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PREVALENCE AND RISK FACTORS OF EAST COAST FEVER (ECF) IN THE COPPERBELT AND CENTRAL PROVINCES OF ZAMBIA

BY

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DECLARATION

I David Chipuku Chabala declare that the work presented in this dissertation, is my own work, carried out with the help of people acknowledged and that this work has not been submitted before for the award of a degree at any University.

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DAVID CHIPUKU CHABALA

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DATE

CERTIFICATE OF APPROVAL

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Supervisor Signature

Examiner.....Sign.....Date.....

Examiner.....Sign.....Date.....

Examiner.....Sign.....Date.....

DEDICATION

This dissertation is dedicated to my dear mother, friend and teacher, Mrs. Beatrice Kabanshi Chabala, for her unfailing love, support, inspiration and encouragement right from my early school days and for making it all possible. For all that I am and will ever be, I owe it to you.

ABSTRACT

East Coast fever (ECF) is an infectious tick-borne disease of cattle, caused by a protozoan parasite *Theileria parva*. It is a disease of major economic importance in Zambia as it is the main cause of cattle morbidity and mortality. Despite its economic importance, the epidemiology of ECF in Zambia is poorly understood, thereby making ECF prevention and control difficult. Further, there is limited published literature on this disease in Zambia, with the little available research concentrating on Southern and Eastern provinces. Such literature is mostly based on serological techniques such as indirect fluorescent antibody test (IFAT) which have limited sensitivity and specificity.

Thus, this study was conducted to determine the prevalence and associated risk factors of ECF in Copperbelt and Central provinces of Zambia. The study was cross sectional in design. Multistage cluster sampling was used involving district, veterinary camp, herd and individual animals. The provinces and districts were selected based on their vast potential for livestock production and the previously reported incidence of ECF. From each district, two veterinary camps were randomly selected. From each camp herds were randomly selected from which individual animals were randomly sampled. Samples were collected from Mpongwe and Masaiti districts (Copperbelt province) and Kapiri Mposhi and Chibombo districts (Central province). Samples were examined for presence of schizonts on giemsa stained lymph smears.

The lymph smear examinations revealed that 6.4% (95%, CI=4.9-7.9) of the samples were positive for *T. parva* schizonts. In Central province, the overall prevalence was 6.7% (95%, CI=4.0-8.2), while on the Copperbelt province it was 6.1% (95%, CI=4.0-8.2). Among the districts in these provinces, Kapiri Mposhi did not record any schizont positive cattle, while Masaiti recorded 2.4% (95%, CI=0.5-4.3). Mpongwe had a prevalence of 9.7% (95%, CI=6.0-13.4) and Chibombo had the highest prevalence at 13.6% (95%, CI=9.4-17.9). Risk factors that were identified to be associated with ECF were the district, frequency of veterinary service provision, tick control frequency, age and previous experience of ECF.

The results indicate that ECF is prevalent in Copperbelt and Central provinces and hindering livestock production. There is hence the need for concerted efforts to control ticks and prevent ECF transmission through farmer sensitization, routine, regular, mandatory and supervised dipping and spraying of cattle and stringent livestock movement control.

Key words: East Coast fever, microscopy, prevalence, risk factors, schizonts, *Theileria parva*.

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LIST OF ABBREVIATIONS

Anon	Anonymous
ANOVA	Analysis of variance
Bm 86	*Boophilus microplus* 86 k DA antigen
Bp	base pairs
^0C	Degrees Celsius
CLW	Community livestock worker
DNA	deoxyribonucleic acid
DRC	Democratic Republic of Congo
DVOs	District Veterinary Officers
ECF	East Coast fever
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
GDP	Gross Domestic Product
Go Taq	Thermus aquaticus DNA polymerase
IFAT	Indirect fluorescent antibody test
ITM	Infection and treatment method
LAMP	Loop mediated isothermal amplification
LD_{70}	70% Lethal dose

NALEIC	National Livestock Epidemiology and Information centre
PCR	Polymerase chain reaction
p67	T. parva 67 kDA sporozoite antigen
PVOs	Provincial Veterinary Officers
RBC	Red blood cell
SPSS	Statistical package used for statistical analysis
TBDs	Tick-borne diseases
VA	Veterinary assistant

CHAPTER ONE

1.0 INTRODUCTION

East Coast fever (ECF) is an infectious tick-borne disease (TBD) of cattle caused by a protozoan parasite *Theileria parva.* It is mainly transmitted by the African brown ear tick *Rhipicephalus appendiculatus* and characterized by lymphadenopathy, pyrexia, laboured breathing and often causes mortality (Fandamu *et al*., 2005a; Mtambo *et al*., 2008).

East Coast fever has been recorded across eastern, central, and southern parts of Africa from 11 countries which include: Kenya, Uganda, Tanzania, Burundi, Rwanda, Malawi, Mozambique, southern Sudan, Democratic Republic of Congo (DRC), Zambia and Zimbabwe (Anon., 2013a; Fandamu *et al*., 2005a; Gacholi *et al*., 2012). East Coast fever was also reported in Comoros between 2003 and 2004 for the first time and is believed to have resulted from the importation of immunized cattle from Tanzania that were fed upon by naïve ticks and subsequently transmitted the infection to the local cattle population (Gacholi *et al*., 2012). Currently 28 million heads of cattle in the region are at risk and the disease kills at least one million cattle per year (Gacholi *et al*., 2012).

In Zambia, ECF is one of the most economically important diseases of cattle and was first reported in 1922 in the Northern Province (Billiouw *et al*., 2005a; Chizyuka *et al*., 1985; Fandamu *et al*., 2005a; Mtambo *et al*., 2008). Initially, it spread to Eastern and Southern provinces and later spread to cover the whole country except for Luapula, Western and North-western provinces, thus a greater part of the national herd is at risk (Chizyuka *et al*., 1985). The impact of the disease is due to high morbidity and mortality as well as production losses and costs related to the control of ticks and the disease (Anon., 2010a; Gacholi *et al*., 2012; Makala *et al*., 2003). This has resulted in loss of sources of livelihoods for most farmers, especially the

rural small scale resource poor households (Gacholi *et al*., 2012; Norval *et al*., 1992). East Coast fever has further been a restraint to the genetic improvement of indigenous breeds of cattle, as it prevents the introduction of more productive exotic breeds and has hindered growth of the livestock sub sector which is an important component of Zambian agriculture (Gacholi *et al*., 2012; Simuunza *et al*., 2011).

1.1 STATEMENT OF THE PROBLEM

Despite the importance of this disease to the cattle industry, there is inadequate systematic documented data on the prevalence of ECF in most parts of Zambia including Copperbelt and Central provinces (Simuunza *et al*., 2011). Although Simuunza *et al* (2011) described the epidemiology of tick-borne diseases (TBDs) in Zambia's Lusaka, Central and Eastern provinces, the sample size for the former province was very low and it is likely that this study did not give a clear view of the status of ECF in the three provinces. Fandamu *et al* (2005a) also described the prevalence of ECF in Southern province using indirect fluorescent antibody test (IFAT) as the diagnostic tool. However, when using serological methods cross reactivity is known to occur among similar pathogens (Billiouw *et al*., 2005a). Additionally, this study did not fully address the risk factors associated with the occurrence of ECF in the Province. For effective disease control planning, there is need to have adequate information on the epidemiology of the diseases. This study was therefore aimed at determining the prevalence and risk factors associated with ECF in Copperbelt and Central provinces of Zambia.

1.2 GENERAL OBJECTIVES OF THE STUDY

To determine, the prevalence and risk factors of ECF in Copperbelt and Central provinces of Zambia**.**

1.3 SPECIFIC OBJECTIVES OF THE STUDY

(1) To determine, the prevalence of ECF in Copperbelt and Central provinces by microscopy.

(2) To determine, the risk factors associated with occurrence of ECF in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GEOGRAPHIC AND CLIMATIC CHARACTERISTICS IN ZAMBIA

Zambia is located almost in the centre of southern Africa with a population of about 14.8 million people and lies between latitudes 24^0S and 34^0S and longitudes 8^0E and 18^0E. The country is surrounded by eight neighbours, namely: Angola to the west, Botswana and Zimbabwe to the south, Namibia to the southwest, Mozambique to the southeast, DRC to the north, Malawi to the east and Tanzania to the northeast. It has a land mass of 75.26 million hectares of which 0.92 million hectares are water bodies. The country is administratively divided into ten provinces namely: Luapula, Northern, Muchinga, Eastern, Southern, Central, Lusaka, Copperbelt, Western and North Western (Anon., 2014c).

Zambia has a tropical climate which is modulated by high altitude (1,000-1,600m) which makes it cool and pleasant and it has three distinct seasons as follows, hot and dry season from September to November, hot and wet season from December to April and cool and dry season from May to August. Average annual rainfall for the country is about 600 to over 1000 mm/year with rainfall increasing from the South to the Northern part of the country (Muleya *et al*., 2013; Simuunza *et al*., 2011).

Wide seasonal variations in temperature occur across the country. October is the hottest month and July is the coldest (Muleya *et al*., 2013; Simuunza *et al*., 2011). Day time temperature ranges between 23^0C to 31^0C and may drop to as low as 5^0C in the night during June and July (Mtambo *et al*., 2008; Simuunza *et al*., 2011). Mean minimum temperature ranges between 5-10^0C in the Southern and Western parts of the country and 10-13^0C in Northern and Eastern parts of the country. The mean maximum temperature ranges from 30-

35^0C in the Southern part of the country to 25-30^0C in the Northern and Eastern parts of Zambia (Mtambo *et al*., 2008; Simuunza *et al*., 2011).

