of Wards, Villages and number of households sampled

Wards	Villages	Number of households sampled
Chakwale	Chakwale	4
Chakwale	Leshata	2
Mikumi	Mufilisi	4
Mikumi	Magoma	3
Mikumi	Ihombwe	3
Mikumi	Vikweme	2
Mikumi	Mikumi	2
Msingisi	Msingisi	4
Msingisi	Mnadani	4
Gairo	Gairo	3
Kilosa	Manzese	3
Kilosa	Magomeni	2
Madoto	Mbwade	3
Msingisi	Ngoto	3
Ludewa	Parakuyo	4
Ludewa	Twatwatwa	2

3.3 Data Collection

3.3.1 Socio-economic survey

The aim of this study was to determine the level of awareness of livestock keepers to ticks and tick-borne diseases especially East Coast Fever (ECF). Techniques used to collect social-economic data were individual interviews using semi-structured questionnaire (Appendix 1) and focus group discussion. These techniques are widely used and recognized as effective way of getting valid and detailed information from local communities (Bayer and Waters-Bayer 1994). The selected farmers were individually interviewed using questionnaire which targeted the household heads or their representatives. Both closed and open-ended questions were included in the questionnaire administered to the respondents in order to seek information on household socio-economic characteristics such as age, education, experience on cattle keeping, major activities, herd size, common tick species, control measures and farmers perception on immunization against ECF. Focus group discussion were conducted with the livestock

owners in a group of twelve farmers to seek additional information such as cattle production constraints, impact of ECF immunization and tick seasonality. This was done in Mikumi and Gairo as it was only possible in community areas such as the livestock markets. A few farmers whose cattle were grazing far at that moment were also interviewed.

3.3.2 Blood samples collection

During socio-economic survey, blood samples were collected from cattle in the same households where the questionnaire were administered. A total of 382 blood samples were collected from animals of different age (136 calves, 109 yearlings and 136 adult) between January 2013 and May 2013. Blood was collected by jugular venipuncture using 10-ml vacutainer tubes (Becton Dickson Vacutainer Systems, England).Verified correct labeling of the tubes was done after which they were kept in cool boxes with ice for some few hours, and at the end of the day refrigerated in Laboratory. The blood samples was centrifuged at 3000 rpm for 20 minites to obtain sera. Approximately 2 milliliters aliquots in cryotubes, were made from each sample and stored in a freezer at -20 °C until the testing time. No parasitological examinations done due to the shortage of funds but this can be planned for future studies.

3.3.3 Sera testing

The enzyme-linked-immunosorbent assay (ELISA) as described by (Katende *et al.*, 1998), was used to estimate prevalence of ECF in the study area. The system adopted was based on indirect Enzyme Linked Immunosorbent Assay (indirect ELISA) using *T. parva* kit batch number NDEC 12#123 from International Livestock Research Institute (ILRI-Nairobi Kenya). The kit is designed to detect specific antibodies against *Theileria parva*. *Theileria parva* specific recombinant antigen from ILRI - Nairobi Kenya was bound on

the surface of the walls of microplates and the free spaces on the walls of the microplates and the non specific sites on the bound antigen were blocked using blocking buffer supplied together with the kit. The presence of antibodies specific to *T. parva* was tested by addition of the test sera to the wells. Anti-bovine IgG1 monoclonal antibody (MoAb) conjugate to horse raddish peroxidase (HRP) from ILRI was then added to demonstrate that the initial antigen and antibody reaction took place. The amount of the second antibody bound on the first antibody determines the strength of the signal.

The reaction was revealed by the addition of the substrate /chromogen containing 1% hydrogen peroxidase substrate and 40 mM 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS) from ILRI as chromogen in sodium citrate buffer pH 4.0. The intensity of the colour, which developed following hydrolysis of the substrate by the enzyme and the oxidation of the chromogen by the oxygen liberated from hydrogen peroxide, was determined by using an ELISA reader (Multiskan Dichromatic Version 1.03) with 405 nm filter. Results were recorded in the computer, connected to the reader, and runs ELISA data interchange (EDI version 2.1.1) software programme. The raw data obtained from the ELISA reader were expressed as percentage positivity (pp) relative to a strong positive standards. The results were expressed as percent positivity (pp) as follows: pp = (optical density of test serum/optical density of strong positive) x 100 (According to Wright *et al.,* 1993). A sample was considered positive if the PP value was 20 or above, hence, animals were classified as positive or negative depending on whether the pp values were above or below 20.

