# MOLECULAR DIVERSITY OF *THEILERIA PARVA* IN KILOSA DISTRICT, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF ... REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.



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## ABSTRACT

*Theileria parva* is an apicomplexan protozoan parasite causing East Coast fever in Eastern and Central Africa. A study was conducted between February and May 2012 in six villages in Kilosa district in Tanzania to determine the diversity of the *T. parva* parasite circulating in the district. Also data of the disease cases reported to Veterinary investigation centres (VIC's) and compiled by the Ministry of Livestock Development and Fisheries, were analyzed to gain insight into the current status of ECF in Tanzania. To determine the diversity, convectional Polymerase Chain Reaction (PCR) was used to amplify antigenic genes coding for sporozoite surface protein (p67) using DNA extracted from cattle blood samples naturally infected with *T. parva* and *T. parva* isolated from ticks. A total of 100 cattle blood and 95 tick samples were collected. PCR products (banding Patten) were analyzed to characterize *T. parva* parasite that occur in cattle and ticks. Analysis of the PCR products suggested the existence of two groups of *T. parva* parasites with band sizes 800 and 900 bp respectively. This study has found that there is *T. parva* allele in Kilosa that is not in the imported ECF trivalent vaccine ("Muguga cocktail") currently in use. Three year records (2008-2010) of the disease indicate lower prevalence of ECF compared to prevalence reported by other previous studies. The low prevalence observed in this study suggests that reports from Vic's cannot give good estimates of ECF prevalence due to under reporting, poor diagnosis, poor records keeping and others in the field. It was recommended that more research has to be conducted so as to establish the complete list of parasite circulating in Kilosa district and the region.

## DECLARATION

I **Leila Robin** 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 in any other institution.

..... 04/10/2023 .....

**Leila Robin** **Date**

(Msc Student)

The above declaration is confirmed by,

..... .....

Elikira N. Kimbita **Date**

(Supervisor)

## COPYRIGHT

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## LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| %: | Percentage |
| °C: | Celsius temperature |
| AEZ: | Agro-ecological Zone |
| Bp: | Base pair |
| COSTECH: | Commission for Science and Technology |
| DNA: | Deoxyribo-nucleic acid |
| ECF: | East Coast Fever |
| ELISA: | Enzyme linked immunosorbent assay |
| FAO: | Food and agriculture organization |
| IFAT: | Indirect fluorescent antibody test |
| ITM: | Infection and treatment method |
| MLD: | Ministry of Livestock Development |
| MLDF: | Ministry of Livestock Development and Fisheries |
| PCR: | Polymerase Chain Reaction |
| PIM: | Polymorphic immunodominant molecule |
| RFLP: | Restriction fragment length polymorphism |
| VIC: | Veterinary Investigation Centre |
| RBC: | Red blood cell |
| SUA: | Sokoine University of Agriculture |
| TVLA: | Tanzania Veterinary Laboratory Agency |
| TBD: | Tick Born Disease |

# CHAPTER ONE

## 1. 0 INTRODUCTION

### 1.1 Background

Theileriosis caused by *Theileria parva* is one of the important disease constraining the development of livestock industry in Tanzania and causes mortality and performance losses particularly in highly susceptible taurine breeds and their crosses (Kivaria, *et al*, 2007). Economically, it is the most important tick-borne disease (TBD) in East and Central Africa, in particular *Theileria parva* infection, generally known as East Coast fever (ECF) (Mbassa *et al.*, 2009a). Although *T. parva* causes other forms of theileriosis including January disease in Zimbabwe and Corridor disease in South Africa, East Coast fever which is found in East Africa countries remains as the major form of theileriosis in Tanzania. The actual ECF losses are caused directly by death of animals, whereby the mortality may exceed 90% (Sibeko, 2009) or indirectly through the costs of control and reduced production capability (Mbassa *et al.*, 2009a). Losses due to ticks and the disease transmitted by ticks in Tanzania is estimated to reach US$ 364 million annually, mainly due to death of more than 1.3 million cattle; 68% caused by theileriosis, 13% by anaplasmosis, 13% by babesiosis and 6% by cowdriosis (Kivaria, 2006a; Kivaria *et al.*, 2007).

In susceptible cattle, ECF is characterised by enlargement of superficial lymph nodes, a sustained fever and diverse clinical signs associated with invasion of non-lymphoid tissues with parasitized lymphoblasts. ECF causes high mortality with death occurring approximately three weeks after infection, mainly as a result of severe pulmonary oedema. The early ECF control attempts were based on tick control (Dolan, 1999) largely through application of acaricides by dipping, use of spray race, or by hand spraying.

Recently, "pour on" or "spot on" formulations have been introduced (Fandamu, 2005). Infection and treatment method (ITM) is another method of controlling ECF through immunization basing on injecting *T. parva* sporozoites of infected *Rhipicephalus appendiculatus* ticks and simultaneously treating the animal with 30% oxytetracycline (Di Giulio *et al.*, 1997; Mbassa *et al.*, 1998a; Mbassa *et al.*, 1998b). The most widely used immunizing stock is the 'Muguga cocktail' (Radley *et al.*, 1975) composed of *T. parva* Muguga, Kiambu 5 and Serengeti-transformed stocks. However, the ITM is facing controversies concerning not only its economic implication, as one has to continue using acaricide against other tick-borne diseases (Kivaria *et al.*, 2007), but also the duration of immunity (Mbassa *et al.*, 2009b).

Control of theileriosis in East Africa has proven to be difficult principally because of the lack of epidemiological information. A gradient-based *T. parva* prevalence has been reported from a single district of Kenya (Gitau *et al.*, 2000), the Eastern province of Zambia (Billiouw, 2005), Mbale district in Uganda (Rubaire-Akiiki *et al.*, 2004), and from Comoro (Geysen *et al.*, 2004). In Tanzania, what we have now is a collection of epidemiological information and assumptions from work done long ago as a result, in most cases control efforts have not been commensurate to the magnitude of the disease problem.

## 1.2 Problem statement and justification

In Tanzania, the current status on the prevalence of theilerial infection is unknown although ECF is considered to be major threat to the cattle industry. Furthermore, vaccination against ECF does not seem to produce the expected results since immunized cattle have been observed to succumb to ECF.

Vaccination protection engendered by ITM is strain specific, but the question remains on our local parasite strains are they genetically similar to 'Muguga cocktail' vaccine? Knowledge of *T. parva* strains will provide the necessary baseline information on the strain structure of the parasite in the field; this will assist in making decision on whether to continue using the vaccine or develop our own vaccine. Furthermore, prevalence will reveal the proportion of animals that get infected with ECF in cattle population.

There is therefore a need to establish the current status of the disease and *T. parva* parasites that are circulating in cattle in the field, so as to plan rational control programme against ECF.

Thus, the present study will report on the prevalence of ECF, in association with the molecular characterization of *T. parva* parasite isolated from cattle and ticks in Kilosa district in Morogoro region Tanzania using *T. parva* P67 gene banding pattern.

## 1.3 OBJECTIVES

### 1.3.1 General objective

The overall objective of this study is to determine the diversity *Theileria parva* parasite in Kilosa district.

### 1.3.2 Specific objectives

i. To identify *Theileria parva* alleles that exist in cattle in Kilosa, Morogoro

ii. To assess prevalence of ECF infections in Tanzania.

iii. To compare *Theileria parva* alleles in cattle and ticks.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The epidemiology of East Coast Fever

#### 2.1.1 The Parasite: *Theileria parva*

*Theileria parva (T. parva)* is haemoprotozoan parasite that is responsible for causation of East Coast fever (ECF) of cattle and transmitted by the tick *Rhipicephalus appendiculatus (R. appendiculatus)* (Billiouw *et al.*, 2002; Geysen *et al.*, 1999; Minjauw *et al.*, 1997).

By far it is the most pathogenic and economically significant *Theileria sp.* in eastern, central and southern Africa (Norval *et al.*, 1992). The waterbuck (*Kobus deffasa*) and buffalo (*Syncerus caffer*) are the natural reservoir hosts of *T. parva* (Stagg *et al.*, 1994).