2.2 LIVESTOCK PRODUCTION IN ZAMBIA

Agricultural production is an important source of livelihood for a greater part of the population in Zambia, estimated at 1.5 million households. It promotes economic growth, reduces poverty and creates employment (Anon., 2013b) and is anchored on both communal and commercial production systems. The agricultural sector contributes about 11-16% to the country's Gross Domestic Product (GDP), of which 35% is from livestock production. It employs the largest proportion of the population especially in the rural areas (Anon., 2013b; Mulumba *et al*., 1999).

The major types of livestock reared in Zambia include; cattle, goats, sheep, pigs and poultry (Pegram *et al*., 1986; Muleya *et al*., 2013; Mulumba *et al*., 1999). Cattle are the most important livestock reared. The common cattle breeds in Zambia include (i) European (*Bos taurus*), (ii) Indigenous (*Bos indicus*) and (iii) crosses of indigenous and *taurine* breeds. The main commercial cattle breeds found in Zambia include Friesians, Herefords, Boran, Jersey and AfriKander, while the common indigenous breeds are (i) Barotse cattle, a long horned Sanga type in Western province, (ii) Tonga cattle, a medium horned Sanga type in Southern and Central provinces, (iii) Angoni cattle, a short horned Zebu type in Eastern province (Pegram *et al*., 1986; Muleya *et al*., 2013; Mulumba *et al*., 1999). According to the most recent statistics (Anon., 2014c), the Zambian cattle population is estimated at 4,005,560.

About 85% of livestock production is under the traditional farming system (Mulumba *et al*., 1999; Simuunza *et al*., 2011). Traditional farming is a preserve of the rural households and is characterised by low productivity (Chilonda *et al*., 1999). One of the reasons for low productivity of cattle in this sector is cattle diseases (Chilonda *et al*., 1999). The extensive

production system results in a lot of herd inter-mixing which makes control of spread of disease difficulty (Chilonda *et al*., 1999). Other constraints to livestock production in Zambia include use of unimproved breeds, limited access to credit, poor livestock management and extension services (especially in remote rural areas), fluctuating prices, low value addition and failure to attract adequate private sector investment. Poor rural infrastructure (roads and dip tanks), poor feed/pasture and inadequate water especially in the dry season also contribute to the low productivity of cattle in the country. Further, traditional attitudes that emphasize livestock numbers rather than returns negate growth of the industry coupled with limited investment in community and public infrastructure such as breeding centres, disease control laboratories, markets, modern abattoirs and quarantine stations. Farmers have been reluctant to invest in preventive veterinary care and improved nutrition, due to low market incentives and cultural practices (Anon., 2010a; Anon., 2013b; Muleya *et al*., 2013).

Livestock diseases have a serious impact in Zambia as they reduce the livestock population and deprive farmers of their livelihood and increase the cost of livestock production both at individual and national levels. The diseases have reduced the export potential and earnings for the country due to international livestock movement and trade restrictions. Indeed, Zambia's potential for livestock production especially in the traditional farming sector has not been fully attained mainly due to the prevalence of animal diseases (Mulumba *et al.,* 1999).

Tick borne diseases are among the most economically important livestock diseases in Zambia leading to the high morbidity and mortality and reduced livestock productivity (Makala *et al*., 2003; Simuunza *et al*., 2011). They are generally distributed across the whole country especially in the livestock rearing areas such as the Eastern, Central, Southern, Lusaka, Copperbelt and Western provinces (Akafekwa *et al*., 1976; Chilonda *et al*., 1999; Makala *et al*.,

2003; Muleya *et al*., 2013; Simuunza *et al*., 2011)**.** The important TBDs of cattle in Zambia include *theileriosis* (East Coast fever), *babesiosis*, *anaplasmosis* and *heart water.* East Coast fever causes considerable economic losses in Eastern, Central and Southern African region, with annual losses estimated at US$ 168 million (Mukhebi *et al*., 1992; Siegel *et al*., 2006). There is therefore a need to better understand the epidemiology of ECF in the country so that appropriate control measures can be applied at local and national levels.

2.3 AETIOLOGY OF ECF

East Coast fever is caused by *Theileria parva,* a protozoan parasite of domestic cattle and wild buffalo. It is the most important *Theileria* species in Africa, south of the equator (Fandamu *et al*., 2005a; Mtambo *et al.,* 2008). The classification of *Theileria parva* has for a long time been controversial (Irvin *et al*., 1987). However Levine *et al* (1980) classified the parasite as belonging to Phylum Apicomplexa, Class Sporozoea, Subclass Piroplasmia, Order Piroplasmida, Family *Theileridae*, Genus *Theileria* and species *Theileria parva*. Other members of the *Theileria* genus include *T. mutans, T. taurotragi, T. velifera, T. buffeli* and *T. annulata* (Fandamu *et al*., 2005a; Mtambo *et al.,* 2008).

Uilenberg (1976) and Lawrence (1979) proposed a trinomial system of classification of the three forms of *T. parva*; (i) *T. parva parva* for parasites causing classical ECF, (ii) *T. parva lawrencei* for parasites causing Corridor disease and (iii) *T. parva bovis* for parasites causing January disease. However, this classification was abandoned as the molecular characterization and cross immunity data did not support the existence of these subspecies within the *T. parva* complex (Conrad *et al*., 1987, 1989; Allsopp *et al*., 1989).

Adl *et al* (2005) revised the classification based on morphology, biochemistry and molecular phylogenetics and *T. parva* was designated as protists rather than protozoa but still remained a

member of Apicomplexa. However, the Taxonomy of protozoa and other protists is still undergoing dramatic, controversial changes and rearrangements. Presently *T. parva* is generally classified as protozoa partly because of its ability, unique among protozoan parasites, to transform bovine lymphocytes which they invade into uncontrollable cancer like proliferation (Kaba *et al*., 1998).

2.4 LIFE CYCLE OF *THEILERIA PARVA*

The schematic representation of the life cycle of *Theileria parva,* the causative agent of ECF is shown in Figure 1. The parasite has a complex life cycle which includes an asexual stage in the mammalian host and a brief sexual stage in the invertebrate host (tick). It has several distinct morphological developmental stages in both the tick and mammalian hosts (Fawcett *et al*., 1982; Mtambo *et al.,* 2008).

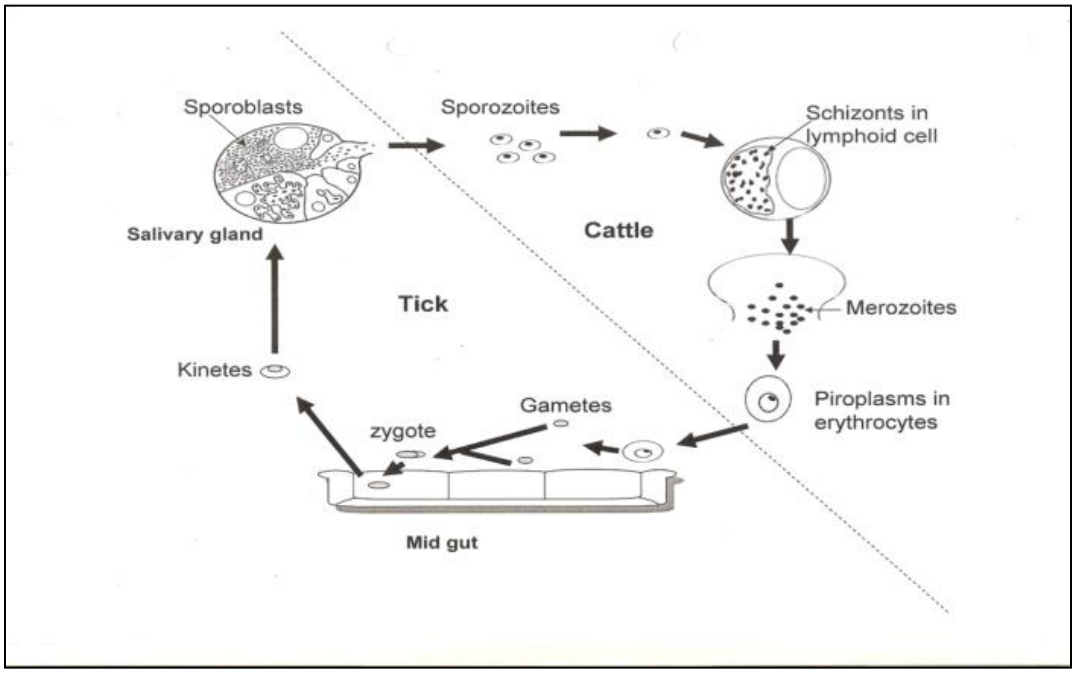

Figure 1. Schematic diagram of the life cycle for *theileria parva*: (Simuunza *et al* 2009).

The infectious stage of the parasite, the sporozoite, is introduced into the bovine host in the saliva of the ticks feeding as nymphs or adults after having acquired the infection as larvae or nymphs respectively (Mtambo *et al*., 2008). They enter the lymphoid cells where they develop into schizonts and are disseminated throughout the body by the normal circulation of the host lymphoid cells. After a period of growth and division in the host lymphoid cells, the schizont gives rise to numerous uninucleate merozoites which leave the lymphoid cells to invade erythrocytes (Fandamu *et al*., 2005a; Mtambo *et al*., 2008). The intraerythrocytic stage of the parasites, the piroplasms, are ingested during blood meal by feeding ticks and released in the gut lumen. There they differentiate into macro and microgametocytes which fuse to form a zygote (Figure 1). The resulting zygote enters the lining of the gut epithelium where they develop into motile kinetes. The kinetes migrate through the gut wall into the haemocoele and make their way to the salivary gland where they become intracellular and transform into infective sporoblasts (Fawcett *et al*., 1982; Oura *et al*., 2005).