3.3.4 Data analysis

Data derived from questionnaires were coded and recorded into the spreadsheets for statistical analysis. The data were analyzed using Epi-Info version 6.04 b (Epi-info, 1996)

statistical software and the following descriptive statistics were generated: means, standard deviations, frequencies and percentages. For ELISA data, sero-prevalence was computed as the percentage of animals tested positive. The chi-square test in Epi-Info version 6.04 b (Epi-info, 1996) statistical software was used to assess the significance of differences in sero-prevalence among different age groups.

CHAPTER FOUR

4.0 RESULTS

4.1 Respondent Socio-economic Characteristics

Social - economic characteristics of the population are shown in Table 2. Fifty three (53) respondents were interviewed in Kilosa District, 48 (90.6%) were males and 5 (9.4%) were females. More than half of respondents 28 (54.7%) had age of less than 40 years and others 25 (45.3%) were aged above 40 years. Almost half of the respondents 26 (49.1%) had no education at all while 25(47.1%) had primary school education and few 2 (3.8%) had an adult learning education. The major economic activities in the study areas is livestock keeping 42 (79.2%) with few farmers 11(20.8%) who practice agro-pastoralism. The majority 28 (52.8%) of the respondents reported that they had been keeping cattle for more than 20 years and 25 (47.2%) farmers said that they had 10-20 years of keeping cattle.

Table 2: Respondent socio-economic characteristics

Variable	Percent
Age of the respondents	
≤ 40 years	54.7
> 40 years	45.3
Level of education	
No education	49.1
Adult education	3.8
Primary education	47.1
Cattle keeping experience	
10 -20years	47.2
< 20 years	52.8
Major activities	
Livestock Keeping	79.2
Livestock and farming	20.8

4.2 ECF Knowledge and Methods Used to Control Tick

Responses on ECF knowledge and methods used to control ticks are shown on Table 3. The majority of the farmers 36 (62.3%) reported that they were not able to identify exactly which tick transmit ECF while others 17(39.6%) were able to mention common ticks in the areas for example brown ear ticks which are found mainly in the ears (*Rhipicephalus appendiculatus*) and *Amblyomma* ticks. The brown ear ticks are commonly found on both calves and adults while the other ticks are mainly seen on adult animals. Most of the respondents interviewed said that they used conventional methods for controlling ticks. Out of 53 farmers interviewed, 52 (98.1%) said that they apply acaricide to control ticks, while 1(1.9%) replied that she did not use acaricide to control ticks. For those who used acaricide, hand spraying was the commonest method of application. About the use of other control measures apart from acaricide majority, 41(77.4%) said that they didn't know, while few 12 (22.6%) said that they used other means such as treating their animals when they fell sick.

Table 3: Respondent responses on ECF knowledge and methods used to control ticks

Variable	Percent
Ability to differentiate tick species	
Able	37.7
Not able	62.3
acaricides use	
Apply	98.1
Do not apply	1.9
Knowledge on the Causes of ECF	
Know	60.4
Do not know	39.6
Use of other control measures	
Yes	75.5
No	24.5
Use of vaccine to control ECF	
Yes	17.0
No	83.0
Why not vaccinate their cattle	
Not affordable	90.6
Affordable and efficient	9.4

On the other hand most of the farmers 44(83.0%) declared that they were not aware of immunization and few 9 (17.0%) said that they were aware of immunization but it was very expensive and therefore they could not afford.

4.3 Livestock Production Constraints

Focus group discussions were conducted in which a total of twelve farmers were involved and the responses are shown in Table 4. More than half 83.3% (10) of the respondents declared that major sources of household income in Kilosa District were sales of livestock and livestock products; while a few 16.7% (2) said that crop products contributes to the household income. Therefore livestock products were reported to be the major source of income.

Table 4: Cattle production constraints

Variables	Percent
Major source of income	
Livestock products	83.3
Livestock products and crops	16.7
Cattle production constraints	
Livestock diseases	41.6
Shortage of water and forage in draught seasons	58.4
Price of veterinary drugs	
Affordable	25.0
Not affordable	75.0
How to attend a sick animal	
Slaughtering	16.6
Treating	83.4
Widely used drug	
Oxtetracycline	58.3
Others	25.0
Non	16.7
Which season tick are abundant	
Dry season	0.0
Wet season	100

Majority of the farmer (58.4%) who participated in group reported that shortage of forage and water were the main constraint for livestock production, while 41.6% (5) said that livestock diseases were the most important constraint. About the veterinary drugs 75% (9) reported that were expensive and few 25% (3) said that are not expensive. On how to attend the sick animals most 83.4% (10) of them declared that they treat their animals while few 16.6% (2) said that they slaughter them. Most of the farmers (58.3%) said that they treat their animals using oxytetracycline. All 12 respondents said that ticks are abundant during the rainy season.