#### 2.1.2 Vectors of *Theileria parva*

The three-host metastriate ticks *Rhipicephalus appendiculatus* and its closely related *Rhipicephalus zambeziensis* are principle field vectors and to a lesser extent *Rhipicephalus duttoni* (Madder *et al.*, 2003). Being highly susceptible to *T. parva* infection, *R. appendiculatus* ticks are efficient transmitters of this disease 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). Climate condition, vegetation and host availability are the factors known to determine the distribution of *R. appendiculatus/R. zambenziensis* which in turn determine the distribution of the parasite itself (Lawrence, 1991). The vector ticks are very numerous in tropical areas, particularly East Africa. This problem is compounded by communal pastoral grazing of livestock and sharing of pastures between domestic and wild animals. The models for predicting the impact of climate change on tick-borne

diseases and tick distribution predicts the increase in the number of cattle at risk becoming infected by *Theileria parva* (Olwoch *et al.*, 2008).

### 2.1.3 Hosts of *Theileria parva*

Cattle, *Bos indicus*, *Bos taurus*, African buffalo, *Syncerus caffer* (Zweygarth *et al.*, 2009) waterbuck, *Alcelaphus buselaphus*, elands, *Taurotragus oryx* Egyptian buffalo, *Bubalus bubalus*, roan antelope (Oosthuizen *et al.*, 2009) and sable antelope are known to be the hosts of *Theileria pava*. The water buffalo (*Syncerus caffer*) is the natural reservoir host of *T. parva* and presence of the parasite in this animal does not result in disease (Pellé *et al.*, 2011). A study conducted in livestock-wild life 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.*, 2008, 2009a).

### 2.1.4 The life cycle of *Theileria parva* in the mammalian host

Transmission of *T. parva* is only achieved by infected nymph or adult ticks. The infected nymph or adult transmit infection during feeding when sporozoites in its salivary glands have matured (Stagg et *al.*, 1981). The sporozoites enter lymphocytes and differentiate into schizonts inducing a lymphoproliferative disorder (Stagg *et al.*, 1980). The sporozoites entry process is not orientation-specific as has been observed with other apicomplexan parasites (Shaw, 2003). In the lymphocytes, the schizonts later differentiate into merozoites that invade red blood cells (RBCs) and develop into piroplasms (Conrad, Denham & Brown 1986).

***In the vector tick***

Piroplasms in the erythrocytes of the mammalian host are ingested during tick feeding. The sexual stage of development of *T. parva* occurs in the gut of the tick (Melhorn and

Schein, 1984). In the tick gut lumen gametogenesis and fertilization take place resulting in the production of a zygote. The zygote invades the gut cell and remains there throughout the tick moulting cycle and develops into a single motile kinete. Kinete escape the gut cell and invade the salivary glands through the haemolymph. The parasite remains in the salivary gland until transmitted to another mammalian host when the resulting post-moult nymph or adult feeds. The tick feeding initiate rapid sporozoite development and infective sporozoites are released during later stages of feeding (Norval *et al.*, 1992).

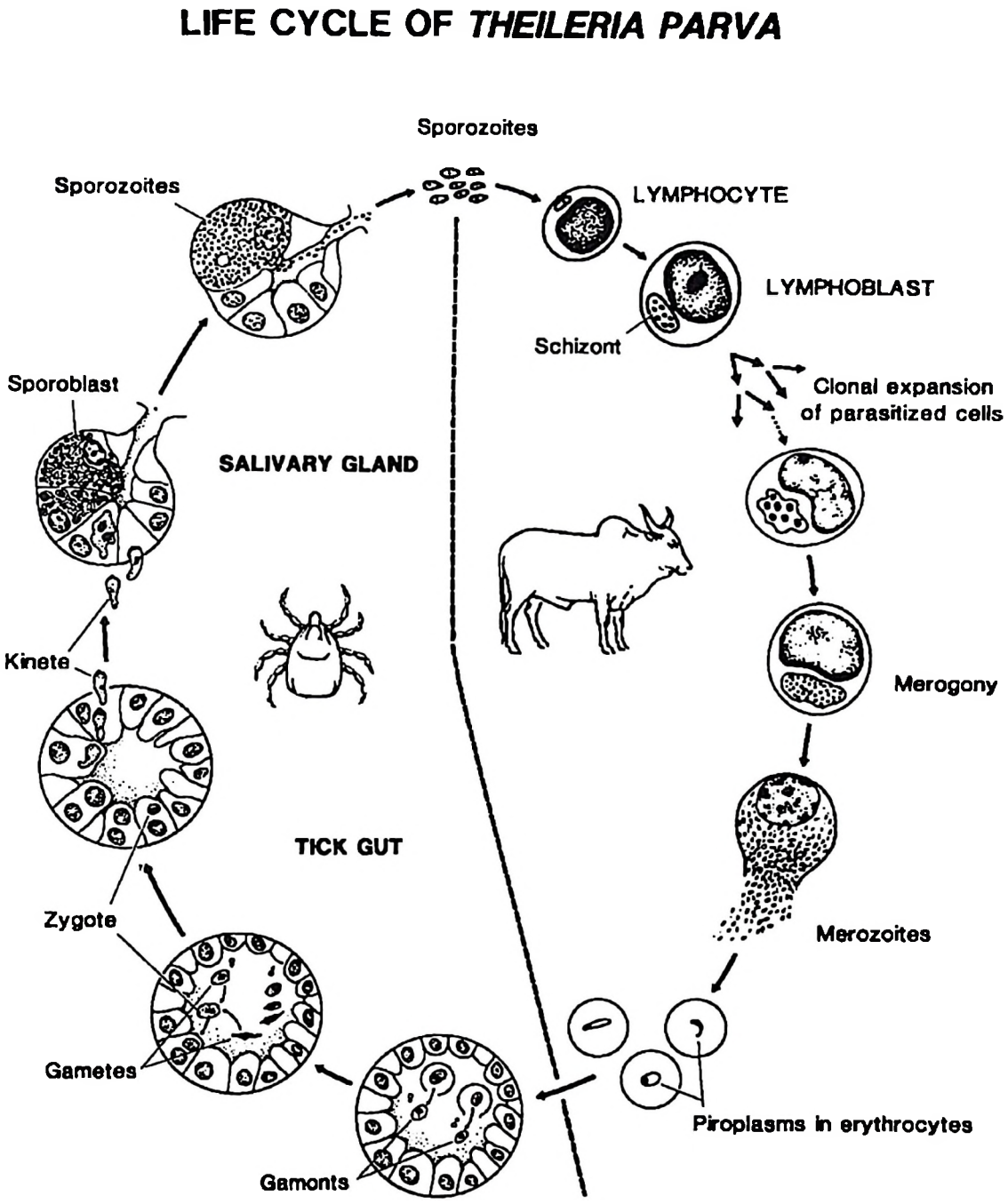


**Figure 1: The lifecycle of *Theileria parva***

Source: Bazarusanga, *et al.*, 2007.

## 2.2 Major disease syndromes caused by *T. parva* infection

East Coast fever, Corridor disease and January disease are disease syndromes caused by *T. parva* infection in cattle. These disease syndromes results from tick transmission of the parasite from infected cattle or wild animal particularly buffalo to susceptible cattle. Cattle-to-cattle transmission results in East Coast fever and January disease while buffalo-to-cattle transmission of *T. parva* results in Corridor disease.

### 2.2.1 East Coast fever (ECF)

ECF is a fatal disease of cattle caused by the cattle-derived strains of *T. parva* (formerly known as *T. parva parva*). It is particularly severe in exotic *Bos Taurus* cattle, but it also causes high mortality in young zebu (*Bos indicus*) cattle in pastoralist systems (Di Giulio *et al.*, 2009). The parasite is transmitted through tick infection from infected cattle to susceptible cattle. The type of stock of *T. parva* parasite involved can modify the course of the clinical disease (Mbogo *et al.*, 1996).

