BOVINE TICK BORNE DISEASE OCCURRENCE IN SOUTHERN RIFT VALLEY OF KENYA

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DECLARATION

Declaration by the candidate

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DEDICATION

Special dedication to mum and my sisters

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ABSTRACT

Tick borne protozoan diseases, are among the most important diseases affecting the productivity of livestock worldwide and result in high economic losses. A prerequisite for the control of these diseases is to study their epidemiology by mapping their distribution and seasonality of transmission relative to their vectors. The main objective of the study was to determine the prevalence and transmission of tick borne protozoan diseases in bovids in Southern Rift valley. The specific objectives were to determine the prevalence and distribution of tick borne protozoan diseases in three sub counties in relation to age and gender, determine the effects of temperature and humidity on vector abundance and the effectiveness of the vector control measures employed by farmers. Purposive sampling was used in site selection and a total of 196 blood serum samples were collected from 100 randomly selected farms in the three sub counties. Geimsa staining and ELISA Test techniques were employed to identify the parasites while the tick vectors were collected by flagging and identified by use of morphological features and key guides. The results showed that the overall TBDs prevalence rate by microscopy in the three sub counties was 29.6% though it varied among the sub counties, Kericho West (25%) Bureti (36%) and Bomet central having 27.47%. An overall TBDs prevalence rate of 66.84% by serology was observed and more females 116(59.18%) were infected than males 15(7.65%) but the association of gender with seropositivity was significantly indifferent ($p > 0.05$). Based on age bovids >1 year were more affected 126 (56.12%) compared to bovids < 1 year 70 (10.7%) and this was statistically insignificance ($p > 0.05$). Comparison of weather variables and vector abundance, showed that temperature was negatively correlated ($r = -0.235$) while relative humidity was positively correlated ($r = 0.216$) to vector abundance. Two tick genera (*Boophilus* and *Rhipicephalus*) were identified in the region with varying abundances. Majority of the farmers employed hand spraying (77%) in the control of tick vectors however, hand sprayed cattle (55.6%) were more affected than dipped cattle (11.2%). However the difference in effectiveness of the two methods was statistically insignificant ($p > 0.05$). Three tick borne diseases were diagnosed in the region and these were Theileriosis and Babesiosis The results showed that babesiosis(18.38%) was more prevalent especially in Bomet (27.47%) probably due to warm conditions, grazing system (communal) and host availability and Theileriosis was more prevalent in Kericho. It was concluded that tick borne protozoan diseases are still a problem to livestock farmers and there was a need to improve tick control efforts in the region.

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ABBREVIATIONS AND ACRONYMS

AEZ- Agro-Ecological Zones

DPBS- Dulbecco's Phosphate Buffer Saline

ECF-East Coast Fever

ELISA- Enzyme Linked Immunosorbent Assay

EBTS-Ethylbenz-thiozoleline-6-sulphuric acid

HBP-Histamine Binding Protein

HRP- Horseradish Peroxidase

IFAT- Immunoflorescent Antibody Test

IHA- Indirect Haemaglutination Assay

ITM- Integrated Treatment Management

LAT- Latex Antibody Test

OD- Optical Density

PP- Percentage Positivity

PCR- Polymerase Chain Reaction

PIM- Polymorphic Immunodominant Molecule

R^2-Coefficient of determination

R-spearman rank coefficient of correlation

Rmp –Revolutions per minute

TBDs-Tick Borne Diseases

TBPs- Tick Borne Pathogens

TG- ROC- Two-Graph Receiver Operating Characteristic

VIL- Veterinary Investigation Laboratory

OPERATIONAL TERMS

Diapause - a period of suspended development in an insect during unfavorable environmental conditions characterized by cessation of growth and reduction of metabolic activity.

Cyclopropagative - transmission of an arthropod transmitted disease where the causal organism undergoes cyclical changes and multiplies in the arthropod.

Ecdysis- is the molting of the cuticular in invertebrates.

Endemic instability- A state in which only a small proportion (<30%) of the cattle in a Population become infected and immune by six months of age leading to build up of susceptible population and , therefore, clinical disease is experienced across all age groups.

Endemic stability- A state in a cattle population where the majority (>70%) of the Population becomes infected by 6 months of age and little or no clinical disease occurs.

Engorged - fully filled with blood.

Epidemic – a disease affecting a large population at the same time.

In star - each developmental stage between molts.

Merogony - a form of sexual reproduction whereby a parasitic protozoan replicates its own nucleus inside its host cell and then induces cell segmentation.

Parasitemia- presence of parasites in the blood.

Piroplasm - protozoan parasite of the phylum Apicomplexa. They divide by binary fusion and as sporozoan parasites they posses sexual and asexual phases

Preimmune –preceding an immune response

Quisensence - a state of temporary dormancy.

Schizogony -asexual reproduction by multiple segmentation characteristic of sporozoans

(Multiple fusion of a trophozoites or merozoites)

Sexual dimorphism-phenotypic difference between male and female of the same species (size, appearance and ornamentation).

Sporogony- sporulation involving multiple fusion of a sporont resulting in the formation of sporocyts and sporozoites

Transovarial transmission- type of transmission where there is transfer of pathogen to succeeding generation through invasion of the ovary and infection of the egg.

Transtadial transmission – type of transmission where by the pathogen remains with the vector from one life stage to the next also considered as vertical or horizontal transmission.

Zoo noses - a disease that can be transmitted to humans from animals

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Tick- borne diseases (TBDs) of livestock constitute a complex of several diseases whose etiological agents may be protozoal, rickettsial, bacterial or viral. They are present throughout the world, but are most numerous and exert their greatest impact in the tropical and subtropical regions (McCosker, 1981).

East Coast fever (ECF) is the most important tick-borne disease of cattle in Eastern, Central and Southern Africa (Norval *et al.,* 1992). It is caused by a protozoan, *Theleria parva* which is transmitted by the brown ear tick *Rhipicephalus appendiculatus*. Other less-important tick-borne diseases in cattle include benign theileriosis caused by *Theileria mutans,* Anaplasmosis caused by *Anaplasma marginale* and cowdriosis caused by *Erlichia ruminantiun*. ECF is prevalent in large areas of East and Central Africa where it causes major economic losses through morbidity and mortality (Perry &Young, 1992). The disease is an important constraint to the improvement of the livestock industry in large areas of East, Central and Southern Africa (Norval *et al.,* 1992).

Babesiosis is caused by *babesia* parasites which are intra-erythrocytic protozoa with worldwide distribution (Angus, 1996). The main species of babesia that infect cattle in Kenya is *Babesia bigemina* with the main tick vector being *Boophilus microplus* (Freidhoff, 1997).