4.4 Prevalence of Serum Antibodies

Prevalence of serum antibody for *Theileria parva* is shown in Table 5, 6 and Fig. 2. A total of 382 animals were tested for the presence of antibody to *Theileria parva*. Overall 31 animals tested positive hence 8.1% prevalence of antibodies to *Theileria parva.* The age of the animals did not significantly ($P > 0.05$) affect the prevalence of antibodies to *Theileria parva*. However, slightly higher seroprevalence was observed in the adults (9.6%) compared to yearlings (8.2%) and calves (6.6%).

Table 5: Prevalence of serum antibody to *T. parva* among age groups

Age of the animal	No of animals sampled	No of positive samples	Sero prevalence	P value in Chi-square test
Adults	136	13	9.6	
Yearlings	110	9	8.2	0.304
Calves	136	9	6.6	

Table 6: Prevalence of serum antibody to *T. parva* among adults and calves

Age of the animal	Number of samples	Positive samples	Sero prevalence	P value in Chi square
Adults	136	13	9.6	0.375
Calves	136	9	6.6	

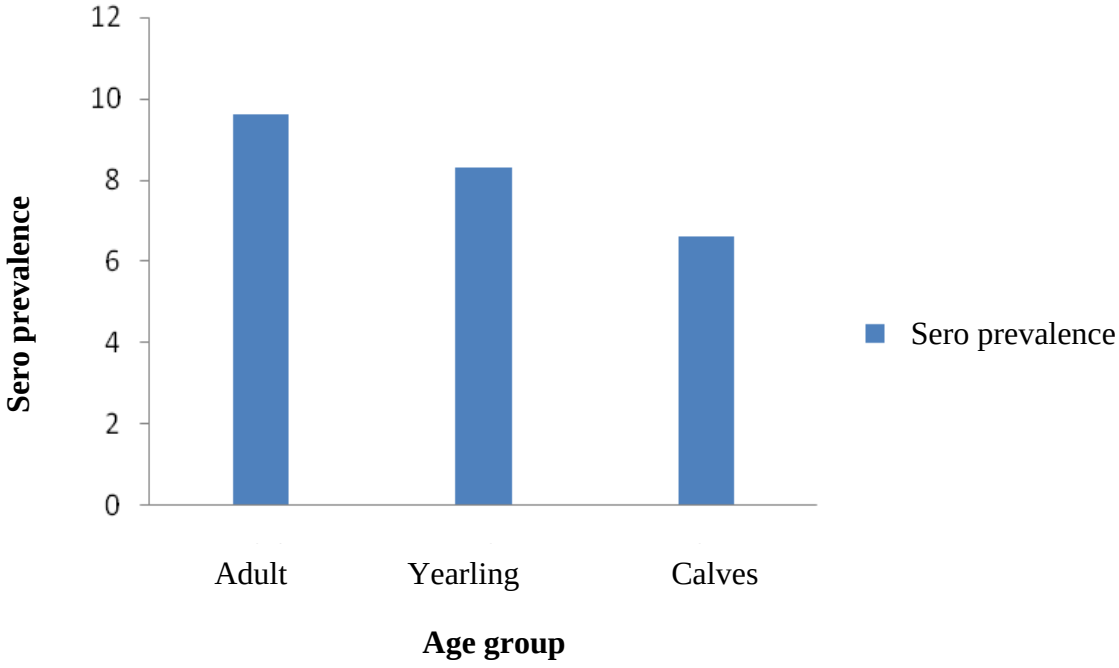

Figure 2: Prevalence of serum antibody to *Theileria parva* in the study area

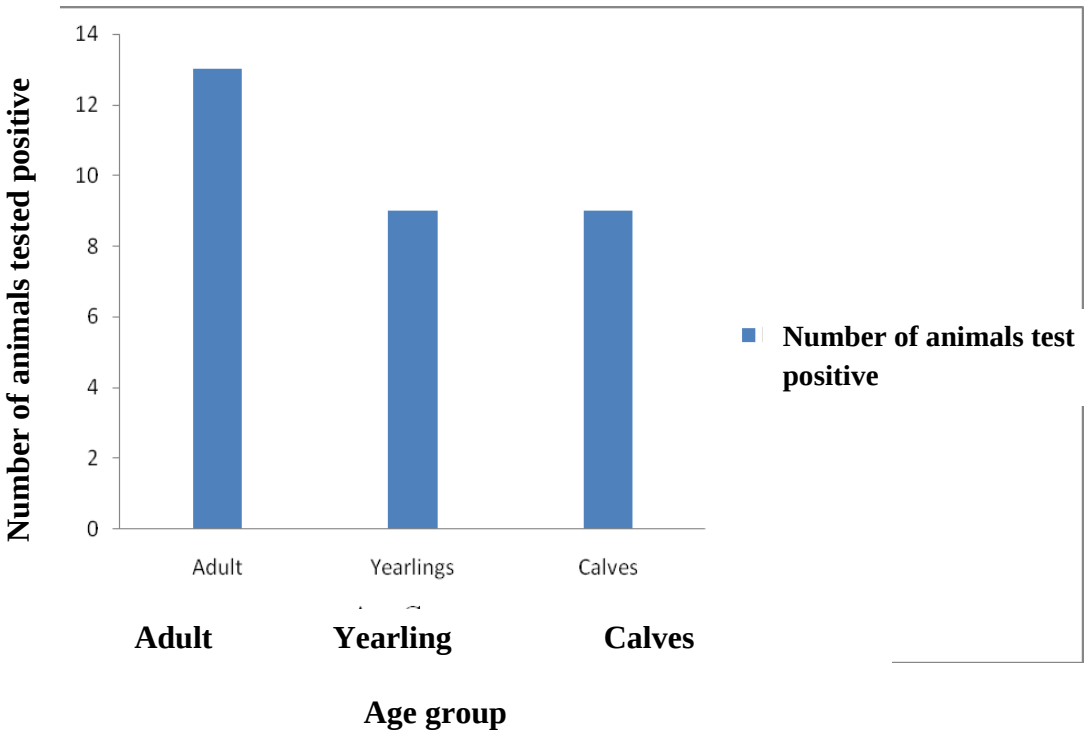

Figure 3: Number of animals tested positive

CHAPTER FIVE

5.0 DISCUSSION

5.1 Production Constraints

The present studies have shown that Tickborne Diseases (TBDs are the most important diseases that affect cattle production in Kilosa. This corroborates previous observations by Swai *et al*. (2007), Kivaria *et al*. (2007) and Chenyambuga *et al*. (2010) who reported that livestock diseases were ranked above the other production constraints. Shortage of pastures/grazing land and water especially in the dry season, and high price of veterinary drugs were important constraints to cattle production in the study area. Swai *et al*. (2005), and Chenyambuga *et al*. (2010) reported that the major cattle production constraints are diseases, shortage of forages and water during the dry season, expensive veterinary drugs and lack of livestock market.