The pathology associated with East Coast fever arises from invasion of lymphoid and nonlymphoid tissues by parasitized lymphoblasts (Katzer *et al.*, 2007; McKeever, 2007), Death of infected cattle is a result of this lymphoproliferation (Rocchi *et al.*, 2008), membrane 'leakage' and infiltration of lymphoid and non-lymphoid organs by parasitized lympoblasts interfere with the normal function of these organs, the lungs in particularly, resulting in the death of the animal (Ndungu *et al.*, 2005). Normally there are haemorrhages in mucous membranes and heart, subcutaneous and pulmonary oedema, froth in lungs, trachea and nostrils, kidney infarcts and lymphocyte deaths (Mbassa *et al.*, 2006). There is serous fluids effusion in body cavities, necrosis of Peyer's patches and hydropericardium. Haemorrhages and ulceration may be seen throughout the

gastrointestinal tract particularly in the abomasum and small intestine, and the lungs show interlobular emphysema.

There is a marked variation in the susceptibility of cattle to infection; a proportion of animals may recover, however the recovery process might be prolonged. Recovered animals may remain emaciated and unproductive for months. ECF is controlled through tick control using acaricides, immunization by infection and treatment method as well as using chemotherapy with antitheilerials which are available in the market. Early diagnosis is important in order to have successful treatment.

### 2.2.2 Corridor disease

Corridor disease is another form of theileriosis which was first diagnosed in 1953 in a corridor of land between Hluhluwe and iMfolozi Game Reserve in South Africa, hence the name Corridor disease (Neitz *et al.*, 1955). It occurs in southern and east Africa especially in areas where there is contact between cattle and infected buffalo in the presence of *Rhipicephalus appendiculatus, Rhipicephalus zambeziensis* or *Rhipicephalus duttoni* (Blouin and Stoltsz, 1989). The disease is caused by infection with buffalo-derived strains of *T. parva* (formally known as *T. parva lawrencei*). Transmission of the disease occurs in cattle sharing the same grazing ground with infected buffalo in the presence of the tick vector, resulting in buffalo-to-cattle transmission.

Corridor disease is an acute, usually fatal disease of cattle resembling ECF. The pathogenesis and pathology of Corridor disease are very similar to those of ECF (Oura *et al.*, 2011), although buffalo derived *Theileria parva* infection which result into Corridor disease is characterized by low schizont parasitosis and piroplasms parasitaemia (Pellé *et al.*, 2011). 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 sign (Lawrence *et al.*, 1994a). Corridor disease is generally regarded as self-limiting (Oura *et al.*, 2011) as cattle usually die in the acute stage before the parasite develops into erythrocytic piroplam stage which might be picked up by the tick (Uilenberg, 1999). Corridor disease is still a serious threat in areas where there are common grazing grounds between cattle and infected buffalo and where the tick vectors, *Rhipicephalus appendiculatus*, *Rhipicephalus zambeziensis* and *Rhipicephalus duttoni* occur (Uilenberg, 1999). 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).

### 2.2.3 January disease (Malignant Rhodesian theileriosis/ Zimbabwean theileriosis)

January disease is the type of theileriosis common in Zimbabwe which emerged after eradication of ECF in the country. The name January disease is attributed to the strict seasonality of the disease occurrence which is between December and March, coinciding with the seasonal activity of adult *R. appendiculatus*. It is an acute, frequently 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).

## 2.3 The epidemiological status of ECF

Disease incidence and prevalence are the minimum data sets used to assess the epidemiological status of ECF. Adequate data on disease incidence are difficult to obtain and need prospective studies with close animal supervision (Norval *et al.*, 1992). The common method used to detect a case definition of ECF under field conditions is based on a combination of clinical signs and the demonstration of schizonts/piroplasm in microscopic examinations. However, reports from several countries were based on the

presence of piroplasm alone which has confused the disease incidence data. It is for instance not possible to discriminate between piroplasms of *T. parva* from those of other *Theileria spp.* which frequently occur in mixed infections in the field (Norval *et al.*, 1992). In addition, detection of piroplasm alone may merely indicate that an animal is a carrier. A long lasting carrier state following recovery gives rise to low numbers of erythrocytes infected with *Theileria* piroplasm (Neitz, 1957, Paling *et al.*, 1991) and detection of piroplasms in stained-blood smears may be difficult and impractical for large-scale surveys.

Recently, the prevalence and case fatality rate due to East Coast fever (ECF) were estimated in 1402 dairy cattle in 87 small herds in the Dar es Salaam region of Tanzania, using a capture-recapture method. Following application of the capture-recapture method, the estimated number of clinical cases and deaths was 625 (CI (95%) 617-633) and 401 (CI (95%) 384-418), respectively. The respective prevalence and case fatality rates were 45% (CI (95%) 41-48%) and 64% (CI (95%) 60-68%) (Kivaria and Noordhuizen, 2010)

### 2.4 Detection of *Theileria parva* infection

Diagnostic tests currently used for detection of *T. parva* infection include conventional, serological and molecular methods.

#### 2.4.1 Conventional methods

Conventional diagnosis of *Theileria* parasites has mainly been based on microscopic examination of blood and lymph node smears for the presence of the parasites which could be differentiated from other blood parasites by morphological and staining properties. The method is routinely used for detection of Babesia, *Theileria* and *Anaplasma* species in acutely infected animals (Morzaria *et al.*, 1999). This method is the

method of choice for early and rapid treatment of the disease. However, it has major limitations in that *T. parva* schizonts and pirolasm are difficult if not impossible to differentiate from those of other *Theileria* species which may occur simultaneously within the same blood sample (Norval *et al.*, 1992). One important epidemiological limitation is that microscopic examination is less sensitive in detecting piroplasm in carrier animals. Following recovery from ECF, the parasitaemia in carriers is often below the threshold of detection by microscopy.

### 2.4.2 Serological methods

There have been a variety of serological tests described for *T. parva* piroplasm antigens. These include capillary tube agglutination (CA) (Ross and Lohr, 1972), indirect immunofluorescent antibody (IFA) test (Burridge, 1971), indirect hemagglutination assay (IHA) (Duffs and Wagner, 1974) and Enzyme-linked immunosorbent assay (ELISA) (Katende *et al.*, 1998). Although the sensitivity and specificity of the ELISA surpasses that of IFA test (Katende *et al.*, 1998), the IFA test remains widely used in the diagnosis of *Theileria* parasites and has also been the main tool in many assessments of endemic stability of *T. parva* infections.

### 2.4.3 Molecular techniques

Detection of *T. parva* infections in carrier animals has always been a challenge when using parasitological and serological methods. Until recently, experimental tick transmission of the parasite of infected animals to susceptible animals was the definitive method of a determining a carrier state. However this approach is expensive and time consuming and can also be intermittent (Dolan, 1986). The advent of molecular diagnosis has led to the discovery of molecular techniques ranging from the classical single polymerase chain reaction (PCR) to more sophisticated techniques based on the DNA

probes. Their use in diagnosis has improved the sensitivity and specificity that previous diagnostic tests lacked over the years.

*PCR-based RFLP assays*

Recently, semi-nested Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) assays based on the *T. parva* p104 and 18S rRNA genes have been used for detection of *T. parva* infections (Geysen, 2000; Bazarusanga *et al.*, 2007; DeDeken *et al.*, 2007). Although the 18S rRNA-based assay is effective in detecting *T. parva* DNA, the addition restriction enzyme digestion, which often requires overnight incubation makes this assays time-consuming and laborious. Therefore these assays suffer the same limitation as the PCR-based hybridization assays.

*Real-time PCR assay*

Real-time PCR has greatly improved molecular detection and diagnosis of organisms belonging to the same genus (Nicolas *et al.*, 2002; Kares *et al.*, 2004). This technique allows not only the accurate detection and quantification of specific DNA in various biological samples but also allows differentiation of species or strains of several medically important pathogenic organisms by melting curve analysis of fluorescent hybridization probes (Nicolas *et al.*, 2002). The use of LightCycler (Roche Diagnostics) allows fast real time monitoring of a PCR, where amplification and detection can be accomplished in one closed capillary tube, which minimizes contamination problems.