2.5 VECTORS OF *THEILERIA PARVA*

The only known field vectors of *T. parva* are *R. appendiculatus*, *R. zambeziensis* and to a lesser extent *R. duttoni. R. appendiculatus* and *R. zambeziensis* are three-host ticks and are the principal vectors of *T. parva* (Fandamu *et al*., 2005a; Mtambo *et al*., 2008; Siegel *et al.,* 2006). Larva feed indiscriminately on a variety of hosts whereas nymphs show preference for ungulates and adults feed on large mammals such as cattle and buffalo. To become infective the tick must feed on an infected host and moult to the next stage (Billiouw *et al*., 2005a).

R. appendiculatus has been recorded in 17 countries of Eastern, Central and Southern Africa, ranging from south of Sudan to South Africa (Mulumba *et al.*, 1999). The distribution of *R. appendiculatus* and *R. zambeziensis* is influenced by several factors which include climate,

vegetation and host availability (Fandamu *et al.,* 2005a). *R. duttoni* is a common tick species in Angola and has also been reported in the Democratic Republic of Congo. Little is known of the factors that limit the distribution of this tick species (Gomes *et al*., 1991).

In parts of Africa where there is a well-defined rainy season, there is an obvious association between the onset of rains and adult *R. appendiculatus* tick activity and in these regions only one generation of ticks is observed per annum (Short *et al*., 1981). In other parts of Africa where rains may be present throughout the year, adult ticks may be present all year round (Mc Culloch *et al*., 1968; Newson 1978). In many parts of Zambia there is only one generation of ticks per year (Pegram *et al*., 1986). However, the Eastern and Northern parts may have a second generation of adult ticks which coincides with first wave of nymphal activity in the period May-June. The second wave of nymphs become active in September (Billiouw *et al*., 2005b). Adult ticks occur from December to April, larvae between March and May and nymphs between May and September (Pegram *et al*., 1986). *R. zambeziensis* is a more important vector of *T. parva* in several hotter and arid areas of Central and Southern Africa and includes some of the drier parts of Zambia, Mozambique, Zimbabwe and South Africa as well as the non-desert areas of Botswana and Namibia (Madder *et al*., 2005; Mtambo *et al*., 2008).

2.6 HOSTS OF *THEILERIA PARVA*

Theileria parva is a parasite of domestic cattle, African buffalo (*syncerus caffer*), water buffalo (*bubalus bubalis*), and water buck. Symptomatic infections are only commonly seen in cattle and water buffalo. *Theileria* species have been found in most wild bovidae in Africa. They have also been reported in wild animals on other continents (Anon., 2009b; Mtambo *et al*., 2008).

2.7 PATHOGENESIS OF ECF

Severity of the clinical signs depends on the lymphoid stage of *T. parva* and inherent virulence of the strains and host reactions (Nambota *et al*., 2000). Lymphadenopathy due to schizogony in the lymphoblasts is a dominant feature and occurs first in the lymph nodes near the inoculation site, usually the prescapula lymph node (Siegel *et al*., 2006). The rate of macroschizont multiplication determines virulence of the disease. If it is low or moderate, depending on the parasite strain and host resistance, there are chances that immunity will develop and prevent clinical cases of ECF (Nambota *et al*., 2000; Siegel *et al*., 2006).

Schizogony is associated with transformation of the infected cells to a state of uncontrolled proliferation. By associating with the mitotic spindle, the parasite divides in synchrony with the host cell, resulting in each daughter cell inheriting the infection. This stage of parasitic division is associated with the severity of the pathology and clinical signs seen in *T. parva* infections (Rocchi *et al*., 2006). Some pro-inflammatory cytokines have been found to be up regulated during the acute phase of *T. parva* infections when the parasite is proliferating and are thought to contribute to the severity of the disease (Yamada *et al*., 2009). Initially the disease is characterized by swelling of the lymph nodes draining the ear surface which is the predilection site of the vector ticks. The incubation period is usually between 7 to 10 days after infection with fever (39.5-42^{o} C), which is a consistent feature of the disease from about day 10 post infection. Schizont infected cells disseminate from lymph nodes to other organs including the interstitial tissues of the lungs, kidneys and the gastro-intestinal tract which leads to infiltration of inflammatory cells and cell damage leading to symptoms such as bloody diarrhoea and laboured breathing. Lung tissues are damaged by inflammatory cell infiltration, leading to severe pulmonary oedema which eventually results in the death of infected animals (Gwamaka *et al*., 2004).

2.8 CLINICAL SIGNS AND PATHOLOGICAL FINDINGS

East Coast fever is characterized by lymphadenopathy, pyrexia, dyspnea and frothing due to interstitial pneumonia and pulmonary oedema. Other signs may include subcutaneous oedema, diarrhoea, lacrimation and mortality. Petechiation on mucous membranes, inappetance, ceasation of rumination, salivation, serous and nasal discharge, rapid and weak heartbeat, and intestinal ulceration may be exhibited (Mtambo *et al*., 2008; Siegel *et al*., 2006).

Animals which recover either naturally or after treatment with theilericides develop long lasting immunity giving complete protection to homologous challenge, but may remain susceptible to some heterologous strains (Billiouw *et al*., 2005b; Kariuki *et al*., 1995).

2.9 EPIDEMIOLOGY OF EAST COAST FEVER (ECF)

Distribution of *T. parva* is closely related to that of its principal vector *R. appendiculatus,* which in turn is influenced by climatic conditions *(*Mtambo *et al*., 2008). The climate influences tick population dynamics and transmission of *T. parva*, creating a wide range of epidemiological situations for different areas (Lessard *et al*., 1990; Norval *et al*., 1991; Randolph *et al*., 2010). Based on rainfall and temperature three different transmission scenarios of *T. parva* have been described and are determined by the number of tick generations per year (Norval *et al*., 1991). In East Africa the adult ticks are present throughout the year and this leads to year round transmission (Norval *et al*., 1991). The climate in central Africa does not favour the presence of adult tick throughout the year and therefore dry season and wet season adult peaks occur and determine the transmission pattern (Norval *et al*., 1991). In the southern part of the *R. appendiculatus* range there is only one tick generation every year and the adult ticks are present for only four months which coincides with the rainy season. Peak adult activity occurs during the period January-March and makes the epidemiological situation unstable (Billiouw.,

et al., 2005a, Norval *et al*., 1991; Kariuki *et al.,* 1995). Nymphal transmission (May to August) is also important in these areas, as the nymphs help stabilize the epidemiological situation by ensuring that calves born in the dry season are exposed to the disease early in life (Mulumba *et al*., 2001).

Risk factors associated with *T. parva* transmission include livestock management, livestock movement, abundance of livestock, distribution and abundance of vector ticks**,** and host resistance to both the tick and the pathogen (Salih *et al*., 2007a; Simuunza *et al*., 2011). Two additional factors which are important in the epidemiology of ECF are the host type and density (Norval *et al*., 1988). The African cattle population consists of different breeds which show varying levels of tick resistance and parasite susceptibility (Norval *et al*., 1988).

According to Nambota *et al*. (2000), patterns of ECF epidemiology can be described as follows:

1. Sporadic- where the disease occurs rarely or without regularity in a given population. Animals are generally naïve and may be exposed to the disease as adults leading to severe clinical disease. This may happen during periods of peak tick activity especially in the rainy season.
2. Endemic- where the disease occurs with predictable regularity in a given population, where-by young animals get infected early in life and are immune by six months of age. A state of endemic stability is said to exist when antibody prevalence is high, case fatalities are low and the disease occurring only in young calves (Nambota *et al*., 2000). This state is achieved when *R. appendiculatus* is present throughout the year, with cattle possessing low innate susceptibility to *T. parva* infection and young calves are exposed to a low *T. parva* challenge.

3. Epidemic- where the disease occurs at given time interval, clearly in excess of its expected frequency.

2.10 DIAGNOSIS OF ECF

Diagnosis of ECF is based on history, clinical signs and prevailing conditions in an area but should be confirmed by blood smear and lymph node biopsy examination (Billiouw *et al*., 2005a; Nambota *et al*., 2000). The major diagnostic techniques include Microscopy, Serology (IFAT), Polymerase Chain Reaction (PCR) and Loop-mediated isothermal amplification (Lamp), (Siegel *et al*., 2006).

2.10.1 MICROSCOPY

Blood smears stained with Giemsa can be used to detect *T. parva* parasites and provide a useful adjunct to the clinical assessment. Piroplasms appear in the red blood cells about 5-8 days after detection of schizonts in new infections. Reliance on the presence of piroplasms in stained blood smears for diagnosis early in the course of the disease may therefore result in false negatives. Giemsa stained lymph node biopsy smears can also be used to detect schizonts in infected lympoblasts which is diagnostic of *T. parva* infection (Billiouw *et al*., 2005a; Fandamu *et al*., 2005a). The advantages of microscopy are that it is easy to use and results can be obtained relatively quickly. However, it has low sensitivity and it is difficult to differentiate *Theileria* species based on morphology (Anon., 2009a; Siegel *et al*., 2006). Further, microscopy is time consuming and labour intensive and diagnosis is dependent on skills of the technicians (Billiouw *et al*., 2005a).