Farmers in the Southern Rift Valley region traditionally practice mixed farming, rearing predominantly local breeds of cattle and small ruminants (Peeler & Omore, 1997). However, there is a potential of replacing the indigenous breeds with improved breeds of

livestock as a means of improving livestock production. Already a number of farmers particularly in Kericho County are keeping improved breeds of dairy cattle such as Friesians. The transition from rearing of indigenous to improved breeds of livestock is expected to result into an upsurge of TBDs since the improved livestock breeds are more susceptible to TBDs (Gitau, 1988).

In Kenya, there is considerable recent evidence (Kinuthia, 2001) that the presence and frequency of TBDs obviously depend on the presence and frequency of their vectors, and as these depend to large extent on the climatic factors. It is therefore logical to conclude that climatic changes influence the epidemiology of TBDs and their geographical distribution. Among other possible factors are increase in population of wildlife, agricultural reforms and reduction in accaricide use (Moll, 1986).

Pegram *et al.,* (2000) reviewed the methods applied in the control of ticks and tick borne diseases. Control of ticks and tick borne diseases in Kenya is mainly through the use of acaricides to reduce vector challenge. Several accaricides have been used in the last 50 years in most countries before tick resistance became a problem (George *et al.,* 2004). Subsequently, organochlorides, organophosphates, carbamates, amidines and synthetic pyrethroids have been introduced in roughly that order. Tick resistance to organochloride is now wide spread and these compounds have largely been phased out (Nolan, 1990). Organophosphates are currently the most widely used but still resistance is developing and so their use is likely to decline in future (Spicket, 1998). The amidines and synthetic pyrethroids are becoming more widely used and have a much longer residual effect than other acaricide group (George *et al.,* 2004).

These differences have important implications for both the impacts and control of TBDs (Norval, 1992). With their economic consequences tick borne protozoan diseases have been

ongoing problems and have been the subject of much research activity. In spite of the many intervention strategies that are now available, there has not been any appreciable decrease in these diseases (Wharton, 1983).

Routine clinical diagnoses of protozoan diseases are usually based on microscopic detection of the parasites from collected blood smears. The detection of the parasites has been considered the 'gold standard' for the diagnosis of protozoan parasites. However, the technique is laborious when a large number of blood smears must be simultaneously examined. Furthermore, it is extremely difficult to detect parasites in blood smears during low parasitemia. Alternatively new techniques have been developed for the laboratory diagnosis of these parasites such as, IHA, LAT, IFAT, and ELISA. An ELISA based on the recombinant polymorphic immunodominant molecule (PIM) antigen is a method of choice for epidemiological studies and large scale investigations (Katende *et al.*, 1998). However the antibodies cannot always be detected in long term carriers despite the presences of the parasite (Morzaria, 1999). Furthermore, cross reactivity of the antibodies against other species have limited the specificity of serological test (Katende *et al.*, 1998). Polymerase Chain Reaction (PCR) assay, for the diagnostic detection of protozoan parasites have the potential to provide rapidly qualitative results with higher sensitivity particularly the nested PCR (Bishop *et al*., 2004). These PCR assays have various advantages over microscopy and serological diagnostic test. For example PCR is possible in animals as young as one month of age and data obtained refer to the current prevalence, in contrast to data obtained by serology. However current PCR based assays often require multiple processes to be performed on each sample and moreover, do not provide quantification of parasitemia.

1.2 Statement of the problem

In Africa the livestock industry has been the backbone of most countries' economy because of its products. Approximately 75% of the total population mainly relies on livestock for their products and as a source of income and contributes 26% of the Gross Domestic Production (Anon, 1991). Kenya which occupies an area of 582,670 km^2 has a total of 12 million heads of cattle (Livestock Department, 2010) of which 3 million are dairy breeds. The Livestock industry is ranked as the second most important agricultural income-generating enterprise, unfortunately TBDs particularly protozoan diseases namely Theilerosis and Babesiosis are the major constraints to this industry (Adams *et al.*, 1971). Knowledge of bovine TBDs is incomplete, information on the distribution and prevalence is uncertain, and their economic effects are often under estimated. In 2008, alone 230,000 cases of TBDs were reported in Kenya and many more may have occurred without being reported. Relatively few studies on animal health and production have been carried out in South Rift which has a significant livestock population of 678,934 of cattle (Livestock Department, 2010). The only documented study on the prevalence of TBDs was a cross sectional survey report by Mukhebi, (1985). However, the study was very limited in scope and the data collected was largely qualitative based on a questionnaire. Among the most important documented reasons for the disease occurrence include the following; accaricide resistance, conducive environmental conditions for vector development, inaccurate acaricide dilution ratios and, communal grazing system among others.

Results of this study will provide information on the risk of tick- borne diseases in the region particularly to exotic breeds of livestock. This in turn will be the basis for formulating appropriate control strategies for the tick-borne diseases in the sub counties within the two counties.

1.3 Justification of the study

The study was designed to investigate the prevalence and transmission of tick borne protozoan diseases in Southern Rift Valley of Kenya. These diseases have been endemic and widely spread in Kenya and affect nearly a quarter of the whole country (Moll, 1986).

Since the presence and frequency of TBDs depends on the vector presence and their presence is also modulated by climatic factors, therefore epidemiology by mapping their distribution and study of vector population dynamics need to be done. Dissemination of the appropriate parasite is dependent upon the presences of the appropriate vector

Control measures which have been employed include use of chemotherapy, where tetracycline antibiotic was probably the first compound to be used in 1953 but it was limited to only the early stages of the disease, more effective derivatives of Naphthoquinone (Parvaquone and Buparvaquone) were discovered in late 1970s (Drummond, 1983). Vector control measures which have been employed include use of acaricides (Pyrethroids, Avemectins, Organophosphates, Amitraz etc) of which some vectors have developed resistance and are detrimental to the environment as reviewed by George, *et al.,* (2004). Acaricide are commonly applied by dipping or spraying, dipping being considered the most effective means of application. As a pre requisite for proper vector control, the most effective method should be identified and used.

In Rift valley and in particular the Southern part, domestic animals graze on a vast area utilizing different pastures. This prompted the need to study the prevalence and distribution of bovine tick borne protozoan diseases, study the impact of weather variables in relation to

vector abundance and vector control strategies employed by farmers. The information will be of importance in the upcoming planned vaccination against TBDs and future vector control programmes.

1.4 Objectives

1.4.1 General objective

The general objective of this study was to investigate the occurrence of bovine tick borne protozoan diseases and relate them to weather variables and the vector control strategies employed by farmers in Southern Rift Valley of Kenya.

1.4.2 Specific objectives

The specific objectives were:

1. To determine the prevalence and distribution of tick borne protozoan's by microscopy and serology in relation to age and gender.