## 2.5 Impact of treatment and Control methods on the epidemiology of *Theileria parva*

### 2.5.1 Tick control

Theileriosis has mainly been controlled by means of tick control. Tick control was first implemented in southern Africa and has been achieved through various combinations of

pasture spelling, control of cattle movement and acaricide application (Dolan, 1999). Pasture spelling was abandoned as it was found impractical because of the lengthy periods this method involved (18months). Cattle movement control and acaricide application were retained, and were made compulsory through vetirinary legislation in southern African countries to which *T. parva* had spread (Norval *et al.*, 1992). However, the rapid raising costs of acaricides has led to the development of other control methods, including vaccines against ticks, slow release devices, more efficient means of topical application acaricides, manipulation of hybrid sterility between closely-related tick species and the use of pheromones to disrupt mating or to attract ticks and so improve the efficiency of acaricide treatment. Other methods including biological tick control using predators and pathogens, tick-killing or repelling plants, habitat modification and resistant hosts have been studied and, in some instances, tested but have not yet been implemented on a large scale (Norval *et al.*, 1992). Development of resistance from using acaricides for maintaining effective tick control remains a major concern (Dolan, 1999)

### 2.5.2 Immunization

Immunization by the method of infection and treatment became a reality when sporozoite stabilates were produced which allowed cattle to be infected with a predetermined dose (Cunningham *et al.*, 1973). Tick stabilates are now available for *T. parva, T. annulata and T. taurotragi.* The simultaneous administration of stabilate and long-acting tetracycline has made the infection and treatment a practical method.

The main epidemiological problem with this method of immunization has been the *T. parva* strain differences in the field (Radley *et al.*, 1975). There was hope that the Muguga Cocktail, a combination of three stocks (*T. parva* Muguga, *T. parva* Kiambu 5 and *T. parva* Serengeti (transformed)), would be a universal stabilate and would induce a broad protection in immunized animals. However, the Muguga triple cocktail vaccine failed to

protect against Corridor disease (Radley *et al.*, 1979). Since then many countries have been cautious about using foreign stocks because of persistent carrier infections and introduction of new strains into an area.

The results of a study by Geysen *et al.* (1999) indicate that in southern Zambia parasites with a different genotype from that of local parasites, introduced as part of the immunisation campaign in the late 1980s and early 1990s, have become established and are causing disease. On the other hand, there appears to be some evidence of homogeneity of the *T. parva* stocks in many areas of Africa (Irvin *et al.*, 1989; Mutugi *et al.*, 1990a, 1990b) and some more broadly immunizing stocks such as *T. parva* Boleni from Zimbabwe and *T. parva* Marikebuni from Coast Province in Kenya, may give good protection against field challenge. An in vitro method of determining whether one stock of *T. parva* will protect against another is urgently needed to avoid the expensive and time consuming cross-immunity experiments (Norval *et al.*, 1992).

### 2.5.3 Chemotherapy

Three effective compounds are available for ECF: halofuginone, parvaquone and buparvaquone. The drugs are expensive and must be administered early in disease. There are no reports of resistance to the drugs but they are only on the market since the mid-80s and therefore only limited selection pressure has been exerted.

ECF-infected cattle that have been treated and recover remain carriers of *T. parva*, depending upon the parasite, for extended periods (Dolan 1986). The widespread use of chemotherapy has increased the number of carriers. Parasites are surviving treatment and the threat of resistance cannot be denied since these are the parasites that eventually will be transmitted by recovered cattle (Dolan, 1999). In South Africa the use of chemotherapy

was banned because of the risk of the carrier state developing in cattle infected with buffalo-derived *T. parva* (Potgieter *et al.*, 1988).

### 2.6. Molecular characterization of *Theileria parva* stock

The PCR methods have proven to be able to characterize and distinguish *T. parva* from multiple *Theileria spp* infections in field samples (Geysen *et al.*, 1999, Ogden *et al.*, 2003).

Characteristic and unique DNA banding patterns have been detected in several *T. parva* stocks. Size polymorphisms displayed by *T. parva* antigen genes, PIM, p104, p150 and p67, have been used to develop several molecular tools for characterization of *T. parva* stocks, exploiting the variable regions of these genes (Geysen *et al* ., 1999, Bishop *et al* ., 2001).

Characterization of the p67 gene sequence has revealed the presence of 129 bp deletion in the central region in cattle-derived *T. parva* isolates and this deletion is not present in buffalo-derived parasites in East Africa (Nene *et al.*, 1996; Nene *et al.*, 1999). Consequently it has been assumed that all cattle-derived isolates have the deletion while buffalo-derived isolates lack the deletion.

The cattle-derived *T. parva* stocks, Muguga, Marikebuni and Boleni characterized by Nene *et al.* (1996) all had the same p67 allele with a 129 bp deletion while there was no deletion in central region of the gene in buffalo-derived stock.

### 2.7. Four p67 alleles reported in East Africa

In a recent study, size differentiation and sequence variation of the central region of the *T. parva* antigen gene, p67, were used to characterize South African *T. parva* field samples

(Sibeko *et al.*, 2010). Four alleles were revealed, alleles 1 and 2 identical to those previously detected in East Africa (Nene et al 1996), cattle-derived isolates associated with East Coast fever (ECF) with a 129 bp deletion in the central region (allele 1), compared to buffalo-derived isolates with no deletion (allele 2). Other two novel alleles include, one with a different 174 bp deletion (allele 3), the other with a similar sequence to allele 3 but with no deletion (allele 4).

Sequences characteristic of allele 1 and 2 were obtained from the 0.9 and 1.1 kb amplicons, respectively, and allele 3 and 4 sequences were obtained from the 0.8 and 1.0 kb PCR products, respectively.

### 2.8. Overview on East Coast Fever (ECF) in Tanzania

Tanzania has the third-largest cattle population in sub-Saharan Africa, estimated at 19.2 million, with indigenous cattle accounting for more than 98% (FAO Livestock statistics, 2009). The agro-pastoral and pastoral livestock sector accounts for 70% of the national herd. In 1997, the overall costs of ticks and tick-borne diseases amounted to US$ 64.7 million and mortality associated with ECF resulted in losses of US$ 35.1 million (Mcleod and Kristjanson, 1999). ECF is responsible for up to 70% of deaths in 6–8-month-old calves (Melewas *et al.*, 1999, Homewood *et al.*, 2006, Lynen *et al.*, 2006) and represents a major constraint to the livelihoods of pastoralists and agro-pastoralists.

#### 2.8.1 Physical characteristics of Tanzania

Tanzania is located on the eastern coast of Africa south of the equator between latitudes 1° 00' S and 11° 48' S and longitudes 29° 30' E and 39°45'. Eight countries – Kenya and Uganda in the north, Rwanda, Burundi, Democratic Republic of Congo and Zambia in the west, Malawi and the Republic of Mozambique to the south—share boundaries with

Tanzania. The eastern side of Tanzania is a coastline of about 800 km long marking the western side of the Indian Ocean.

Tanzania, with a total of 942,784 km², is the third largest country in the GHA region (after Sudan and Ethiopia), the others being Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Sudan, and Uganda. Out of the total area, water bodies cover 61, 495 km² (6.52%).

Based on altitude, rainfall pattern, dependable growing seasons and average water holding capacity of the soils and physiographic features, Tanzania has 7 main agro-ecological zones. These are Coastal; Eastern plateau and mountain blocks; Southern highlands; Northern rift valley and volcanic high lands, Central plateau; Rukwa-Ruaha rift zone; and inland sedimentary plateau, Ufipa plateau and western highlands.