2.10.2 SEROLOGY

The most commonly used serological assay in recent years for *T. parva* has been the indirect fluorescent antibody test (IFAT) (Bazarusanga *et al*., 2008). IFAT has been widely used in serological surveys in Africa and greatly contributed to understanding the epidemiology of *Theileria parva* infections (Bazarusanga *et al*., 2008; Billiouw *et al*., 2005b; Fandamu *et al*., 2005b). However, it has its drawbacks. Firstly IFAT is cumbersome to carry out and is dependent on subjective observation of degrees of fluorescence. Secondly it lacks specificity in that *T. parva* does cross react with *T. taurotragi* and *T. annulata,* thereby complicating the diagnosis *(Anon., 2009a; Anon., 2010c; Siegel et al., 2006).*

2.10.3 POLYMERASE CHAIN REACTION (PCR)

Polymerase chain reaction uses specific primers and a thermostable DNA polymerase to amplify specific DNA sequences up to several million folds. This technique is highly sensitive and therefore useful in detecting low levels of parasitaemia (Anon., 2009a; Ogden *et al*., 2003; Phillip *et al.,* 1980). Apart from the increased sensitivity, another advantage over IFAT is that PCR primers are specific for *T. parva* and they do not amplify DNA of closely related parasites. Polymerase chain reaction can also detect schizonts in lymph node biopsy before they can be detected by light microscopy (Anon., 2009a; Ogden *et al*., 2003; Phillip *et al.,* 1980). PCR is a valuable means of distinguishing species, strains and stocks of pathogenic protozoan parasites which are difficult or impossible to distinguish morphologically or serologically (Anon., 2009a; Ogden *et al*., 2003; Phillip *et al.,* 1980). The disadvantages are that it requires sophisticated equipment, highly trained personnel and is quite costly and therefore its use in laboratory practice is not common especially in rural endemic regions (Anon., 2009a; Ogden *et al*., 2003; Phillip *et al.,* 1980).

2.10.4 LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Loop-mediated isothermal amplification test is a novel gene amplification method that is rapid, simple, highly sensitive and useful in the detection of protozoan, viral, bacterial and fungal pathogens (Thekisoe *et al*., 2007; Nakao *et al*., 2010). Unlike PCR, Lamp reaction can be conducted using a simple laboratory heat block or water bath for incubation at a constant temperature of between 60-65^{0}C and this removes the need and cost for a thermal cycler. The results can be visualized by the naked eye (white turbidity in reaction tubes) or by addition of fluorescent dyes visualized under ultra violet light (Thekisoe *et al*., 2007; Nakao *et al*., 2010). Lamp reagents are relatively stable and the Lamp method has the potential to be used in clinical laboratories and in the field (Nakao *et al*., 2010). A lamp method has been developed which is specific to *T. Parva* and is based on the p104 gene, which is immune-dominant and specific to *Theileria parva* (Skilton *et al*., 2002).

2.11 ECF CONTROL

Measures employed to control ECF include tick control, livestock movement control, immunization and chemotherapy (Mtambo *et al*., 2008).

2.11.1 TICK CONTROL

The major method of controlling the tick vector is by application of acaricides to the surface of an animal through dipping, spraying or hand washing to kill the ticks. In conditions of heavy tick infestation or high disease incidence, the frequency of acaricide application can be as often as twice a week (Allan *et al*., 2011; Lawrence *et al.,* 2004; Makala *et al.*, 2003). Tick control through use of acaricides has become less reliable, unacceptable and unsustainable for a number of reasons. These include high cost of acaricides, high costs of construction and maintenance of dip tanks and spray races, water shortages, tick resistance to acaricides and

contamination of the environment or food chain with acaricide residues (Anon., 2010a; Nambota *et al*., 1994; Fandamu *et al*., 2005a). Strict acaricide application results in highly susceptible cattle populations as cattle are not exposed to the parasite and when tick control breaks down enormous losses are bound to occur (Kocan *et al*., 2000; Lawrence *et al*., 1980).

Other methods of tick control include the use of tick resistant breeds of cattle and anti-tick vaccines (Canales *et al*., 2009; Popara *et al*., 2013; Rodriguez-Valle *et al*., 2012). Genetically tick resistant breeds are a vital component of many tick control strategies. However it may be difficult to breed for tick resistance while at the same time preserving some of the desirable traits, or resistance may simply fail to develop in some hosts (Simuunza *et al*., 2011; Wilkinson *et al*., 1962).

Anti-tick vaccination is premised on controlling tick infestations through immunization of hosts with selected tick antigens. Initial trials were based on the concept that ticks feeding on an appropriately immunized host might ingest antibodies specific for antigens in the digestive tract and reproductive organs of the tick, resulting in deleterious effects on the feeding and reproductive behaviour (Canales *et al*., 2009). There is currently only one tick antigen, Bm86 against *R. microplus,* which is commercially marketed (Canales *et al*., 2009; Popara *et al*., 2013; Rodriguez-Valle *et al*., 2012). Advantages of the anti-tick vaccines are that they are cost effective, reduce environmental contamination and prevent selection of drug resistant ticks due to repeated acaricide usage (Canales *et al*., 2009). Development of vaccines against ticks using multiple antigens that could target a broad range of tick species may prevent or reduce transmission of other pathogens (Canales *et al*., 2009). Unfortunately, however, no vaccine is available for the control of the vectors of *T. parva.*

2.11.2 LIVESTOCK MOVEMENT CONTROL

This method depends on an active and reliable veterinary service delivery system where disease outbreaks are quickly diagnosed, reported and affected areas placed under restricted livestock movement. In Zambia, movement of cattle is controlled by livestock movement permits issued by Government Veterinary Officers **(**Anon., 2010b; Nambota *et al*., 1994). These restrictions are necessary to limit the spread of cattle diseases in general and ECF in particular. Livestock movements within ECF endemic areas are allowed, but movements from endemic areas to non-endemic areas are only allowed on the following conditions: (Anon., 2010b**;** Nambota *et al*., 1994).

(i) Animals to be moved must test negative by immunofluorescent antibody test, lymph and blood smear microscopy.

(ii) Cattle can only be allowed to move within seven days after the test. If they overstay for more than seven days, they should be subjected to a new set of tests.

(iii) Animals are treated with an acaricide before they are moved to ensure that they are tick free.

(iv) Animals are subjected to compulsory quarantine at destination under close veterinary supervision for a minimum period of 26 days. They are checked for any signs of ECF by the local veterinary officer before they can be allowed to mix with other animals.

(v) Animals for slaughter must be branded with slaughter brands and must be slaughtered under veterinary supervision within 24 hours of their arrival at destination. Livestock movement control is generally focused on cattle and therefore other *Theilerial* hosts and wild animals are still able to move without restriction and may therefore spread the disease (ECF). Compulsory

tick control like livestock movement regulations is generally unpopular with farmers and therefore difficult to enforce (Anon., 2010a; Nambota *et al*., 1994).

2.11.3 IMMUNIZATION

Safer, cheaper and more sustainable methods based upon immunization are being advocated for (Anon., 2010a; Nambota *et al*., 1994; Fandamu *et al*., 2005a). There are two methods of immunization, namely (i) infection and treatment method (ITM) and (ii) use of subunit vaccines (Anon., 2010a; Radley *et al.,* 1975).The ITM method is based on the fact that cattle, which recover from ECF, are immune to homologous challenge (Anon., 2002; Radley *et al.,* 1975). Cattle are given an infective dose of *T. parva* parasites, and simultaneously injected with long acting tetracycline to control the infection. Due to the different immunogenic stocks or strains of *T. parva*, it is necessary to conduct various immunity trials before starting an immunization campaign in a new area. This is done to avoid the introduction of new strains into a given area as antigenic variation or diversity can also lead to new infections or carrier state (Nambota *et al*., 1994). Since the stabilate contains live sporozoites of the parasite, it is important to immunize with local strain (or cocktail of local strains) which protect against all the strains present in the target area. It is also important to know the epidemiological status of the disease in the area before deciding to immunize (Anon., 2010b; Lawrence *et a*l., 2004). The main disadvantage of this method is that live vaccines need the maintenance of a cold chain facility which is a formidable obstacle with the poorly developed infrastructure of rural Zambia where the vaccine is mostly needed (Makala *et al*., 2003). The practical limitations imposed by using live parasites for vaccination against *theileriosis* have led to efforts aimed at developing alternative vaccines based on defined parasite antigens. This work has largely focused on understanding the immune responses that mediate protection against ECF, with a view to using

immune probes to identify candidate antigens for vaccination (Morrison *et al*., 2006). Antigens expressed in the different stages of parasite development have been identified and tested for immunogenicity (Kaba *et al*., 1998; Morrison *et al*., 2006).

The genes encoding the respective sporozoite proteins in *T. parva* and *T. annulata* have been identified using specific antibodies (Morrison *et al*., 2006). The *T. parva* product is a 709 amino acid polypeptide, commonly known as p67. The gene that encodes it is present as a single copy gene and entirely conserved in all cattle derived isolates (Morrison *et al*., 2006). It produced a high antibody titre in cattle, but offered partial protection under field conditions (Mtambo *et al*., 2008). Several recombinant forms of p67 have been generated in E. coli and these were evaluated extensively in laboratory immunization and challenge experiments. These had generally taken the form of three to five monthly immunizations using proprietary adjuvant formulations, followed by sub-cutaneous needle challenge with an LD_{70} of cryopreserved sporozoites two to three weeks after the final boost (Morrison *et al*., 2006). These experiments resulted in about 70% of immunized animals being protected from severe disease (Morrison *et al*., 2006). Although currently in the experimental stage recombinant vaccines offer hope for the future of the livestock industry in Zambia (Makala *et al*., 2003).