2. To correlate temperature and relative humidity to vector abundance in the study area.

3. To determine the effectiveness of vector control measures employed within the study area.

1.5 Hypothesis

H_O: prevalence and distribution of tick borne protozoan diseases are independent on age and gender.

H_O: There is no correlation between temperature and relative humidity to vector abundance

CHAPTER TWO

LITERATURE REVIEW

2.1 Ticks and Tick borne diseases

Ticks are obligate haematophagous ectoparasaites of the wild, domestic animals and human that are classified in the subclass Acari, order Parasitiformes, suborder Ixodida, (hard ticks) and are distributed from Arctic to the tropical regions of the world (Levine, 1971). Despite efforts to control tick infestation, ticks and the pathogens they transmit, continue to be a serious constraint to human and animal health (De La Fuentes & Kocan, 2006).

Ticks were the first arthropods proved to be vectors of protozoan diseases. Smith & kilborne proved for the first time in 1893 that *Boophilus annulatus* was the vector of *Babesia bigemina*, the causative agent of Texas fever. *B .annulatus,* a native of Southern USSR, Middle East and Mediterranean area and has moved South wards to Africa (Hoogstraal, 1979). Tick borne protozoan diseases have proved over years to be of great threat to the animal industry throughout the world. For example in the Old Testament, Murrain plaque of Egyptian cattle appeared to have been babesiosis (Smith & Kulborne, 1893).

Ticks transmit a great variety of infectious agents than any other group of hematophaguos arthropods and they surpass all other arthropods in number and a variety of diseases they transmit to domestic animals, and they are ranked second to mosquitoes as vectors of human diseases (De La Fuentes & Kocan, 2006).

Hard ticks progress through larval, nymphal, and adult stages, of which all require a blood meal. For the majority of hard ticks of medical and veterinary relevance (including Ixodes species, Dermacetor, and Amblyoma), a three stage life cycle including host seeking, feeding, and off host molting (for egg laying), is the most common developmental pattern, whereas some ticks such as *Rhipicephalus microplus* (formerly *B microplus*), undergo a single host life cycle (Appendix IV). Ticks feeding on a pathogenic – infected vertebrate host imbibe these pathogenic microorganisms, and once ingested, the pathogen life cycle differs depending on the pathogen. In the mid gut, pathogens such as *Babesia* species and *Rickettsial* species immediately invade both the tick ovaries and salivary glands via the hemolymph (Chavin, 2009). *Theileria* species exhibit a similar cycle in the vector but without ovarian invasion (Bishop, *et al.*, 2004).

The unique number of disease agents which ticks transmits both to their host and transovarially to their off springs increases the potential health hazard many folds since they are both vectors and reservoirs of these diseases (Brown, 1990). The annual global cost of controlling ticks and TBDs together with the loss to mankind of significant amounts of animal protein due to cattle death or diminished productivity, is in the range of thousands of millions of dollars (Mukhebi, 1985)

Several events that occurred during the final decade of the 20 th century suggest a rise of tick borne infestation worldwide. These events include the recent National and Regional epidemic of known disease such as Kyasanur Forest Disease in India, and Rocky Mountain spotted fever in Arizona and US among others (Anonymous, 1984). Globally, the recognized number of distinct and epidemiologically important transmitted diseases by ticks have considerably increased for the last 30 years (Paddock *et al.*, 2008), and majority

of the emerging diseases are zoo noses that are predominantly vector borne (Salman & Pena, 2013). The incidence of TBDs has increased dissapropriately in relation to other emerging diseases and peaks at times of severe weather events and climatic anomalies (Githeko, 2008), a reflection to sensitivity and reliance of arthropod on permissive conditions.

Although advances in molecular technology have contributed to the identification of these pathogens, rapidly expanding pathogen diagnosis and increasing incidence have raised concern about the accuracy of the case counts and epidemiological reports (Bishop *et al* ., 2004). The problem of analyzing the incidence of tick borne pathogens is the concurrency of the factors affecting the whole system such as climate driving the cycle of ticks, availability, occurrence and seasonal patterns of competent reservoirs**.** All these factors should be analyzed with increasing levels of complexity that obscure the relationship between climate and the final impact of tick borne parasites.

Ticks and tick borne pathogens have coevolved molecular interactions involving genetic traits of both the tick and the pathogen that mediate their survival and development (Riek, 1963). These mechanisms are not well defined and the impact of environmental factors such as climate adds additional complexity to their study. However, the complexity of tick pathogen relationships emerging show that it is difficult to describe a single effect on the consideration of social and biological events affecting the transmission of tick borne pathogens**.**

2.2 Protozoan diseases and pathogen transmission

Tick borne protozoan parasites of the phylum Apicomplexa, class sporozoa, subclass piroplasma, cause diseases in a wide variety of domestic and wild animals and sometimes in man (Duh *et al.,* 2008). They are transmitted by the Ixodid ticks often referred to as "hard" ticks due to the presence of a tough outer scutum, which provides sexual dimorphism in ticks as the scutum completely covers the idiosoma of an adult male, whereas the female is partially covered, revealing the alloscutum. This feature allows the female tick to engorge during a blood meal **(**Soneshine, 1992).

Once inside the tick, intestinal, salivary or ovarian barriers must be crossed, and multiple distinct cell types must be invaded for pathogenic multiplication to occur. During tick infection and transmission Tick Borne Pathogen (TBP) must also adapt to specific physiological and behavioral characteristic particularly with regard to blood feeding via the saliva, and for certain pathogens, they can be transferred to the next tick generation via transovarial transmission (Natali, 2004). This vertical transmission is an absolute necessity for those TBP infecting single – host tick species such as *R. microplus* **(**Chavin, 2009). In most transmission cases, pathogens present in tick salivary glands cells invade vertebrate host at the skin site where ticks have salivated during feeding (Natali, 2004), some factors present in the saliva are then used by microorganism to increase their pathogenicity and evade the host immune response. For example salp 15 (salivary gland protein) and HBPs (Histamine Binding Protein) which modulates vascular permeability and enhances blood flow, which in turn facilitates tick engorgement (Dai, 2010).

The most widely accepted classification scheme placed all of these tick borne pathogens in two families, *Babesidae* and *Theileridae* with single genus *Babesia* and *Theileria* respectively (Levine, 1971). Piroplasms (do not produce a pigment formed from the host cell hemoglobin) are tick transmitted blood cell parasites of vertebrates occurring in RBCS, lymphocytes and other blood system cells (Brown, 1990). Their shape may be piriform, amoeboid, round, oval, comma, and ring shaped or characteristic pear shaped (*Babesia* species) (Wright, 1988).