**Table 1: Agro-Ecological Zones of Tanzania.**

| S/N | Zone | Altitude m/sea level | Rainfall pattern | Dependable growing season (months) | Physiographic |
|---|---|---|---|---|---|
| 1 | Coastal (C) | < 100 to 500 | Bimodal and unimodal | 3 to 10 | Combination of coastal lowlands, uplands, undulating and rolling plains |
| 2 | Eastern plateau and mountain blocks ( E ) | 200 to 2000 | Predominantly unimodal | From < 2 to 7 | Many physiographic types, ranging from flat areas, undulating and rolling plains, hilly mountain, plateau to mountain blocks |
| 3 | Southern highlands ( H) | 1200 to 2700 | Unimodal l | 5 to 10 | Composed of flat to undulating rolling plains and plateau, hilly areas and mountains |
| 4 | Northern rift valley and volcanic high lands ( N ) | 900 to 2500 | Unimodal | < 2 to 9.5 | Ranges from flat to undulating plains, hilly Plateau to volcanic mountains |
| 5 | Central plateau ( P ) | 800 to 1800 | Unimodal | 2 to 6 | Composed of flat plains, undulating plains, plateau and |

| S/N | Zone | Altitude m/sea level | Rainfall pattern | Dependable growing season (months) | Physiographic |
|---|---|---|---|---|---|
| | | | | | some hills |
| 6 | Rukwa-Ruaha rift zone (R) | 800 to 1400 | Unimodal | 3 to 9 | Composed of flat terrain, rocky terrain and complex terrain |
| 7 | Inland sedimentary plateau , Ufipa plateau and western highlands (SUW) | 200 to 2300 | Unimodal | 3 to 9 | Composed of undulating plateau, strongly dissected hills, dissected hilly plateau and undulating rolling plains. |

Source: De Pauw, 1984

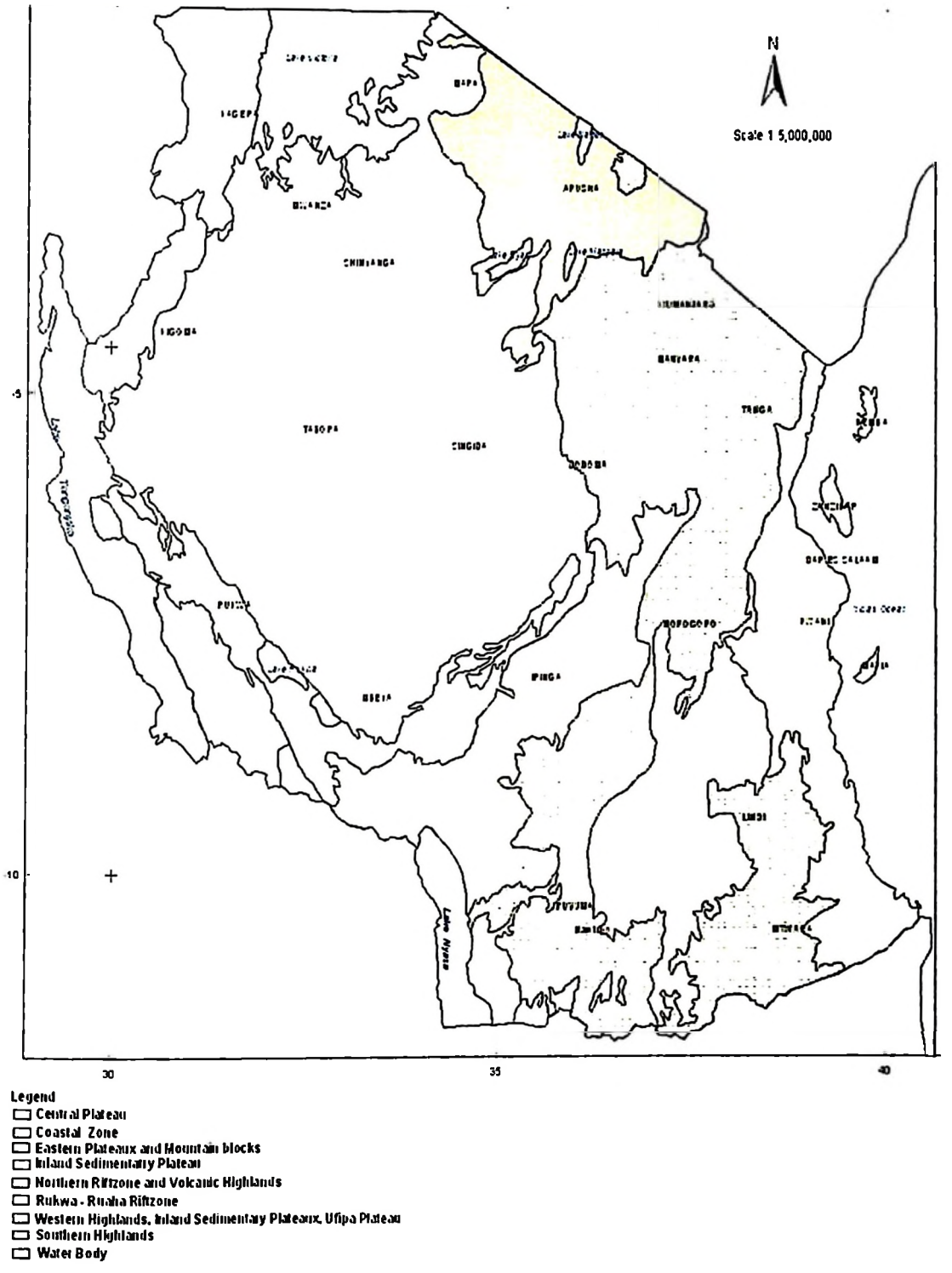


**Figure 2: Map of Tanzania showing Agro-ecological Zones**

(Map prepared by MALD 2005 source De Pauw, 1984)

### 2.8.2 The history of East Coast Fever

East Coast fever (ECF) has been recorded in Tanzania since the beginning of the century, but its history among traditional herdsmen is much older.

### 2.8.3 Control methods

The official Tanzanian policy for control of ECF and other tick-borne diseases is tick control. This policy has evolved from one of compulsory free dipping in the late sixties and early seventies to the introduction of a dipping fee in the 1980s.

Efforts to immunise cattle in Tanzania against ECF by the infection-and-treatment method started in 1976, using the 'Muguga cocktail'. Trials in the Pugu area of the Coast Province produced very satisfactory results. This early work could not be continued because of the breakup of the East African Community in 1977.

In 1990 vaccination against ECF was re-introduced in the country, now using Lilongwe-produced ECF vaccine. Cattle vaccination against ECF was started in the Southern Highlands, where the disease is believed to be endemic. After a satisfactory demonstration of the vaccine's efficacy, the programme was extended to the northern part of the country in Arusha, Kilimanjaro and Tanga Regions and later to the Coast Region (including Dar-es-Salaam). Plans are being made to expand the programme to the regions around Lake Victoria. To date, over 5000 cattle have been vaccinated against the disease, with 3% of these requiring treatment and 1% dying. Generally, it has been accepted that under careful management and administration the trivalent ECF vaccine is efficacious and protects animals against ECF. Farmers in Tanzania are ready to use the tick-borne diseases (TBD) vaccine because they believe that it will be an insurance against cattle losses and that they will be able to save money on the acaricides by cutting down the frequency of dipping.

### 2.8.4 Disease Surveillance and Monitoring

There are seven (7) Veterinary Investigation Centres (VICs) that are located strategically in the seven agro-ecological zones of the country. Animal diseases information flows from the Districts to the VICs and then to the Director of Veterinary Services.