2.11.4 CHEMOTHERAPY

Early diagnosis of the disease is important for effective treatment of ECF. Drugs that are effective against ECF include Parvaquone and Buparvaquone (Minjauw *et al*., 1999; Mtambo *et al*., 2008). Previously, most of the available chemotherapeutic agents were only effective, if given early in the course of the disease, especially before the onset of respiratory signs (McHardy *et al*., 2004; Mbwambo *et al*., 2002). However, there is now a parvaquone preparation that contains an antidiuretic drug (Frusemide) which is quite effective even when

the animal is showing respiratory signs (McHardy *et al*., 2004; Mbwambo *et al*., 2002). When effectively applied, chemotherapy leads to a higher proportion of immune animals in a population but this measure also increases the number of carriers which are a source of the parasites for the infective ticks (Lawrence *et al*., 2004; Mtambo *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

This study was carried out in Mpongwe and Masaiti districts (Copperbelt province) and in Kapiri Mposhi and Chibombo districts (Central province), Zambia (Figure 2). These areas were chosen because of their vast potential for livestock production and the previously reported incidence of ECF (Anon., 2010b; Nambota *et al.,* 1994). For Mpongwe district, lymph node biopsy samples were collected in December 2011, and in January, February, August and December 2012. Lymph node biopsy samples from Masaiti district were collected in February, March and December 2012. Lymph node biopsy samples from Kapiri Mposhi district were collected in June 2012, while lymph node biopsy samples from Chibombo district were collected in July and August 2012.

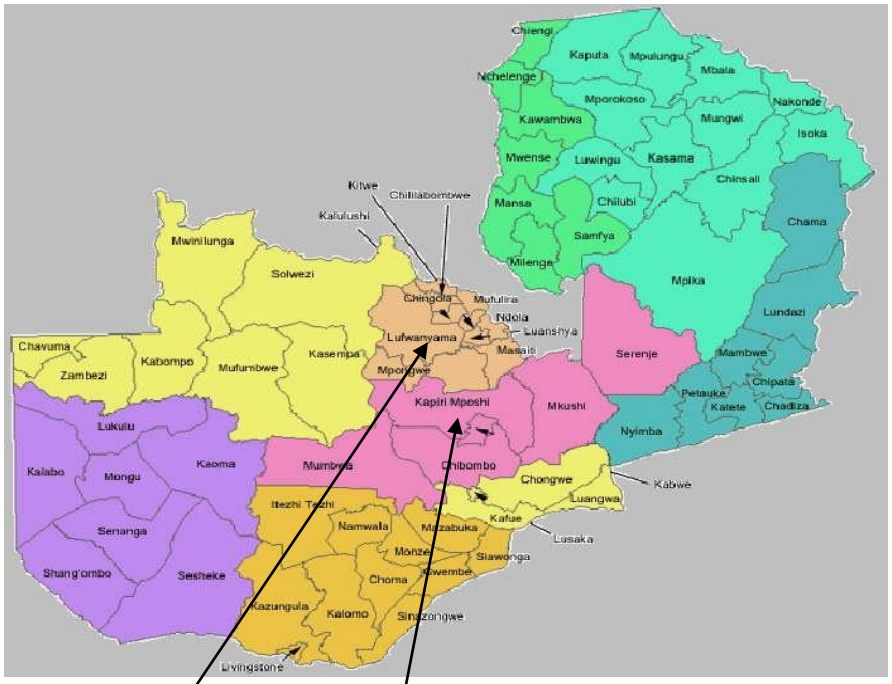

Figure 2. Map of Zambia showing Copperbelt Province and Central Province (Anon 2015)

3.2 STUDY DESIGN AND SAMPLING

The study was cross sectional in design. Multistage cluster sampling was used. First, the provinces and the districts were selected and then from each district, two veterinary camps were randomly selected. From each camp, herds were randomly selected and from each herd individual animals were randomly sampled. The sample size was determined as previously described by Martin *et al.,* (1987), using the formula: $n=Z^2(1-P)p/L^2$

Where:

n = the required number of individual animals to be examined,

Z = the value for the 95% confidence level,

P = a known or estimated prevalence (20%),

L= the allowable error of estimation (5%).

Therefore $n=1.96^2 (1-0.2)0.2/0.05^2=246$ cattle.

Since lymph node biopsy samples were collected from four districts, this gave a presumed total of 984 animals for the whole study area. Lymph node biopsy was collected from the prescapula lymph node of 997 cattle from which lymph smears were made.

At the same time, a semi-structured questionnaire was administered to each household from which animals had been sampled to collect information on farming system, management, ECF immunisation status, tick burden, breed, age and sex. Tick burden was estimated for each of the sampled cattle by counting the number of ticks on one side of the animal and then multiplied by two. Tick burden was classified as nil (0 ticks), low (1-10 ticks), medium (11-20 ticks) and high (20 or more ticks).

3.3 LABORATORY ANALYSIS

3.3.1 SMEAR PREPARATION FOR MICROSCOPY

Smears were prepared whilst in the field from lymph node biopsies for microscopic examination. The smears were made by placing a drop of lymph node aspirate onto one end of a clean and labeled slide. The slides label was checked with the samples to make sure it was the same. The edge of another clean slide (pusher slide or spreader) was brought in contact with the drop and the drop was allowed to bank evenly behind the spreader. The angle between the pusher slide and specimen slide was maintained at 45^0. The spreader was then pushed with the right hand to the left in a smooth and quick motion. The smear covered about half of the slide and consisted of a head, middle part and tail (anon., 2014a; anon., 2014b). The pusher slide was discarded in a biohazard discard bag and the smear allowed to air dry in a dust free area. The smears were fixed in methanol for 3-5 minutes, dried and placed in the freshly prepared Giemsa stain solution diluted with water at 1: 9 (giemsa: water) for 30 minutes, washed in distilled water, then air dried and microscopically examined for presence of *T. parva* parasites under oil immersion at a magnification of X100 and at least 10 sites of the slide were examined (Aiello *et al*., 1998; Salih *et al*., 2007b).

3.4 DATA ANALYSIS

Data from the questionnaire and laboratory lymph smear examination was entered into Microsoft Excel spreadsheets and later transferred to SPSS version 16 (IBM, USA) for analysis. Descriptive statistics were calculated for each of the variables included in the study. Binary logistic regression was used to quantify the effect of each risk factor on cattle being *T. parva* positive. All variables with $p \leq 0.250$ in the univariate analysis were included in the model. Criteria that was used to determine whether the model fitted the data was a non significant

Hosmer and Lemeshow test ($p>0.05$) and a significant ($p\leq0.05$) Omnibus Test for Model Coefficients. All statistics were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 DESCRIPTIVE STATISTICS

Of these samples collected from 997 heads of cattle, 12.4% (95%, CI=10.4-14.5) came from the commercial farms and 87.6% (95%, CI=85.5-89.6) came from the traditional farming system. A few of the farms reportedly had no access to veterinary care, with 9.2% (95%, CI=7.5-11.0) of the sampled cattle falling in this category, whereas most of the farms [90.8% (95%, CI=89.0-92.6)] had access to veterinary services. None of the farms from which samples were collected practiced ECF immunisation for their cattle.

Most of the farms (99.3%, 95%, CI=98.78-99.82) reportedly practiced tick control. The majority of farms [95.9% (95%, CI=92.7-99.1)] practiced tick control throughout the year, but a few [3.4% (95%, CI=0.5-6.3)] practiced strategic tick control as they believed the risk of tick borne diseases was high during the period November-July.

Some farms [19.6% (95%, CI=13.2-26.0)] reported having recorded mortalities in their cattle in the previous 12 months, but most farms [80.4% (95%, CI=74.0-86.8)] did not record any mortalities. The period January to March recorded 123 mortalities, followed by the period April to June which recorded 77 mortalities.

Of the 997 heads of cattle sampled, 5.7% (95%, CI=3.6-6.4) were owned by women, 91.1% (95%, CI=89.33-92.9) were owned by men and 3.2% (95%, CI=2.11-4.3) were owned by institutions such as Heifer International and Training Institutions. Based on age, 27.1% (95%, CI=24.3-29.8) were calves (cattle up to one year old) and 72.9% (95%, CI=70.2-75.7) were adults (cattle more than one year old). The majority of the cattle sampled [54.0% (95%, CI=50.9-57.1)] were females and 46.0% (95%, CI=42.9-49.1) were males. Of these cattle sampled, 12.1% (95%,

CI=10.1-14.1) were commercial breeds, 5.6% (95%, CI=4.2-7.0) cross breeds and 82.3% (95%, CI=79.9-84.7) local breeds.

The average number of cattle (herd size) in the study area was 51.4 (95%, CI=51.3-51.5) per household. The average birth rate across the farms was 10.6 (95%, CI=10.5-10.7) calves, while the average number of animals received in the previous 12 months per farm was 0.5 (95%, CI=0.4-0.6) and these animals came from within Mpongwe, Lufwanyama, Southern province or Central province. The average number of animals sold or given out during the previous 12 months in the different farms was 2.4 (95%, CI=2.3-2.4). Tick burden was classified as nil (0 ticks), low (1-10 ticks), medium (11-20 ticks) and high (20 or more ticks), (Table 1).

Table 1: Tick burden observed on cattle in the study area

Tick burden	Cattle number	Percentage %	95% Confidence interval
Nil (0 ticks)	114	11.43	9.5-13.4
Low (1-10 ticks)	147	14.74	12.5-16.9
Medium (11-20)	681	68.31	65.4-71.2
High (≥20 ticks)	55	5.52	4.1-9.9

4.2 OVERALL PREVALENCE OF ECF

The lymph smear examinations revealed that 6.4% (95%, CI=4.9-7.9) of the samples were positive for *T. parva* schizonts (Figure 3). In Central province, the overall prevalence was 6.7% (95%, CI=4.5-8.9), while on the Copperbelt province it was 6.1% (95%, CI=4.0-8.2).