2.2.1 Theilerosis

2.2.1.1 Parasites and distribution

This is a tick transmitted protozoan disease, the most important species being *Theileria parva* which infects cattle and wild bovidae and is the causative agent of East Coast Fever (ECF) *(*Theiler, 1969). Thirty years ago, Callow,(1984) revised the classification based on morphology, biochemistry and molecular phylogenetics, in which *T. parva* (and other unicellular eukaryotes) are designated as protists rather that protozoan but still remain members of Apicomplexa.

This parasite is confined to East, Central and Southern Africa, and has been reported in 11 countries in the region (Figure 2.1): Kenya, Uganda, Tanzania, Burundi, Ruanda, Mozambique, Malawi, Sudan, Congo, Zambia, and Zimbabwe (Norval *et al.*, 1992). Its distribution in Uganda coincides with the presence of its vector, the brown ear tick, *Rhicephalus appendiculutus* (Mattyssi, 1989). ECF was also reported in Comoros between 2003 and 2004 for the first time (Martin, 2007). The later incident was suggested to result from the importation of immunized cattle from Tanzania, which were fed upon naïve ticks

that subsequently transmitted infection to susceptible local cattle population (Norval *et al*., 1992**).**

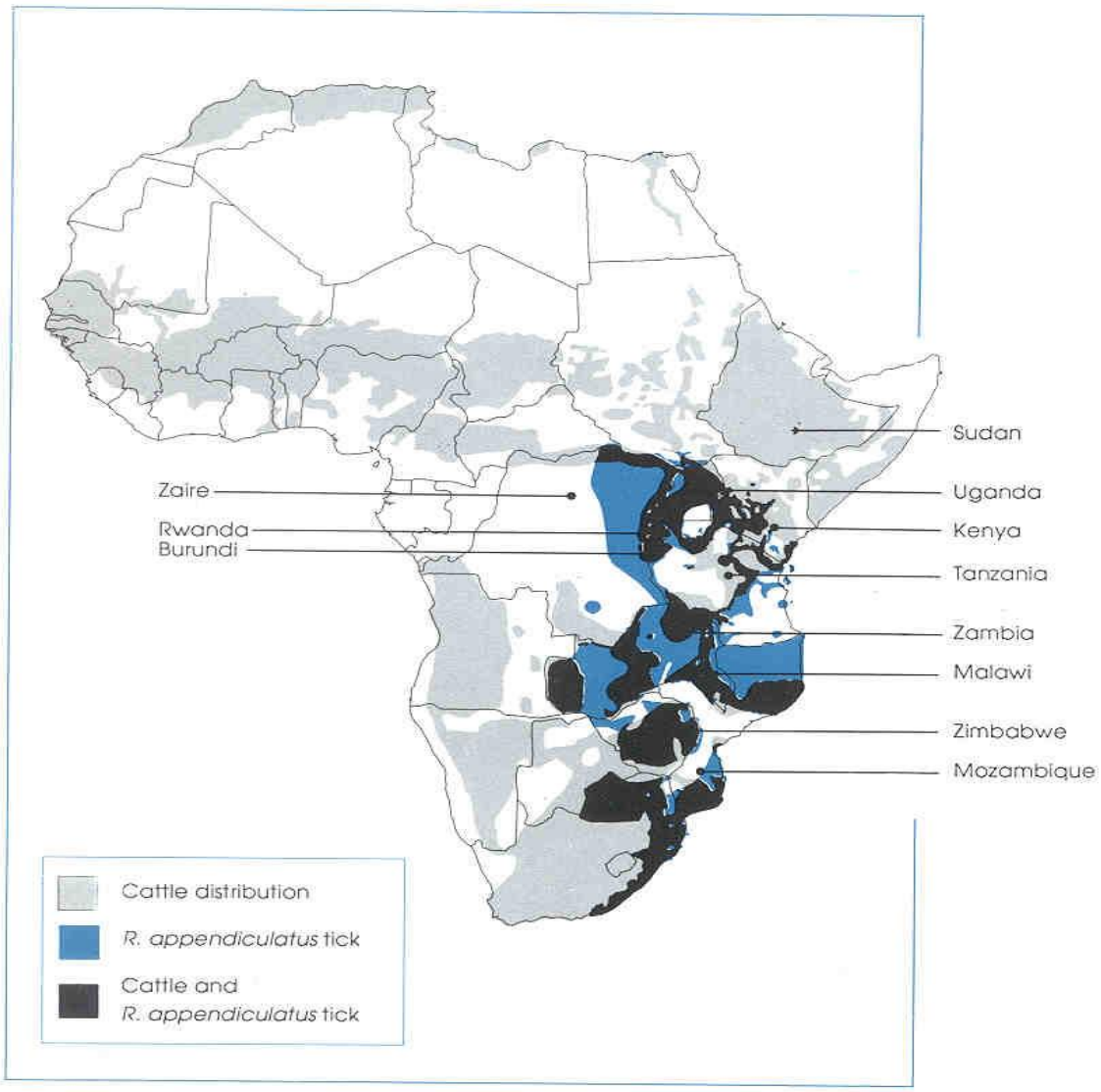

Figure 2.1: Map showing the distribution of *Rhipicephalus appendiculatus* in Africa,

(Source: Norval *et al*., 1992).

About 28 million cattle in Kenya are at risk of theileriosis and the disease kills at least 1 million cattle per year (Mukhebi, 1985). In Kenya *T. parva* infection poses significant

threat to livestock sector in two ways; through the economic impact of the disease from cattle morbidity and mortality and the production losses in production systems as well as from the costs of measures taken to control ticks and diseases.

The cost of acaricide application which is the primary means of tick control was estimated to range between 6-36 US Dollars per adult animal in Kenya (George *et al.,* 2004). The disease further prevents the introduction of the ECF susceptible but more productive exotic breeds of cattle, hampering the development of the livestock sector considerably (Gitau *et al.,* 1998).

Another species of *Theileria* that is widely spread in tropical and subtropical areas of the world is *T. annulata,* causing Tropical Theilerosis (Uitenberg & Camus, 1981). Taxonomically there are seven species of *Theileria* namely *T. parva parva* (causal agent of ECF), *T. parva Lawrenci (*causal agent of corridor diseases), *T. mutans (*casual agent of Tropical Theileriosis). *T.ovis* and *T. hiicri* (causal agents of Ovine Theilerosis) in Africa, Europe and Asia, *T. orientallis* (causal agent of oriental theileriosis) and *T. taurotragi* (causal agent of Benign Africa theileriosis) (Appendix I)

ECF is the most virulent disease compared to all other theilerioses presumably because of the rapid division of the parasites which occurs in the lymphoid cells and outpaces the immune response (Brown, 1990). Clinical disease occurs in stressful situation or when the host is debilitated by other parasitic organisms and malnutrition. The disease is characterized by swelling of lymph nodes beginning with the parotid the lymph node closest to the ear or eyelid surface which is the predilection feeding sites of the vector ticks. Later lymphadenopathy is generalized (Coles, 1986).