The majority of livestock farmers were aware of ECF, but most of them considered ECF as not a big problem. The same observation was made by Chenyambuga *et al*., 2010 who reported that the farmers around Lake Victoria considered their breeds to be resistant to ticks and ECF, the reason given being that animals always carry ticks without getting sick. This is because adult cattle were regarded as tolerant to ticks and they can harbour a large number of ticks without being very much affected. Also when the adult animal show signs of the disease the use of chemotherapy such as oxytetracycline can suppress the condition. This is in agreement with Chenyambuga *et al*. (2010) and Ocaido *et al*. (2005) who reported that many farmers attach little importance to ECF since it is considered a disease of calves for the reason that these are the ones that suffer most severely.

Majority of the farmers in Kilosa District knew different types of ticks (brown ear ticks, blue ticks and bont ticks), but they were not aware of the one which transmits ECF. They reported that tick were abundant after rain season and that it is the time when most diseases also show up. This also was observed by Ndamukong (1993) who reported that large numbers of ticks, mainly *R. appendiculatus,* were active during the rainy season. The amount of rainfall is the principal stimulus to *Rhipicephalus appendiculatus* activity (Yeoman, 1966). The behavior of active unfed ticks (questing) has been reported to be affected by many external factors of the environment and the physiological state of the ticks (Punyua *et al*., 1991). *Rhipicephalus appendiculatus* activity is not only determined by season, but also hydration status of the tick is also the most important determinant factor (Punyua *et al*., 1991).