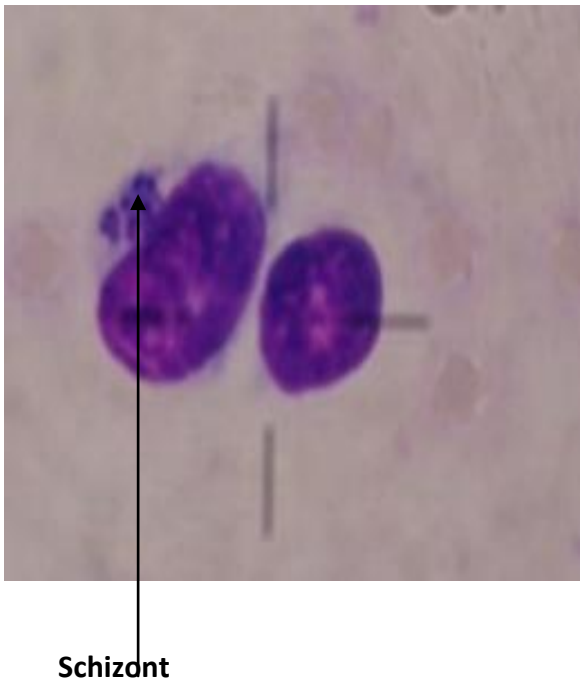

Figure 3. Schizont in a reactive lymph node shown by the arrow

Among the districts in these provinces, Kapiri Mposhi did not record any schizont positive cattle, while Masaiti recorded 2.4% (95%, CI=0.5-4.3). Mpongwe had a prevalence of 9.7% (95%, CI=6.0-13.4) and Chibombo had the highest prevalence at 13.6% (95%, CI=9.4-17.9). No schizont was detected in Chibwe, Chilumba (Kapiri Mposhi District) and Mutaba (Masaiti District) veterinary camps. Chibombo Central East had a prevalence of 9.5% (95%, CI=2.7-16.3), 15.3% (95%, CI=10.0-20.6) in Chibombo Central West, 4.4% (95%, CI=-1.6-10.4) in Kashiba, 3.2% (95%, CI=0.7-5.7) in Masaiti Central and 10.9% (95%, CI=6.6-15.2) in Mpongwe. The prevalence among the chiefdom's was, Chipepo 0%, Lesa 9.2% (95%, CI=4.6-13.8), Liteta 13.6% (95%, CI=9.4-17.9), Malembeka 9.9% (95%, CI=3.4-16.4), Mushili 2.4% (95%, CI=0.3-4.5) and Ndubeni 5.8% (95%, CI=-0.6-12.2), (Table 2).

Table 2: Schizont prevalence according to geographical areas

Variable	Categories	N	Prevalence (%)	95% CI	p value
Province	Central	505	6.7	4.5-8.9	0.390
	Copperbelt	492	6.1	4.0-8.2	
District	Chibombo	250	13.6	9.4-17.9	<0.001
	Kapiri Mposhi	255	0	-	
	Masaiti	245	2.4	0.5-4.3	
	Mpongwe	247	9.7	6.0-13.4	
Camp	Chibombo CE	74	9.5	2.7-16.3	<0.001
	Chibombo CW	176	15.3	10.0-20.6	
	Chibwe	109	0	-	
	Chilumba	146	0	-	
	Kashiba	45	4.4	-1.6-10.4	
	Masaiti Central	190	3.2	0.7-5.7	
	Mpongwe	202	10.9	6.6-15.2	
	Mutaba	55	0	-	
Chiefdom	Chipepo	255	0	-	<0.001
	Lesa	153	9.2	4.6-13.8	
	Liteta	250	13.6	9.4-17.9	
	Malembeka	81	9.9	3.4-16.4	
	Mushili	206	2.4	0.3-4.5	
	Ndubeni	52	5.8	-0.6-12.2	

The prevalence for female cattle was 6.1% (95%, CI=4.1-8.1) and 6.8% (95%, CI=4.5-9.1) for male cattle. Calves had a higher prevalence (11.5%, 95%, CI=7.7-15.3) than older cattle (4.5%, 95%, CI=3.0-6.0). Prevalence according to cattle breeds was as follows, commercial breeds 0.8% (95%, CI=-3.0-4.6) cross breeds 17.9% (95%, CI=7.5-27.9) and, local breeds 6.5% (95%, CI=4.8-8.2), (Table3).

Table 3: Schizont prevalence according to host factors

Variable	Categories	N	Prevalence (%)	95% CI	p value
Sex	Female	538	6.1	4.1-8.1	0.393
	Male	459	6.8	4.5-9.1	
Age	Adult	727	4.5	3.0-6.0	<0.001
	Calf	270	11.5	7.7-15.3	
Breed	Commercial	121	0.8	-3.0-4.6	<0.001
	Cross	56	17.9	7.5-27.9	
	Local	820	6.5	4.8-8.2	

Cattle under traditional farming system had a higher prevalence (7.1%, 95%, CI=5.4-8.8) than those under commercial farming system (1.6%, 95%, CI=-0.6-3.8), (Table 4).

Table 4: Schizont prevalence according to management factors

Variable	Categories	N	Prevalence (%)	95% CI	p value
Farming system	Commercial	124	1.6	-0.6-3.8	0.009
	Traditional	873	7.1	5.4-8.8	

Prevalence for cattle from owners who reported to have no access to veterinary care was 9.8% (95%, CI=3.7-15.9) and 6.1% (95%, CI=4.5-7.7) for those from owners that reported to have access to veterinary care. Among the cattle from owners that reported to have access to veterinary services, the prevalence in those with weekly access to veterinary services was 8.6% (95%, CI=2.5-14.7) and 6.3% (95%, CI=4.2-8.4) in those that accessed services monthly. Cattle for which veterinary services were rarely accessed had a prevalence of 5.1% (95%, CI=2.6-7.6) and 9.0% (95%, CI=3.4-15.6) for those whose frequency of veterinary services was not defined. The prevalence among cattle serviced by a community livestock worker was 0%, 6.2% (95%, CI=4.6-7.8), for cattle serviced by a veterinary assistant and for cattle with unknown service providers it was 8.7% (95%, CI=3.3-14.1), (Table 5).

Table 5: Schizont prevalence according to veterinary service provision

Variable	Categories	N	Prevalence (%)	95% CI	p value
Access to vet. Service	No	92	9.8	3.7-15.9	0.126
	Yes	905	6.1	4.5-7.7	
Frequency of vet. Service	Unknown	100	9.0	3.4-15.6	0.383
	Every week	81	8.6	2.5-14.7	
	Monthly	520	6.3	4.2-8.4	
	Rarely	296	5.1	2.6-7.6	
Service provider	Community livestock worker	3	0	-	0.671
	Veterinary assistant	890	6.2	4.6-7.8	
	Unknown (or not serviced)	104	8.7	3.3-14.1	

None of the cattle on which tick control was not practiced were found positive for schizonts, while for those on which tick control was practiced the prevalence was 6.5% (95%, CI=5.0-8.0). Cattle that were dipped to control ticks had a significantly higher prevalence (19.3%, 95%, CI=9.1-29.6) than those that were sprayed (5.7%, 95%, CI=4.2-7.2).

Prevalence among cattle on which tick control was done twice a week was 7.7% (95%, CI=-6.8-22.1) and 5.3 (95%, CI=3.1-5.5) among cattle on which it was done weekly. Cattle on which tick control was done once every two weeks had a prevalence of 6.8% (95%, CI=4.7-8.9) and 0% for cattle with tick control frequency of every three weeks, while cattle on which tick control was irregular the prevalence was 16.0% (95%, CI=1.7-30.4)**.** Prevalence for cattle with all year round tick control was 6.2% (95%, CI=4.7-7.7) and 12.5% (95%, CI=2.3-32.8) for cattle on which strategic tick control was practiced. (Table 6)

Table 6: Schizont prevalence according to tick control method and frequency

Variable	Categories	N	Prevalence (%)	95% CI	p value
Practice tick control	No	6	0	-	0.671
	Yes	991	6.5	5.0-8.0	
Tick control method	No tick control	6	0	-	0.671
	Chemical	991	6.5	5.0-8.0	
Method of application	Dipping	57	19.3	9.1-29.6	0.001
	Spraying	934	5.7	4.2-7.2	
Tick control frequency	No tick control	6	0	-	0.256
	Every 3 weeks	2	0	-	
	Every 2 weeks	556	6.8	4.7-8.9	
	Irregular	25	16.0	1.7-30.4	

	Every week	395	5.3	3.1-5.5	
	Twice per week	13	7.7	-6.8-22.1	
Tick control period	No tick control	6	0	-	0.256
	All year round	951	6.2	4.7-7.7	
	Seasonal	40	12.5	2.3-32.8	

Prevalence was highest among cattle with a high tick burden (18.2%, 95%, CI=8-28.4), followed by those with a medium tick burden (6.8%, 95%, CI=4.9-8.7). For those with low tick burden it was (4.8%, 95%, CI=1.3-8.3) and lastly those cattle that had no ticks it was (0.9%, 95%, CI=-3.0-4.8), (Table 7).

Table 7: Schizont prevalence based on tick burden

Variable	**Categories**	**N**	**Prevalence (%)**	**95% CI**	**p value**
Tick burden	High	55	18.2	8-28.4	<0.001
	Low	147	4.8	1.3-8.3	
	Medium	681	6.8	4.9-8.7	
	Nil	114	0.9	-3.0-4.8	

Prevalence among female owned herds was 8.8% (95%, CI=1.5-16.2), 6.4% (95%, CI=4.8-8.0) in male owned herds and 3.1% (95%, CI=-2.9-9.1) in those herds owned by institutions, (Table 8).