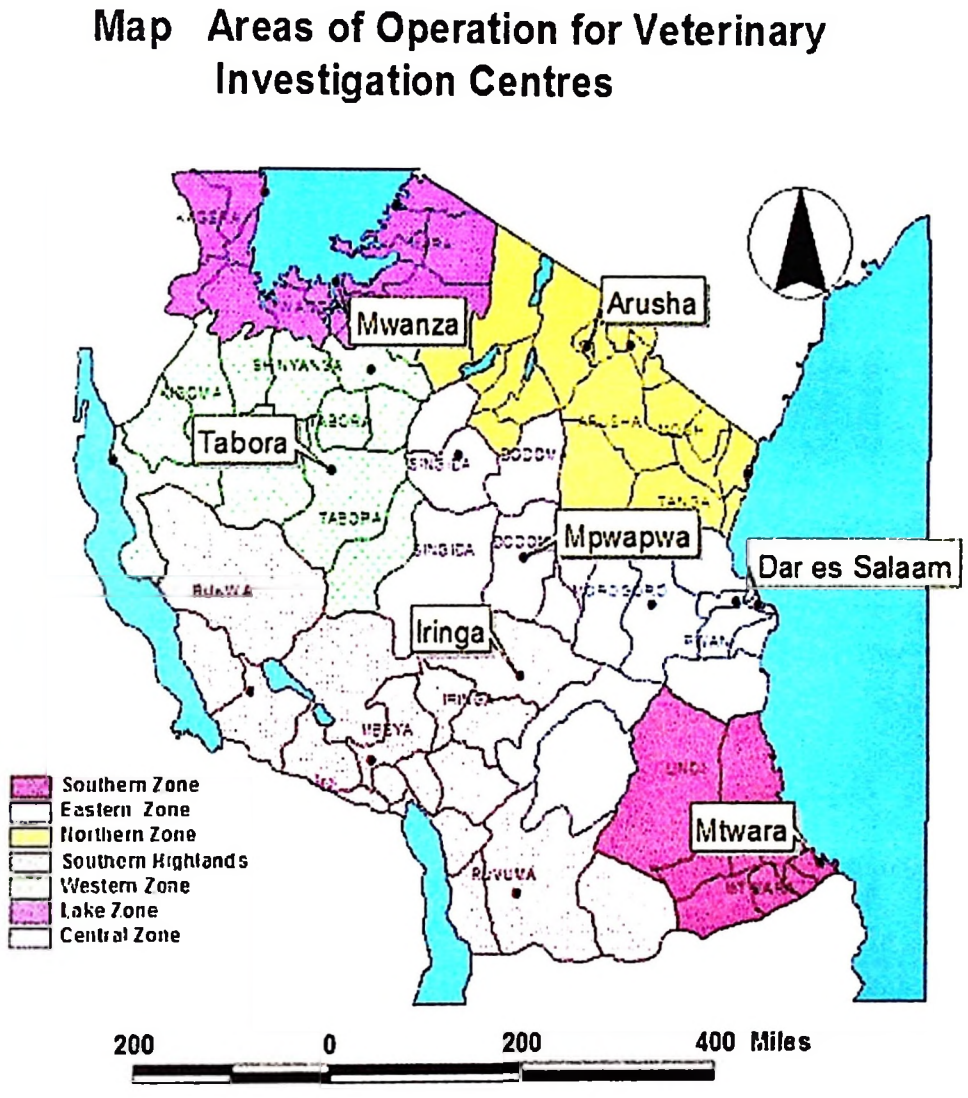


**Figure 3: Location of Veterinary Investigation Centres**

Source: Ministry of Livestock Development, 2006

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study area

The study was carried out in Kilosa, it is one of the one of the six districts of the Morogoro Region in Eastern Tanzania. It is among the most important indigenous cattle area in Tanzania with the history of occurrence of ECF. It is bordered to the North by Manyara Region, to the Northeast by Tanga Region, to the East by Mvomero District, to the Southeast by the Morogoro Rural District, to the South by Kilombero District, to the Southwest by the Iringa Region and to the West by Dodoma Region.

Administratively, Kilosa District is divided into 9 divisions. The divisions are further subdivided into 37 wards, and 168 registered villages. The principal town in Kilosa district is the town also called Kilosa. The study sites were, Magomeni and Manzese in Kilosa town, Madoto in Kimamba B, Mbwade and Parakuyo in Kimamba A in Godes in Msowero, Mabwegere in Kitete and Kwambe in Dumila.

### 3.2 Study design

***Collection of Epidemiological information***

Retrospective study was used to collect the epidemiological information on theileriosis, whereby information on number of cases of ECF reported by Veterinary Investigation Centers (VIC zones) which are located in seven (7) agro-ecological zones for period between 2008-2010 were obtained from Ministry of Livestock Development and Fisheries (MLDF).

*Collection of blood sample from cattle*

Total of 100(Table 2) blood samples were collected in 10 ml EDTA vacutainer tubes (Vacutainer® Dickinson B-D United Kingdom) using 21 gauge needles from traditional herds in Kilosa where cattle theileriosis (ECF) was suspected (purposive sampling) and the criterion for selection of cattle was enlarged lymph nodes. Blood sample was obtained from jugular vein. The tubes were placed on ice in a cool box for storage. Stored Samples were transferred to Sokoine University of Agriculture.

**Table 2: Villages where blood samples were collected from and the number of the blood samples collected from each village.**

| Location | Village | Number of samples |
|---|---|---|
| Kilosa town | Manzese | 16 |
| | Magomeni | 13 |
| Kimamba A | Kimamba A | 24 |
| Kimamba B | Parakuyo | 2 |
| Madoto | Madoto | 6 |
| | Mbwade | 3 |
| Msowero | Godes | 9 |
| Dumila | Kwambe | 27 |
| Kitete | Mabwegere | |
| **Total** | | **100** |

*Collection of Rhipicephalus appendiculatus*

A total of 95 host-seeking (questing) *R. appendiculatus* ticks were collected from herbage by dragging a blanket along the vegetation as described by (Short and Norval, 1981) in the fenced, open and restricted grazing systems, respectively.

Field collection of ticks was conducted in, Kimamba, Madoto, parakuyo, Kwambe, Mabwegere and Mbwade. After collection the ticks were placed in 100ml universal bottles with cotton wool dampened with sterile water. The bottles were placed in cool box maintained at approximately 0 °C by ice packs to prevent death of the ticks and bacteria spoilage. At the laboratory the ticks were stored at -20°C before processing.

**Morphological identification of *R. appendiculatus***

Morphological identification was carried out by means of a stereo microscope (Olympus) using the identification keys of Walker *et al.* (2000).

**3.3 DNA extraction**

Tick DNA samples were obtained by Proteinase-K treatment of homogenized ticks followed by phenol/chloroform extraction and ethanol precipitation as previously described (Hill and Gutierrez, 2003). Ticks were cut into small pieces in a Petri-dist using sterile scalpel blade and transferred in an eppendorf tube to which six hundred (600) µl of Tris(hydroxymethyl)methyl-2-aminoethanesulfonic Acid(TES buffer) was added in it and , mixed gently for 5 min. For DNA extraction 600 µl of phenol: Chloroform: Isoamylalcohol was added and gentle mixed for 5min then centrifuged for 5 min by means of Centrifuge (manufactured by Ependorf Company). The extraction procedure above was repeated for three times followed by extraction with chloroform only. In the extract above one volume out of ten volumes of 3M sodium acetate was added to precipitate the protein and centrifuged for 5 min. Absolute ethanol was added and the

mixture was centrifuged for 5 min then mixed gently by inverting 20times and allowed to stand for 5 minutes. The precipitated DNA was transferred to new eppendorf tube to which 500 µl of 70% ethanol was added to it and centrifuged for 5 min. Ethanol was then removed gently by overturning the tube without disturbing the pellet. The DNA pellet was left to dry at room temperature for 5minutes by on a special laboratory tissue followed by suspension of the DNA pellet in 20 µl of TE buffer and solution stored at -20 °C.

Cattle genomic DNA samples were obtained from 1ml of whole blood using Quick-gDNA™ MiniPrep DNA extraction kit (ZYMO RESEARCH). According to manufacturer recommendations 50 µl of DNA Elusion Buffer was added to the spin column. The mixture was incubated for 5 minutes at room temperature and then centrifuged at 13,500xg for 30 seconds to elude the DNA. The eluted DNA was stored at -20°C.

### 3.4 PCR for selection of *T. parva* positive samples and amplification of the p67 gene from *Theileria parva*

*T. parva* DNA from tick and blood the samples were screened by nested PCR (Takara Thermocycler manufactured in Japan) using p104 gene as it was previously described by Iams *et al.*, 1990. All positives samples were amplified using P67 gene. Table 3 shows pairs of primers used in the first and second rounds of the semi-nested PCR for p104 and p67 gene. The amplification programme for p104 was as follows: Step1: 94°C for 4 min; step 2: 94° C for 1 min; Step 3: annealing temperature of 58°C in the first run and, in the second runs of the semi-nested PCR, 60°C for 45 sec; Step 4: Extension temperature of 72° C for 1 min. Steps2-4 were repeated 39 times in the first runs and 24 times in the second runs. Step 5 was a final extension phase at 72° C for 8 min.