Other clinical features of ECF include; fever (39.5-42°C), severe dyspnoea and frothing at the nostrils due to interstitial pneumonia and pulmonary oedema and, high mortalities **(**Coles, 1986). Animals that recover naturally or after treatment with theilericides have long lasting immunity giving complete protection to homologous challenge, but may be susceptible to some heterologous strains (Morzaria, 1999).

Susceptible cattle undergoing acute infection with *T. parva* produce cytolytic (cell killing) lymphocytes cells which are capable of killing their own infected and uninfected cells. These cells are probably important in mediating the destruction of the immune system which is a prominent feature of the disease. By contrast, cattle which have developed immunity against ECF produce cytolytic lymphocytes which kill only cells infected with *Theileria* schizonts. In this case, the killer cells recognize a parasite – induced antigen on the surface of the parasite cell (Mohammed *et al*., 1975).

Evidence have also accumulated that cattle which recover from ECF produce anti-sporozoite antibodies which play a role in protecting them against subsequent infection and the anti-sporozoite antibodies might also suppress parasite development within the tick.

Recovered or immunized animals remain carriers of infection for life and therefore serve as a source of infection to ticks. However, sometimes ECF does not occur throughout the range of the vector ticks' distribution (Norval *et al.*, 1992). This is because the distribution of the vector ticks even in the countries where they are known to occur commonly is not continuous being influenced mainly by climate, vegetation and host availability. Further, high temperatures (>33°C) experienced in some ecologically marginal areas within the range of the ticks' distribution do not allow development of *theileria* in the vector ticks

(Young & Leitch, 1981). Further still in areas of extreme climatic conditions ECF may fail to establish itself due to low tick numbers for long periods (Speybroeck, 2002).

2.2.1.2 Transmission

East Coast fever (ECF) is transmitted cyclopropagatively and transstadially by a three-host tick**,** *Rhipicephalus appendiculatus* (Theiler, 1969). Briefly, a replete adult female drops off the host and produces a single egg batch after a pre-oviposition period. The eggs hatch into larvae which hardens off and start to quest on pasture. After having been picked up by a host, the larvae feed and drop off the host upon repletion and molt into nymphs (plate 4.5).The nymph cycle through identical series of events resulting in adult ticks. It is accepted that adults, in particular females, have the ability to diapause in order to regulate host finding and feeding, thus ensuring that the most vulnerable stages, namely eggs and larvae, are exposed to favorable climatic conditions (Randoph,1997).

The entire life cycle can be completed in three months but in cooler climates it takes a year to complete. The pattern of seasonal occurrence is regulated by the unfed adults diapauses and do not engage in host seeking until the rains start. In regions close to the equator more than one life cycle can be completed annually and no clear pattern of seasonal abundance may be evident (Suss *et al*., 2008).

Kenya is divided into seven agro-climatic zones (Figure2.2) based on moisture index (Sombroeck, 1982)). The index represents the annual rainfall expressed as a percentage of potential evaporation. Areas with an index >50% are designated as zone I,II,III and are potential for substantial vegetation cover, the zones are characterized by small and commercial systems, other areas with index <50% are designated IV,V,VI,VII (figure 2.2).

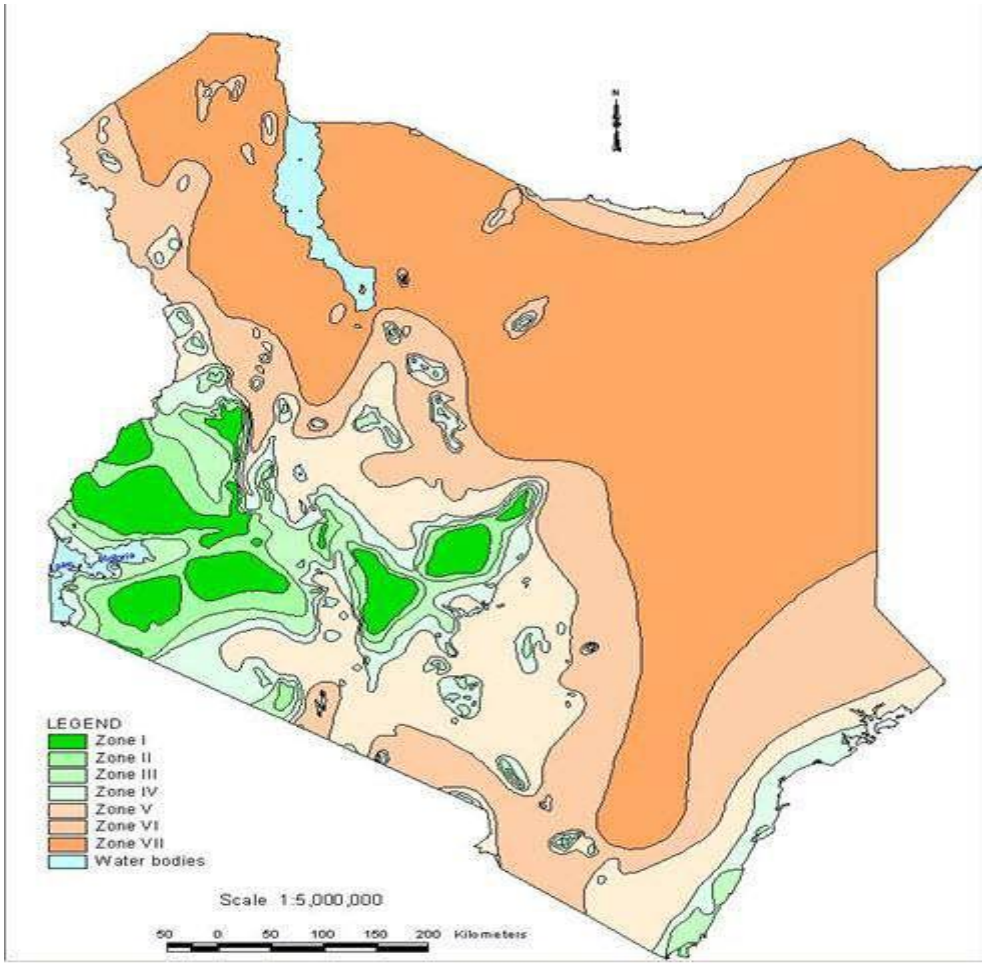

Figure 2.2: Map of Kenya showing the Agro-Ecological Zones.