5.2 Use of Acaricides and Immunization to Control Ticks

More than 95% of the respondents stated that they used acaricide to control ticks. The main method of acaricide application was by hand spraying and most farmers thought that spraying is cheaper, convenient and effective. The same observation was also reported by Mugisha *et al*. (2005) that spraying is the most preferred method for acaricide application in pastoral and agro-pastoral communities. Plunge dips were not in use because most of them were not functioning and most farmers could not afford the cost of acaricides. Furthermore water for running the dips remain an important challenge during the dry season.

In Kilosa District most of the respondents kept indigenous cattle which were considered to be resistant to both ticks and TBDs. ECF affect calves mostly and the animals beyond nine months are not very much affected. There is also evidence that various breeds of cattle exhibit differences in resistance to ticks. This were reported by Norval *et al*. (1988)

and Paling *et al*. (1991) who demonstrated that tick resistant cattle are capable of limiting the level of *T. parva* transmission compared to tick-susceptible cattle.

The resistance to ECF in local animals is also supported by the evidence provided by the respondents that there is usually no need to use veterinary drugs to treat the calves when infected as they could recover without treatment. This indicates that the indigenous cattle in Kilosa District are able to live and produce under tick endemic conditions. Wambura *et al*. (1998) also reported zebu cattle to be relatively resistant to tick infestation compared to crossbred animals, and this resistance is due to mounting of a protective immune response against ticks by the host. This resistance has been naturally selected over generations as a result of the constant infection pressure exerted on the cattle population in the endemic areas. Indigenous cattle are resistance and can tolerance vector-borne diseases and they frequently perform better than exotic breeds under low-input conditions such as climatic stresses, especially during times of drought (Rege and Tawah, 1999).

5.3 Prevalence of Serum Antibodies Against *Theileria parva* Infection

Seroprevalence studies are useful in establishing herd immunity, and therefore evidence of either the need for vaccination or for no intervention. The development of appropriate and effective control strategies for theileriosis is based on the concept of endemic stability (Kivaria *et al.,* 2007).

Prevalence of *Theileria parva* infection in cattle is useful information for determining the response of the animals to natural infection and the levels of endemic stability in the study a area (Chenyambuga *et al*., 2010). Norval *et al.* (1992) describe endemic stability as the ecological balance between host, parasite, vector and environment in which all coexist with the virtual absence of clinical theileriosis. Endemic stability is characterised by a

high (70%) seroprevalence but low incidence of theileriosis, and low theileriosis fatality rates (Norval *et al.,* 1992). Endemic instability on the other hand means an incomplete relationship in which clinical disease occur. Endemic instability is mainly found in the local Zebu cattle maintained under extensive management conditions with little or no efficacious acaricide application and where *R. appendiculatus* can undergo at least two generations annually (Norval *et al*., 1992). Under such condition greater numbers of cattle are reservoirs of *T. parva* at low levels of parasitaemia which is transmissible. Calves born in these areas become immune through natural infection before they are three months old (Moll *et al.,* 1986) and therefore little or no clinical disease occurs.

Prevalence of serum antibody to *Theileria parva* in the study area results were available from 99.2% animals sampled. The missing results arose due to loss of labels and samples during storage and transport to laboratories. A total of 382 out of 384 samples were tested for the presence of antibodies to *T. parva*. The seroprevalence for *T. parva* was less than 70%, suggesting that the cattle in Kilosa district exist in a state of endemic instabiliy as defined by Deem *et al*. (1993), Moll *et al*. (1986) and Norval *et al*. (1992). Prevalence is usually low (63%) in the endemic instability state (Deem *et al* 1993; Medley *et al*., 1993 and Perry and Young, 1995). Endemic stability is likely to exist where the prevalence of serum antibodies to infection is equal or greater than 70% (Lynen *et al.,* 1999). Animals in endemic areas respond to *Theileria parva* infection by mounting humoral responses that decline over months in the absence of challenge.