PCR amplification for characterization of *T.parva* was carried out by using locus encodes p67, a sporozoite surface antigen for which the gene has been characterised previously by (Nene *et al.*, 1992). A total of 48 samples were analysed including 36 *T. parva* positive from cattle and 11from ticks. In addition to these, one DNA sample from cattle-derived *T. parva* stock, Muguga (Brocklesby *et al.*, 1961) was also analysed as reference sample. Primer 613(P67 forward) and 792 (reverse primers) (Nene *et al.*, 1996) were used to amplify the variable region of the p67 gene. Five microliter (5µl) of extracted DNA was used in a 25 µl PCR reaction.

Each reaction contained 2.5 µl of 25mM $MgCl_2$, 2.0 µl of 2.5 mM dNTPs, 0.4 µl of each forward and reverse primer, 11.7 µl of water, 0.5 µl of Taq polymerase enzymes and 5 µl of DNA template. Samples from which no products were amplified after the initial PCR were subjected to re-amplification by nested PCR but with the number of amplification cycles reduced from 40 to 25. In the semi-nested run (second run), 0.5 µl of amplified product from the first run was added to the semi-nested master mix containing the same ingredients and concentration, except that forward primer and quantity of water varied. Thermal cycling (the amplification programme for p67) consisted in an initial denaturation step at 94 °C for 4 min, followed by denaturation at 94 °C for 30 s, annealing at 55°C for 45 s and extension at 72 °C for 1 min. The final extension step was maintained at 72 °C for 7 minutes. The standard detection with ethidium bromide staining was used after electrophoresis of the amplified product together with 100 base pair molecular weight marker.

**Table 3: Primer sequences of semi-nested PCR assays for the p104 and p67**

| Gene | Number of rounds | Primer name and sequence |
|---|---|---|
| p67 | 1 | p67F(5'CAGGTGAAACTACATCGG3') |
| | | p67R(5'TACTCAAAAAAACAAACC3') |
| P104 | 2 | First round |
| | | p104F2(5'CCACCATCTAAACCACCGTT3') |
| | | p104R(5'TAAGATGCCGACTATTGACACCACAA3') |
| | | Second round |
| | | p104nF(5'ACCACCGTTTGATCCATCATTCA3') |
| | | p104R(5'TAAGATGCCGACTATTGACACCACAA3') |

### 3.5 Analysis of the PCR products

Samples were resolved by electrophoresis through 1% agarose gel and visualized by fluorescence under UV light after staining with ethidium bromide. The p67 alleles (bands) obtained during gel electrophoresis were compared with four p67 alleles that have been previously reported in East Africa.

### 3.6 Statistical analysis

Descriptive statistics were used to determine prevalence. Comparison of prevalence between zones was conducted using t- tests).

# CHAPTER FOUR

## 4.0 RESULTS

Table 3 shows cases, deaths and number of cattle at risk of getting ECF. According to annual reports compiled by the Ministry of Livestock Development shows that 13,646 cases of ECF were reported from 2008 to 2010.

**Table 4: Reported cases of ECF in cattle in Tanzania from 2008-2010**

| Zone | cases | death | Population of cattle at risk |
|---|---|---|---|
| Eastern | 7160 | 70 | 85,259 |
| Western | 826 | 190 | 77,577 |
| Central | 771 | 147 | 20,654 |
| Northern | 650 | 15 | 42,825 |
| Southern | 1600 | 112 | 123000 |
| Southern Highland | 1538 | 261 | 364,895 |
| Lake | 1101 | 214 | 78,306 |
| | **13,646** | **1009** | **681,841** |

### 4.1. Estimated prevalence of ECF infection in Tanzania according to seven (7) VIC zones.

Figure one (4) show the prevalence of ECF for three years (2008-2010). The zonal distribution of the disease showed a highest prevalence in the Eastern zone followed by Central zone and the lowest in Southern highland zone. The prevalence of ECF was significantly higher in the Eastern zone compared to other zones ($P<0.05$). There is no significant difference in prevalence between the Lake, Northern and Southern zone but

both zones showed significantly higher prevalence compared to Southern highland zone ($P<0.05$) .(Fig 4) .

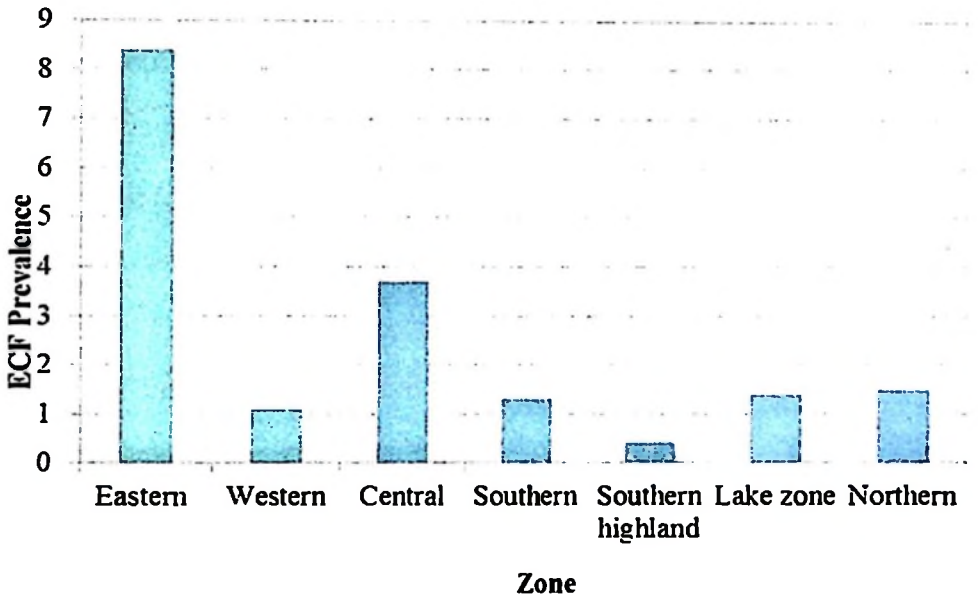


**Figure 4: The prevalence of ECF for three year (2008-2010) according to the seven (7) ecological zones.**

**Table 5: The prevalence of *T. parva* infection in Kilosa as determined by PCR**

| Tissue | Results |
|---|---|
| Blood | 36/100 (36%) |
| Ticks | 11/95(11.6%) |

**4.2 Amplicon analysis by agarose gel electrophoresis**

Out of 100 and 95 blood and tick samples screened, 36 (36%) and 11(11.6%) samples were positive for p104. All samples which were positive for p104 were amplified for p67, out of 36 and 11 p104 amplification positive samples, 7 (19.4%) and 9(81.8%) cattle and

tick samples were positive for p67, the rest of samples showed negative results on amplification of this gene.

Two p67 PCR products of sizes 800 and 900 bp were obtained (Fig 6) from 9 tick and 7 cattle *T. parva* positive isolates. Cattle sample number 1 and tick sample number, 3, 4, 5 6 and 8 have an identical band size to that of Muguga, a *T. parva* stock from Kenya responsible for ECF.

A single amplicon was obtained from all *T. parva* positive isolates obtained from both cattle and ticks (*R. appendiculatus*).

Single amplicon of band size 800 was obtained from 2 tick and 2 cattle samples. Amplicon band size of 900 was obtained from 9 tick and 5 cattle *T. parva* samples.

Fig 5 shows representative Amplicon profiles obtained from amplification of the central region of the p67 gene from ticks (*R. appendiculatus*) and cattle *T. parva* isolates collected from different villages in Kilosa district.