(Source: Sombroeck, 1982)

The vector occurs in areas with over 400mm of rainfall and is found at sea level to over 8,000 feet where vegetation cover is adequate. Areas that are most suitable to these ticks are warmer and humid with landscape (I, II) characterized by a mixture of grass and tree cover (savannah woodland) (Sombroeck, 1982). In Kenya *R. appendiculatus* is found in the Lake Victoria basin (in Nyanza and parts of Western counties), the Kenya coastal region and some parts of the Central and eastern highlands representing zone I,II,IV (Figure 2.3).

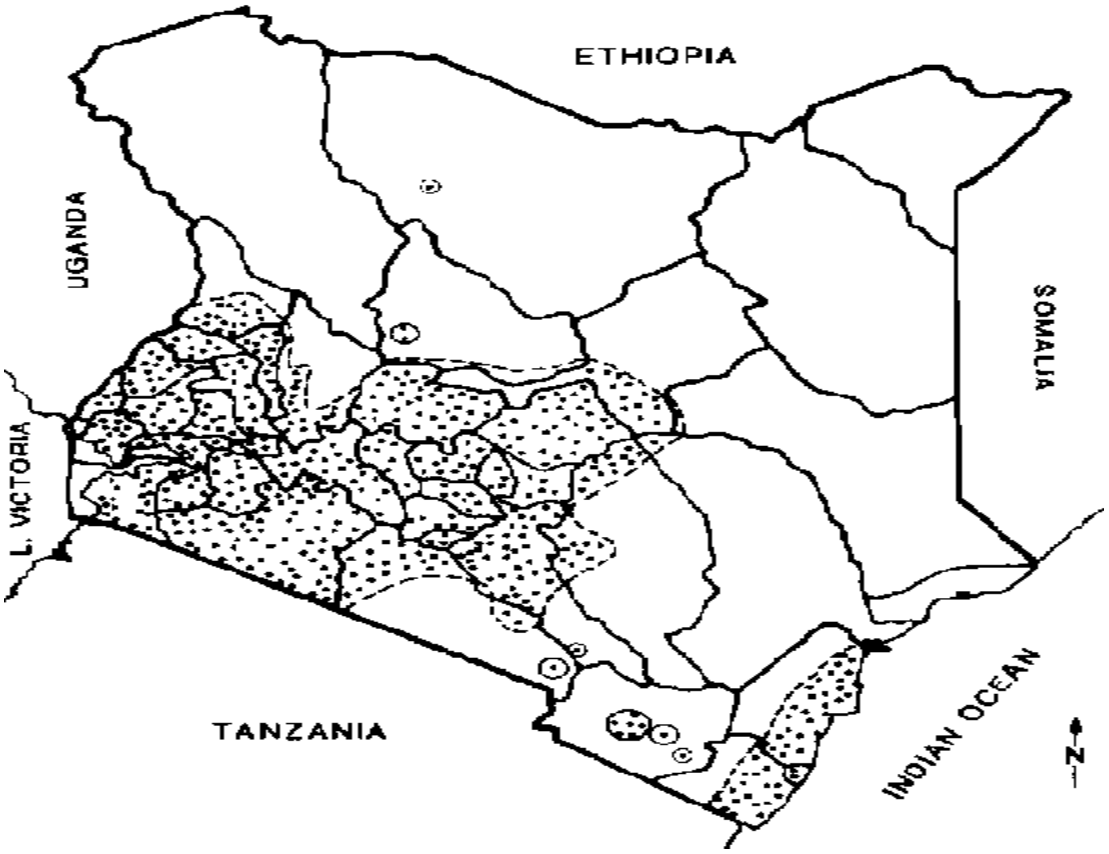

Figure 2.3: Map of Kenya showing the distribution of *Rhicephalus appendiculatus.* (Source: Speybrock, 2002)

Indeed, after ECF was first reported in Kenya in 1904, reports indicate that the disease spreads fast from some of these foci, L. Victoria basin and the coastal region (Norval, 1992). In these initial foci areas low mortality rates were found in the absence of tick control practices (Latif, 1995) indicating endemic stability. At the Coast a seasonal independent, all-year round occurrence of *T. parva* infection was reported (Moll, 1986).

Tick vectors are not found under hot and dry regions, sparse vegetation and open grass land. In the context, of ECF these conditions can be found particularly in semi arid North Eastern and upper parts of Eastern province of Kenya (zone V and VII). Unsuitable areas

also include regions where overgrazing and environmental degradation has occurred and in frosted areas (Sombroeck, 1982).

In cyclopropagative and transstadial transmission, *T. parva* multiplies and undergoes cyclical changes within two developmental stages (nymphs and adult) of the vector (Radostits, 2007). The epidemiological implication of this kind of transmission is the amplification of the vector's competence in parasite transmission and the ability to infect more than one host during the vector's life cycle (Niez, 1965).

The level of infection in a tick population is influenced by ambient temperature, the behavior of *T. parva* (may be low or high piroplasm producing), and the susceptibility of *R. appendiculatus* strains to *T. parva* infections, the presence of infected hosts and whether the infected hosts are clinical cases or carriers (Young & Leitch, 1981).

Ticks acquire higher infections when they feed on clinical cases than carrier cases (Gray, 2002)**.** The latter present extremely low parasitaemias (piroplasm) hindering the development of high infections in ticks (Niez, 1965).

Total level of infection in a population of ticks also depends on the numbers (abundance) of feeding stages on such clinical and or carrier hosts. The abundance of feeding stages in an area is in turn influenced by host availability and macro-and micro-climatic requirements of the vector (Norval, 1981).

Climatic conditions vary throughout the range of *R. appendiculatus* and in response ticks have evolved different behavioral and survival strategies. One such behavioral strategy is the diapause phenomenon exhibited by ticks from regions with a marked wet and dry season that enables ticks to delay feeding and hence oviposition so that the most vulnerable

stages of their life cycles are synchronized with the incidence of favorable climatic conditions (Lane & Adderson, 2000).

Within the diapause phenomenon ticks from different geographic areas have evolved different strategies for the initiation and termination of diapause (Belozerow, 1982). These behavioral strategies affect not only their abundance but also the number of generations per year.

The number of generations per year among other factors has an effect on the establishment of endemic stability (Perry *et al*., 1992), which is the epidemiological state of a population in which clinical disease is scarce despite high level of infection (Norval, *et al.*, 1992). As a vector-borne disease, the prevalence of ECF is likely to be largely influenced by varying environmental conditions which in turn influence vector dynamics.