Table 8: Schizont prevalence in relation to livestock ownership

Variable	Categories	N	Prevalence (%)	95% CI	p value
Livestock owner	Female	57	8.8	1.5-16.2	0.591
	Institutional	32	3.1	-2.9-9.1	
	Male	908	6.4	4.8-8.0	

Prevalence among cattle from farmers that had not heard about ECF was 8.0% (95%, CI=4.6-11.5) and 5.9% (95%, CI=4.2-7.6) for cattle from farmers that had heard about ECF. Prevalence for cattle from farmers that reported not to have experienced ECF on their farms was 6.3% (95%, CI=4.0-8.6), while cattle from farmers that reported to have experienced ECF was 6.5% (95%, CI=4.5-8.5), (Table 9).

Table 9: Schizont prevalence in relation to farmer's knowledge and experience of ECF

Variable	Categories	N	Prevalence (%)	95% CI	p value
Heard of ECF	No	238	8.0	4.6-11.5	0.164
	Yes	759	5.9	4.2-7.6	
Experienced ECF?	No	413	6.3	4.0-8.6	0.501
	Yes	584	6.5	4.5-8.5	

Cattle from farms that had not recorded mortality in the previous 12 months had a prevalence of 6.0% (95%, CI=4.5-7.7), while those from farms where mortality was recorded had a prevalence of 7.6% (95%, CI=4.5-10.7), (Table 10).

Table 10: Schizont prevalence in relation to mortality record

Variable	Categories	N	Prevalence (%)	95% CI	p value
Mortality in last 12 months	No	721	6.0	4.5-7.7	0.209
	Yes	276	7.6	4.5-10.7	

Prevalence for cattle from farmers that were not willing to pay for veterinary services was 6.7% (95%, CI=-2.3-15.7) and 6.4% (95%, CI=4.9-7.9) for cattle from farmers that were willing to pay, (Table 11).

Table 11: Schizont prevalence based on farmer's willingness to pay for veterinary services

Variable	Categories	N	Prevalence (%)	95% CI	p value
Willing to pay	No	30	6.7	-2.3-15.7	0.586
	Yes	967	6.4	4.9-7.9	

4.5 FACTORS ASSOCIATED WITH CATTLE BEING POSITIVE TO *THEILERIA PARVA SCHIZONTS*

A step-wise binary logistic regression model was used to determine factors associated with cattle being positive to *T. parva* schizonts. The Hosmer and Lemeshow test was non-significant ($p>0.05$) and the Omnibus Test for Model Coefficients was significant ($p<0.05$), indicating that the model adequately fitted the data.

From the logistic regression model, variables that were determined to be significant predictors of cattle being positive to *T. parva* on lymph node examination were locality (district), frequency of tick control, previous ECF experience and the age (Table 12). Cattle from Mpongwe were 0.20 (95%, CI=0.07-0.56) times less likely to be positive for *T. parva* schizonts than cattle from Chibombo (p=0.002). Cattle on which tick control was irregular were 0.16 (95%, CI=0.05-0.46) times less likely to be positive for schizonts than those on which tick control was done once every three weeks (p=0.002). Cattle on which there was no tick control were 0.34 (95%, CI=0.11-1.05) times less likely to be positive for schizonts than the ones on which tick control was done once every three weeks (p=0.343). Cattle on which tick control was done twice a week were 0.42 (95%, CI=0.19-0.93) times less likely to be positive for schizonts than the cattle for which tick control was done once every three weeks (p=0.421). Cattle from farmers that reported to have experienced ECF were 0.44 (95%, CI=0.24-0.78) times less likely to be positive for *T. parva* than those from farmers who reported not having experienced ECF (p=0.005). Calves were 0.35 (95%, CI=0.20-0.61) times less likely to be positive for schizonts than adult cattle (p<0.001).

Table 12: Maximum likelihood estimates of the binary logistic regression model of factors for the prediction of cattle being positive for *Theileria parva* infection on lymph smear examination

Variable	Category	Odds Ratio	p-value	95.0% C.I for Odds Ratio	
				Lower	Upper
District	Chibombo	-	0.007	-	-
	Kapiri Mposhi	2.32	0.141	0.76	7.09
	Masaiti	0.00	0.994	0.00	-
	Mpongwe	0.20	0.002	0.07	0.56
Tick control frequency	Every three weeks	-	0.008	-	-
	Every two weeks	0.00	1.000	0.00	-
	Irregular	0.16	≤ 0.001	0.05	0.46
	No tick control	0.34	0.062	0.11	1.05
	Weekly	0.00	0.999	0.00	-
	Twice a week	0.42	0.033	0.19	0.93
Experienced ECF?	Yes	0.44	0.005	0.24	0.78
	No				
Age	Calves	0.35	≤ 0.001	0.20	0.61

	Adults				

CHAPTER FIVE

5.0 DISCUSSION

Few studies have previously been done in Zambia to describe the epidemiology of ECF and yet it is an important disease of livestock that continues to affect the income and livelihood of millions of people, (Fandamu *et al*., 2005a; Simuunza *et al*., 2009). The objective of this study was to establish the prevalence of ECF for cattle in Copperbelt and Central provinces and to identify the risk factors associated with its occurrence. The study used microscopic examination of lymph smears to determine the prevalence of ECF and binary logistic regression to determine the risk factors of cattle being positive for *T. parva*.

The study established that ECF was prevalent in Mpongwe, Masaiti and Chibombo districts. The difference in schizont prevalence between the two provinces was not significant. This could be because the two provinces are situated next to one another and receive almost the same amount of rainfall, although the Northern part of the Copperbelt province may receive slightly more. The activity and abundance of the adult tick vectors is dependent on the amount of rainfall (Norval *et al*., 1991; Norval *et al*., 1992). Chibombo district had the highest schizont prevalence followed by Mpongwe and Masaiti districts, while Kapiri Mposhi district did not record any schizont positive cattle. The high schizont prevalence recorded in Mpongwe district is consistent with the high morbidity and mortality observed in various areas of Mpongwe district such as Lukanga, Ntanda, Chamatete, Musofu, Mimpolombo, Ipumbu, Kashiba and Kalukwiso (Anon., 2007). The high prevalence in Mpongwe district may be attributed to the influx of settlers from Southern province which is documented to be endemic for ECF (Chizyuka *et al*., 1985; Nambota *et al*., 1994). They came to Mpongwe district because of the favourable rain patterns and fertile soils, carrying along with them their livestock that could have been

carriers of ECF (Anon., 2007) and further indicates that stock movement controls are not adequately administered in many parts of the country.

Variability in prevalence across provinces up to camp level could be attributed to high variation in spatial distribution of ECF for different areas partly due to a difference in the distribution of the vector *R. appendiculatus* (Bazarusanga *et al*., 2008; Muhanguzi *et al*., 2014) and differences in tick densities and their infection rates, parasite virulence, cattle resistance, tick control strategies, tick control infrastructure and availability of veterinary service (Tembo *et al*., 2012). Geographical factors such as rainfall, altitude, temperature and vegetation cover for the different areas may also account for the differences in prevalence across provinces, districts up to camp level as this affects the presence, growth and survival of the ticks (Fandamu *et al*., 2005a; Pegram *et al*., 1986). The results obtained in this study gave a lower prevalence than the ones determined by Simuunza *et al* (2011) in Eastern, Central and Lusaka provinces of Zambia, Fandamu *et al* (2005a) in Southern province of Zambia and Bazarusanga *et al* (2007) in a study in Uganda. However, it was higher than the prevalence reported by Tembo *et al* (2012) during a study of Mungwi District in Northern province. The reason why the prevalence estimated in this study was lower than that reported by Simuunza *et al* (2011) and Fandamu *et al* (2005a) could be that the diagnostic method used in this study is less sensitive than the ones used in the previous studies. In addition, microscopy was used to determine presence of schizonts in lymph node smears. This stage of the parasite is more visible in animals with clinical disease and may be missed in carriers (Fandamu *et al*., 2005a; Norval *et al*., 1992).

Cattle from the traditional farming systems had a higher prevalence than the ones from the commercial farming system and this may be due to inefficient tick control methods in the traditional sector compared to those in the commercial sector (Anon 2010b). Further, under the

traditional farming system, communal and open grazing is often practiced and this increases the risk of ECF challenge due to increased animal contact and higher chances of tick transmission from animal to animal (Gitau *et al*., 2000; Minjauw *et al*., 1999).

Cattle that were dipped had a higher prevalence of *T. parva* schizonts than those that were sprayed to control ticks. The reasons for this result are not clear but it may be due to inadequacies in the management and use of the dipping facilities and inadequate knowledge on drug dilution and administration as well as inadequate veterinary personnel and resources for effective monitoring and supervision of dipping activities. Further, the schizont prevalence was highest in cattle with a high tick burden which is an indication of high intensity of transmission in cattle infested by high tick numbers and underscores the need for effective tick control.

Cross breed cattle had the highest schizont prevalence, followed by local breeds and commercial breeds had the lowest. This result indicates that the different cattle breeds have different susceptibilities to ECF (Minjauw *et al*., 1999) and agrees with the findings of Oura e*t al* (2005) and Tembo *et al* (2012) who reported a higher ECF prevalence among cross breeds compared to local breeds. The high schizont prevalence among local breeds might be related to the traditional management system which local breeds of cattle are subjected to, where tick control is not consistent. Therefore despite being more resistant local breeds had schizont prevalence second to cross breeds and this result agrees with the findings of Minjauw *et al* (1999) and Simuunza *et al* (2011), who reported a higher prevalence among local breeds. This finding is contrary to that of other studies which have lower prevalence in these breeds of cattle due to high resistance (Gitau *et al*., 2000; Salih *et al*., 2007b). Oura *et al* (2005) reported that commercial breeds are more susceptible to ECF. Results obtained in this study may

indicate effective tick control in the commercial breeds of cattle which are predominantly owned by commercial farmers.