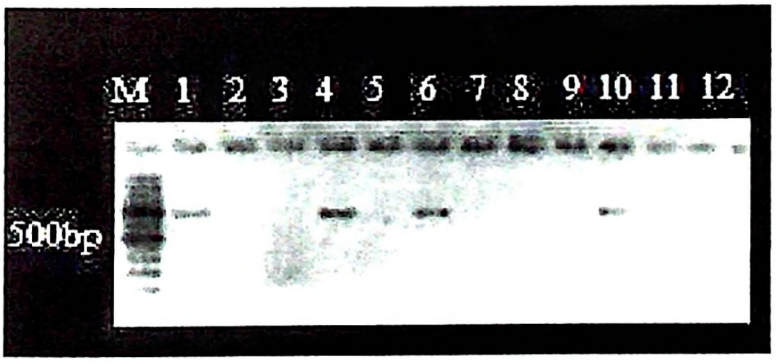


**Figure 5:** Representetive Amplicon profiles obtained from amplification of the central region of the p67 gene from ticks (*Rhipicephalus appendiculatus*) and cattle *T. parva* isolates.
M=1 kb DNA marker, Lane 1= Positive Control, Lane 2, 3,5,7,8, are samples with low parasitaemia.


**Figure 6**: Representative Amplicon profiles obtained from amplification of the central region of the p67 gene from ticks (*Rhipicephalus appendiculatus*) and cattle *T. parva* isolates collected from Godes, Kimamba, Kwambe, Mabwegere, Madoto, Magomeni, Manzese, Kwambe, and Parakuyo. Lanes: M is 1 kbp DNA marker (O'GeneRuler™ 100bp DNA Ladder Plus, Fermentas Life Science, EU), Lane 1, 2 and 3 are *T. parva* isolates from cattle. Lane 4,5,6,7 and 8 are *T. parva* isolates from *Rhipicephalus appendiculatus*. Lane 9= Negative Control (instead of adding template, nuclease free water was added) followed by lane 10 positive control (*T. parva* Muguga) and 1 kb marker.

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Ticks infestation and tick borne diseases in Kilosa

The objective of this study was to characterize *T. parva* stocks isolated from indigenous cattle and ticks in Kilosa district, Tanzania. *T. parva* gene coding for surface antigenic protein p67 was used to discriminate the stocks found in indigenous cattle and ticks in this area. Sampling was done between February and April, the pasture for grazing was available therefore most of the herds were around in the study sites. The tick infestation level was low compared to cold months of June–August according to the cattle owners' explanations. Mbassa *et al.* (2008) reported prevalence close to 100% in calves during cool months of May-July, therefore the low number of ECF cases observed in this study might be due to a problem in timing as sampling was done almost 4 months before the period of high prevalence of disease. Currently tick control measures deployed by Livestock keepers varied from no action, to application of acaricides when sufficient fund becomes available. The main constraints to proper control were found to be lack of infrastructure (service providers, dipping facilities and drug outlets). The most common infectious diseases described by livestock keepers and supported by veterinary records were ECF, anaplasmosis, babesiosis and trypanosomosis. During sampling period, small number of calves and adult animals have prescapular and parotid lymph nodes swollen indicating a clinical sign of theileriosis and other tick borne diseases, a scenario which is different from cool and dry months when most animals are observed to have this sign according to the farmers experience.

### 5.2 Prevalence of ECF infection in Tanzania

The data from this study indicate higher prevalence (8.4%) of the disease in Eastern zone compared to other zones and lowest (0.4%) in southern highlands. This is to be expected as climatic conditions in eastern zone are very favourable for tick survival (Elb and Anastos., 1966; Newson, 1978) allowing intensive host-tick interactions and subsequent challenge of cattle with *T. parva*. This study, as in other previous studies in the East African region (Gitau *et al.* 1997; Rubaire-Akiiki *et al.* 2004; Bazarusanga *et al.* 2007), demonstrated the crucial influence of agro-ecological zones on the variation in tick-borne diseases risk, both spatially and temporally.

Southern and Western Zones are traditionally not livestock areas so there are very few cattle. Probably this might be the cause of the lowest prevalence of ECF in Southern Zone. Furthermore, report from Ministry of Livestock Development and Fisheries shows that for three years no cases were reported from Mtwara rural district. This district has not been engaged in livestock keeping before.

A further limitation of this study was assumptions that all VICs report all cases equally and that all ECF cases in the field were reported to VICs, which may potentially over-estimated or under-estimated the level of the disease to zones. The study conducted in Tarime (north-west Tanzania) between October 2005 and January 2006, prevalence of antibodies to *Theileria parva* was 74.1%, (Chenyambuga *et al.*, 2010). The study conducted in dairy cattle in Dar es Salaam region of Tanzania observed prevalence of 32% due to East Coast fever (ECF). Following application of the capture-recapture method the respective prevalence was 45%, (Kivaria and Noordhuizen, 2010). A cross-section study in the same area in 2001(Kivaria *et al.*. 2005) estimated stratum –specific prevalence of 5% and 24% in vaccinated and non-vaccinated cattle respectively. It is

obvious that the estimates from this study are low and may not reflect the actual situation in the field because appreciable numbers of animals are recovered before diagnoses are made. Most cases also go unreported by many field staff due to inadequate diagnoses.

**5.3 P67 alleles identified in cattle in Kilosa Tanzania**

In this study analysis of p67 PCR products revealed the existence of two *T. parva* alleles in cattle isolates which were similar in term of size and number from those previously reported in South Africa (Sibeko *et al.* 2010). No sample had more than one allele, although other publications have reported samples with up to four alleles (800 bp, 900 bp, 1000 bp, and 1100 bp), indicating a mixture of different stocks of *T. parva* since p67 is a single copy gene (Sibeko *et al.*, 2010).

Analyses of p67 PCR product profiles indicate that among the two alleles, allele with 900 bp occurred more frequently than allele with 800bp.

It was also found that, p67 profile similar to that of Muguga a *T. parva* stock responsible for ECF in Kenya was obtained in this study. Since live vaccination has been recently (early 2012) deployed in Kilosa, *Theileria parva* Muguga stock isolated may be derived from exotic trivalent vaccine ("Muguga cocktail"), but the possibility of being indigenous stock cannot be excluded as Muguga component present in this vaccine is clamed no to stay in vaccinated animal longer than 48 days post vaccination (Oura *et al.*, 2004), hence a low chance of becoming transmitted to ticks although further study to prove this is required.

80.5% blood samples which were positive for p104 they were negative on amplification of p67 gene. The p67 protein is sporozoite surface protein in which its gene is expressed

during the initial stages of development of the parasite and p104 is piroplasm protein in which its gene is expressed during the final stages of the parasites. Since the initial stages of the parasite are found in lymphoid tissues like lymph nodes, when the blood is used as a source of DNA for amplification of p67 it is more likely to fail to detect some positive samples. Therefore p104 is the ideal gene to be amplified during *Theileria parva* screening when blood is used as the source of DNA.

### 5.4 Comparison of P67 alleles identified in cattle and ticks

Two p67 alleles with band size 800 and 900 were also identified from tick *T. parva* positive isolates. Tick samples also contained *T. parva* parasite with p67 alleles similar to those obtained in cattle.

# CHAPTER SIX

## 6.0 CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

Findings from this study revealed two *T. parva* stocks circulating in cattle in Kilosa district with 800 bp and 900 bp alleles. Allele with 900 bp which looks like *Theileria parva* Muguga was the dominant stock isolated. Comparing results obtained from this retrospective study with previous studies on prevalence of ECF in Tanzania, it is obvious the results from this study do not reflect the real situation in the field. For this case reports from VICs cannot give good estimates of ECF prevalence due to under reporting, poor diagnosis, poor records keeping and others in the field.

### 6.2 Recommendations

With this extent of genetic diversity studied by conventional PCR of p67 gene, findings of a parasite that looks like *T. parva* Muguga is of important epidemiology since it could be indigenous but could have been introduced by vaccination process. However, the findings of *T. parva* stocks which is not in the imported ECF trivalent vaccine ("Muguga cocktail") currently in use calls for more research to establish the complete list of parasites circulating in the district and region for efficient control of ECF.

Data on prevalence are important variables in the epidemiology and control of East Coast Fever. Complete records of disease occurrences will provides valuable epidemiological information on the endemic status of ECF in Tanzania, which is central to any future elaboration of strategic control measures.

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