2.2.2 Babesiosis

2.2.2.1 Parasites and distribution

Bovine babesiosis is a tick borne disease in cattle caused by intraerythrocytic protozoan parasites of the genus *Babesia* (Levine, 1971). Two species, *Babesia bovis* and *Babesia bigemina* are the most frequent and important tick borne protozoan's among various bovine *Babesia* parasites and the second most common parasite found in blood of mammals after trypanosomes (Yabsley & Shock, 2013).

The genus *Babesia* belongs' to the phylum Apicomplexa, class sporozoasida, order Eucoccidiorida, suborder piroplasmorina and family Babesiidae, (Levine, 1971). The major *Babesia* species that affect livestock include *B. bigemina, B. bovis, B. divergens* and *B. major* which affects cattle (Uilenberg, 1995). *B.motasi* and *B. ovis* which affects goats and

sheep (Friedhoff, 1997). Pigs are affected by *B. trautman*i and *B. perroncitoi* and finally *B. equi* and *B. caballi* which affect horses (Radostits *et al.*, 2007). The classification of the *Babesia* species is based on the size of the erythrocytic stage and are known as small (1-2.5 µm) and large (2.5-5 µm) babesias (Mackenstedt *et al.*, 1995) both types may occur in domesticated livestock and in wild animals (Appendix III)

The distribution of the causative protozoan parasite is governed by the geographical distribution of the vectors that transmit them (Bock, 2005). Bovine babesiosis is found in most of Africa, Southern Europe, Asia, Central and S. America, and many of the islands in the Caribbean and South pacific countries (McCosker, 1981). They cause a wide range of clinical syndromes in most domestic animals and humans due to difference in virulence within each *Babesia* species (Wright, 1988). Bovine babesiosis is an infections of the tropics and subtropics occurring mostly in countries between 40^0N and 30^0S and are transmitted by the *Boophilus* species (McCosker, 1981).

2.2.2.2 Transmission of Babesiosis

The arthropod vectors of bovine babesiosis are the brevirostrate hard ticks *B. microplus* of the family *Ixodidae* (Uilenberg, 2001). It has been postulated that the vector was introduced to East and South Africa from Madagascar, where it had originally arrived with cattle from Southern Asia (Smith, 1981). In Kenya it is present in coastal region, western and the highlands where moist and warm climatic conditions exist (Kinuthia, 2001).

B. microplus (Plate 4.4) is a one-host tick; all stages are spent on one animal and take about two months to be completed. The eggs hatch in the environment and the larvae crawl up grass or other plants to find a host. They may also be blown by the wind. In the summer, *B. microplus* can survive for as long as 3 to 4 months without feeding (Dagliesh & Sterwart,

1982). In cooler temperatures, they may live without food for up to six months (Gray, 2002).

Ticks that do not find a host eventually die of starvation. Newly attached seed ticks (larvae) are usually found on the softer skin inside the thigh, flanks, and forelegs. They may also be seen on the abdomen and brisket. After feeding, the larvae molt twice to become nymphs and then adults. Each developmental stage (larva, nymph and adult) feeds only once, but the feeding takes places over several days (Friedhollf, 1998).

During feeding, the blood stages of the parasite are ingested during and they undergo sexual and asexual multiplication in the replete female infecting eggs and subsequent stages. The level of infection varies depending mainly on the level of parasitemia of the host at the time the female tick engorges (Callow, 1984). Adult male ticks become sexually mature after feeding, and mate with feeding females. An adult female tick that has fed and mated detaches from the host and deposits a single batch of many eggs in the environment. Typically, these eggs are placed in crevices or debris or under stones and the female tick dies after ovipositing. Ticks in the subgenus *Boophilus* have a life cycle that can be completed in 3 to 4 weeks; this characteristic can result in a heavy tick burden on animals (McCosker, 1981).

Each species of *Babesia* is transmitted by a single tick species which varies from one region to another, thereby causing difference in the epizootiology of babesial diseases (Smith, 1981). *Babesia* parasites are generally transmitted transovarially in which the organism invade the eggs and thus infect the new generation of larvae. This transovarial or vertical transmission enables infections to be disseminated to a large number of progeny (Mackenstedt *et al.,* 1995). *Babesia* parasites can also be transmitted between animals by direct inoculation of blood, by biting insects, and formites contaminated with infected

blood might also acts as mechanical vectors, although of minor importance **(**Friedhollf, 1998).

Since the parasites can be maintained in cattle population by asymptomatic carriers, then recrudensce of parasitemia can occur at irregular intervals. Calves can be infected in utero; however this appears to require pathological changes in the placenta, and transplacental infection is accidental and rare (Mahoney, 1972).The number of ticks in the environment is an important factor which affect the epizootiology of babesiosis (Perry, 1996). Where the tick population is high, most cattle become infected very early in life. In areas less favorable for the propagation of *Boophilus*, although ticks may be constantly present, their numbers are often insufficient to infect a high proportion of cattle in the first year or two of life. The reduction in the transmission rate caused by low vector density is compounded by a tendency of populations to become progressively less infected with parasites as the tick numbers decrease. At times this leads to the complete elimination of *Babesia* from the environment **(**Mahoney, 1969).

In endemic areas three features are important in determining the risk of clinical disease. These are: calves have a high degree of immunity (related to both colostral-derived antibodies and to age) that persists for up to six months (Mahoney & Ross, 1977). Animals that recover from babesiosis infection are generally immune for life (Perrey, 1996). Thus, at high levels of tick transmission all newborn calves will be infected by the age of six months, show few, if any clinical signs and subsequently be immune (Callow, 1977). Finally, some breeds of cattle are inherently more resistant to ticks and the clinical effects of Babesia infection (Bock, 2005).

On recovery from initial infection cattle are immune but harbor the parasite (premune) and may remain carriers for up to two years (Callow, 1977). In enzootic areas, constant reinfection ensure that such cattle remain carriers permanently , although the preimmunity may break down due to some factors such as stress, intercurrent diseases resulting in clinical babesiosis, introduction of susceptible cattle to endemic areas and the incursion of *Babesia* infected ticks into previously tick free areas (Perrey, 1996).

2.3 Effects of climate and other a biotic, biotic factors on ticks development, survival and questing.

Many researchers have been interested in studying what a biotic factors drive the biology of ticks. The potential exists in evaluating patterns of activity resulting from various combinations of climatic conditions, and in thereby obtaining an estimation of risk in climate change scenario (Githeko, 2008, Eisen, 2008). Many reports have correlated environmental traits with empirical data concerning the life cycle, and these results have so far confirmed that the development and questing of ticks among other factors are regulated by weather **(**Perret & Gern, 2004, Lee & Milne, 1951, Randolph, 2000).