Calves had a higher schizont prevalence than adult cattle which is in agreement with the findings of Salih (2007b), who reported high infection of *T. parva* among calves compared to adults. The high prevalence of schizonts among calves, when compared to adult cattle may indicate the susceptibility or low immunity of indigenous calves especially between one month and six months to ECF (Oura *et al*., 2005). Salih (2007b) also reported that calves are at high risk when subjected to infection for the first time with high mortalities in calves of up to 6 months old.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

1. The study established that ECF is prevalent in both the Copperbelt and Central provinces.

2. The risk factors that were identified to be associated with ECF in this study were the district, frequency of tick control, tick burden, age and previous ECF experience.

6.2 RECOMMENDATIONS

1. Concerted efforts from all stake holders must be put in place to control ticks and prevent the spread of ECF in Central and Copperbelt provinces. This should include construction of communal dip tanks and compulsory dipping as outlined in the Animal Health Act, 2010.
2. Disease surveillance and administration of livestock movement control by the Department of Veterinary Services must be improved.
3. There is need for further studies to ascertain the extent of ECF in the Copperbelt and Central provinces using more sensitive tests such Polymerase chain reaction (PCR) and Loop-mediated isothermal amplification test (LAMP).

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8.0 APPENDIX

8.1 Questionnaire

GOVERNMENT OF THE REPUBLIC OF ZAMBIA

Ministry of Livestock and Fisheries Development

A CROSS SECTIONAL STUDY FOR DETERMINING THE PREVALENCE AND RISK FACTORS FOR EAST COAST FEVER IN ZAMBIA

Confidentiality of information supplied

The purpose of this questionnaire is to gather information on the prevalence and risk factors of East Coast Fever in Zambia and therefore all the information provided by respondents will be used only for that purpose.

The information to be collected will be useful in planning and implementing disease control strategies for East Coast Fever and recommend Disease Control measures. This will help increase the livestock figures and positively contribute to the national economy.

A. Identification Information

Identification Number_____

Date of interview_____

Interviewer_____

Province_____

District_____

Camp_____

Chief_____

Village/Farm_____

Crush Pen_____

Name of Owner_____ Gender_____

Number in Household_____ Person Interviewed_____

Number_____

Breeds_____

Farming System:

Commercial……..☐

Traditional……..☐

B. Herd Structure and Size

Category of Cattle	Age	Number
Oxen	Above 3 years	
Bulls	Above 3 years	
Cows	Above 3.6 years	
Heifers	2.6-3.5 years	
Steers	10 months -2.5 years	
Calves	Upto 9 months	
Total		

C. Veterinary Services

Q. 1.

Do you readily access veterinary services? **If the answer is No go to question 4**.

No……☐

Yes……☐

Q. 2.

Name the provider of Veterinary Services in your area.

1. Veterinary Department……☐
2. Community Livestock worker……☐
3. NGO (give name)……☐
4. Others (Specify)_____..... ☐

Q. 3.

Comment on the quality of Veterinary Service you receive.

1. Very good……☐
2. Good……☐
3. No idea……☐
4. Poor……☐
5. Very poor……☐

Q. 4.

Give a reason for your answer to Question 3

……
……

Q. 5.

If you do not receive veterinary services give reasons

……
……
……
……

Q. 6.

What is the distance to the nearest veterinary service provider?

……..

D. HERD DYNAMICS

a. Animals Received

Q. 1.

State the number of calves born to your cows in the last 12 months……

Q. 2.

Did you buy any of the under listed categories of animals in the last 12 months?

If so state the source.

Category of Cattle	Age	Number	Origin/Source
Oxen	Above 3 years		
Bulls	Above 3 years		
Cows	Above 3.6 months		
Steers	2.6 -3.5 years		
Heifers	10 months-2.5		
Calves	Upto 9 months		
Total			

Q. 3.

Did you receive as a gift any of the under listed categories of animals in the last 12 months? If so state the origin.

Category of Cattle	Age	Number	Origin/Source
Oxen	Above 3 years		
Bulls	Above 3 years		
Cows	Above 3.6 years		
Steers	2.6- 3.5 years		
Heifers	10 months - 2.5 years		
Calves	Up to 9 months		
Total			

b. Animals going out

Q. 1.

Did you sale or give out any of the following categories in the last 12 months? If yes, state reason for sale and the destinations.

Category of Cattle	Age	Number	Reason	Destination
Oxen	Above 3 years			
Bulls	Above 3 years			
Cows	Above 3.6			
Steers	2.6-3.5 years			
Heifers	10 moths-2.5 years			
Calves	Upto 9 months			

Q. 2.

If reason for selling of animals in (Q. 1.) was disease, state the disease suspected and give clinical signs observed. _____

Q. 3.

Was the disease confirmed by Veterinary Authorities?

Q. 4.

Did you give out as a gift any of the following categories of animals in the last 12 months? If so state the reason and destinations.

Category of Cattle	Age	Reason	Destination
Oxen	Above 3 years		
Bulls	Above 3 years		
Cows	Above 3.5 years		
Steers	2.6- 3.5 years		
Heifers	10moths-2.5 years		
Calves	Upto 9 months		

Q. 5.

If reason for giving out animals in (Q. 4) above was disease state the disease or clinical signs seen_____
_____
_____

Q. 6.

Was the disease confirmed by Veterinary Authorities……..

E. Slaughters

Q. 1.

Did you slaughter any of the following categories of animals in the last 12 months?

Category of Cattle	Age	Number slaughtered	Month	Reason for slaughter
Oxen	Above 3 years			
Bulls	Above 3 years			
Cows	Above 3.5 years			
Steers	2.6-3.5 years			
Heifers	10months-2.5 years			
Calves	Upto 9 months			
Total				

Q. 2.

If reason for slaughter in (Q.1.) was disease or clinical signs observed and post mortem lesions seen.

Category of Cattle		Clinical Signs	Post mortem Lesions	Disease Suspected
Oxen	Above 3 years			
Bulls	Above 3 years			
Cows	Above 3.6			
Steers	2.6-3.5 years			
Heifers	10 months-2.5 years			
Calves	Upto 9 months			

Q. 3.

Was the disease confirmed by Veterinary Authorities……

F. Mortalities

Q. 1.

Did you record mortalities in the last 12 months?

No……..☐

Yes……..☐

Q. 2.

If answer to question 1. Is "yes", what is the suspected cause of mortality?

……

Q. 3.

How many of each category of animals died in the last 12 months? In which period did these deaths occur? (Tick under the appropriate period in the table)

Category of Cattle	Age	Mortalities	Jan-March	April-June	July-Sept	Oct-Dec
Oxen	Above 3 years					
Bulls	Above 3years					
Cows	Above 3.6 years					
Steers	2.6 -3.5 years					
Heifers	10 months-2.5 years					
Calves	Upto 9 months					
Total						

G. Tick Control

Q. 1.

Do you think ticks are a problem in your area?

No……☐

Yes……☐

Q. 2.

Do you control ticks on your animals

Yes……☐

No……☐

Q. 3.

If your answer to Q. 2. Is "Yes", which method of tick control do you use?

……

Q. 4.

If chemicals are used to control ticks, how are they applied?

……
……..

Q. 5.

If your answer to Q. 2. Is "No" what are your reasons?

……
……
……

Q. 6.

What is your tick control frequency?

1. Once a week……☐
2. Once in 2 weeks……☐
3. Once in 3 weeks……☐
4. Once in a month……☐
5. Irregular……☐

Q. 7.

Is your tick control

1. All year round……☐

2. Restricted to specific periods of the year……☐

Q. 8.

If your answer to Q. 7. Is 2 (Restricted), why do you restrict tick control to specific periods of the year?
……

Q. 9.

If your tick control is restricted to a period or periods, which period is this?

…….

H. East Coast Fever

Q. 1.

Have you heard or experienced a disease called ECF in your herd /area?

No……☐

Yes……☐

Q. 2.

When did you first hear of ECF in your herd/area?

1. 10 years ago……☐

2. 5 years ago……☐

3. 2 years ago……☐

4. Last year (2010)……☐

Q. 3.

When did you last experience ECF?

……

Q. 4.

What clinical signs did you observe?

_____

_____

_____

_____

Q. 5.

Was the disease confirmed by Veterinary Authorities?

No……☐

Yes……☐

Q. 6.

Which category/age group of animals was affected by ECF?

1. Calves……☐
2. Steers and Heifers……☐
3. Cows and Bulls……☐
4. Oxen……☐
5. All age groups……☐

Q. 7.

Which category/age group of animals died more of ECF?

1. Calves (_____)……☐
2. Steers and Heifers (_____)……☐
3. Cows and Bulls (_____)……☐
4. Oxen (_____)……☐
5. All age groups (_____)……☐
6. None……☐

Q. 8.

Which period of the year is ECF a problem in your area?

……

Q. 9.

Do you treat animals against ECF?

No……☐

Yes……☐

Q. 10.

If “yes” to (Q. 9.) what drugs do you use?

_____
_____
_____

Q. 11.

If No to (Q. 10) above, what could be the reason(s) for not treating?

_____
_____
_____

Q. 12.

Are you prepared to pay for Veterinary services in order to protect your animals?

No……☐

Yes……☐

H. Other Relevant Information.

Any other relevant information found by interviewer but not captured in the questionnaire:

……
……
……
……
……
……
……
……
……