The overall prevalence of antibodies to *Theileria parva* in Kilosa District was 8.1%. The prevalence of antibodies to *Theileria parva* was not significantly different between the three age groups ($p > 0.05$). However, slightly higher seroprevalence was observed in the adults 9.6% compared to yealings 8.2% and calves 6.6%. These observations suggest

that calves, yearlings and adult animals are exposed to tick challenge at a low levels so they do not maintain the immunity and sero-positivity. The observed low levels of seroprevalence in this study reflect low levels of exposure of the animals to ticks. When the seroprevalence show low seropositivity findings may provide an entry point for future disease control strategies such as acaricide application, vaccination (Maloo *et al*., 2001). Sometimes factors such as inherent resistance of cattle to ticks and tick borne diseases, virulence of the pathogens and infection rate in ticks can also influence the results (Moll *et al*., 1986). A single cross sectional study can only serve as an indicator of the probability of endemic stability because prevalence can vary with climatic conditions and over time (Gitau *et al*., 1999).

Regarding the presence of antibodies in calves, it is not known whether they were acquired from colostrum or from infection acquired congenitally or after birth. The detection of antibodies to tick-borne disease pathogens in calves of less than three months is possible as at this age the passively transferred colostral antibodies are still in high in the serum of the young animals (Rubaire-Akiiki *et al*., 2004).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

The study reveals that the most important constraints to cattle production in Kilosa District are livestock diseases, followed by shortage of forages and water during the dry season. Among the diseases, tick-borne diseases are the most important diseases that affect cattle production in the district. The majority of the farmers in Kilosa District know that ticks cause diseases but they do not associate ticks with ECF. Most livestock keepers are aware of the disease symptoms of ECF and they use Oxytetracycline to treat these diseases.

Treatment of sick animals is done just from clinical observation without any diagnosis, which is not advisable. Farmers in Kilosa District apply acaricides regularly to control ticks but most of them use hand spray which is very laborious and time consuming especially for those who own big herds. ECF immunization in Kilosa District is not very much practised and most of interviewed farmers were not aware of immunization. Farmers should be sensitised using media, extension workers, and farmers training courses in order to be aware of costs and benefits of ECF immunization.

The ELISA which has been estimated to have a sensitivity of 99% and a specificity of 97 % (Katende *et al.*, 1998) was used to determine sero-prevalence. Any reading of 20 pp or higher was considered positive. The levels of antibody against *Theileria parva* were low in all age groups in the study area, indicating that livestock farming in Kilosa District is carried out under conditions of endemic instability and appropriate tick and theileriosis control strategies are therefore required. Thus, there is a need to develop tick control strategies that can be adopted by farmers in the areas in order to reduce losses due to

ECF. Special attention is to be paid on identifying gaps in our knowledge with particular reference to the epidemiological situation of ECF in Tanzania. In order to help our farmers in their battle against ECF, good diagnosis, treatment and good record keeping are essential and will also avail adequate data on theileriosis.

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APPENDIX

Appendix 1: Questionnaire

1. Date of interview..... (DD/MM/YY)
2. Household's name…….....
3. Contacts.....
4. Age set of respondent……..... (Yrs)
5. District.....
6. Ward …….....
7. Village…….....
8. Gender of respondent:
   - Male (1) [ ]
   - Female (2)
9. Marital status. Single
   - Married (1)
   - Widower (2) [ ]
   - Divorced (3)
10. Level of education
    - None (1)
    - Adult Education (2)
    - Primary School (3) [ ]
    - Secondary School (4)
    - Post Secondary (5)
11. Occupation of respondent.
    - Cattle keeping (1)
    - Employed (2) [ ]
    - Business (3)
    - Others (4)
12. How long have you involved in cattle farming?
    - > 5 years (1)
    - 5-10 years (2) [ ]
    - 11- 20 years (3)

<20 years (4)

13. Cattle system management employed

Communal grazing (1)

Tethering system (2) [ ]

Semi-intensive system (3)

Intensive system (4)

14. Do you know East Coast fever?

Yes (1) [ ]

No (2)

15. Do you know the causing factors of ECF?

Yes (1) [ ]

No (2)

16. Can you differentiate types of tick on your cattle?

Yes (1) [ ]

No (2)

17. Can you tell which one causes ECF?

Yes (1)

No (2) [ ]

18. Do you dip your cattle?

Yes (1) [ ]

No (2)

19. Do you know another means of ECF control apart from dipping?

Yes (1)

No (2) [ ]

20. If yes is it by immunization

Yes (1) [ ]

No (2)

21. If yes is it efficient and affordable

Yes (1) [ ]

No (2)

22. If no what is the main reason

Price (1) [ ]

Not effective (2)

Others (3)

23. Does your herd size increases

Yes (1) [ ]

No (2)

24. If yes what is the reason

Due to immunization (1)

Good management (2) [ ]

Others (3)