Like many other arthropods, ticks are very sensitive to climate. They spend most of their life cycle in the environment, and all life cycle stages are dependent on the complex combination of weather variables for development and survival. Host availability and vegetation significantly modulate the dynamics of tick population (Randolph, 1997). However they have smaller contribution than weather in delimiting tick distribution as it is the major driver to the presence or absence of tick species in a given territory (Eisen, 2008).

Vegetation structure plays a significant role in the presence and absence of ticks. Ticks are often recorded in woodland habitats as this provides a dense shrub layer, habitat with bracken, also provide a suitable habitat (Gray, 2009). Grassland is often considered to be relatively unsuitable for ticks (Sombroeck, 1982). Nonetheless, *R. appendiculatus* are often found in grazed pastures **(**Lane& Aderson, 2000). With respect to vegetation type, tick presence is most likely to occur due to their influence on host presence. As a result, areas that are densely vegetated will provide a more suitable habitat for these hosts, this directly increase the abundance of ticks (Perry, 1992). Though many animals can serve as tick host, there are several host determinants, the suitability and specificity of tick-reservoir host-pathogen relationship is the key to understanding the complex processes conditioning the pathogen transmission by ticks (Randolph, 2009). For example, host availability in time and space is an important determinant of tick bionomics.

Seasonal variation in temperature due to global warming can result in a dramatic impact on how often the host and pathogen – carrying vector come into contact (Perret & Rais 2000). Ticks have a sensory organ, Haller's organ used in detecting environmental stimuli such as carbon dioxide, temperature, and humidity; which is situated on the front tarsi (Walker, 2003). It has been hypothesized that at $<7\ ^{0}$c, ticks remain inactive only venturing out to quest when temperatures increase (Macleod, 1939**).**

Many vector borne diseases are highly susceptible to changes in climate. Suss *et al.,* (2008) found that increasing temperature not only increases distribution, but also linked the rise in temperature to encroachment of ticks into higher altitudes that were previously not colonized by ticks. The following possible changes to tick population due to increased temperatures occur; accelerated tick development cycle, an extension of tick development

cycle, increase in population density and a shift in risk areas. Many of these changes could potentially increase disease incidence (Niez, 1965).

Though surveillance and reports on the changes in the distribution of tick population is generally inadequate, some well documented reports support the slow but apparently continuous expansion of the historical frontiers of some tick species in areas where they were previously absent (Gray *et al,*. 2009). As a consequence, a correlation is formed between increase in temperature and increase in the prevalence of tick borne diseases (Suss *et al.,* 2008)**.**

The potential of changing rainfall has largely been ignored although this may have a greater effect than temperature on the ability of tick populations to establish in invasive events (transportation of an exotic tick species into areas from its native). In addition change in precipitate from year to year affect disease incidence (Randolph, 2000). Multiple studies have shown that there is a correlation between the amount of precipitate and disease incidence. During a year with little precipitate many ticks may die following feeding because of loss of water regulatory control and they never reach adulthood thus lowering transmission (Lane, & Adderson, 2000).

Many ticks are exophilus meaning that they quest in open habitats, not in protected environment as the endophilus (Wharton, 1964). The mortality observed during tick development is regulated by water losses, which in turn are greatly influenced by temperature and the physical properties of the arthropod cuticle (Adderson, 1936). Weather thus affects tick survival mostly during the non parasitic period and their life cycle because

tick survival and host seeking activity are largely inhibited outside certain ranges of temperature and rainfall (Randolph, 1997).

If developing and host seeking ticks suffer mortality at an approximately constant rate in nature, then the lower the temperature, the longer is the developmental cycle and the higher is tick mortality (Lane & Adderson, 2000). Therefore warm weather contributes to increased tick abundance because of fast development and lower mortality and the probability to colonize new territory (Ogden *et al.,* 2004).

Most ixodid ticks are inactive in the lowest layer of the vegetation or litter before they begin to quest (Lane *et al.,* 1995). The combination of a set of suitable conditions which normally involves an activation of temperature triggers the activity causing ticks to climb to the top of the vegetation and quest for the host (Lees & Milne, 1995). During questing ticks may lose water (Lees, 1946) that they normally regain by descending at intervals to the litter zone (Lees & Milne, 1951) where they actively reabsorb water vapour from the atmosphere, (Rudolph & Khull, 1974). After ticks are rehydrated, they are ready to ascend the vegetation and they have varying abilities on water retention or gaining (Kahl & Knull, 1995), and there is an inter specific variability in the management of their water balance.

Extrinsically, tick water balance is affected by saturation deficit of water in the air (affecting water loss) and by relative humidity (affecting the possibility of water gain by active water vapour uptake), and intrinsically, among other points the capability of ticks to find places with favorable microclimate (Wharton, 1964). The energy reserves of the tick and its ability to maintain an acceptable level of water are the factors mainly regulating the short term questing behavior of ticks (Gray *et al.,* 2009).

It has been demonstrated that questing duration of nymphs is inversely related to the saturation deficit, whereas the quiescence is not (Perret & Gern, 2003). When environmental conditions are less desiccating, ticks will quest for longer periods (Lees, 1946). Abrupt decline in the proportion of questing ticks has been shown to coincide with the abrupt increase in saturation deficit, (Perret & Rais, 2000).

Relative humidity has a critical effect on the ability of ticks to absorb water. There is a critical equilibrium above which ticks can actively absorb water and maintain water balance (Wharton 1964), and survive for longer periods irrespective of their saturation deficit. Below this threshold of relative humidity, ticks cannot actively absorb water. Therefore under conditions of low relative humidity ticks cannot rehydrate even if the saturation deficit is low. However, under conditions of high relative humidity for example more than 85% ticks can actively take up water from air and saturation deficit may have a lesser role in regulating questing (Rao *et al.*, 1996). Unfed ticks require a RH of above 80% to survive, with anything less having a detrimental effect on the tick survival (Thornton *et al*., 2000). It is therefore believed that humidity is a fundamental factor influencing tick survival and questing time (Randoph, 2000).

Besides weather or microclimate, photoperiod regulates tick seasonal periodicity by preventing behavioral diapause in unfed ticks or stimulation of questing in unfed species, or by interrupting development (morphogenetic or developmental diapause) in engorged ticks. In both cases the inducing stimulus is the photoperiod, day-night relative duration (or its changes) which are perceived by ticks (Belozerow, 1982). These diapause mechanisms optimize the fate of a given population by enabling them to find a host, feed and enter the molting period before the onset of adverse weather conditions.

Light and photoperiod stimuli also have pronounced effect on the behavior and physiological development of females. Interestingly, it has been found that light levels significantly influence the presence of ticks; it has been predicted that 10EVs, is the optimum light intensity for questing ticks, with intensities above this level significantly reducing the numbers of ticks captured. Perret and Gern (2003), showed that under continual exposure to high intensities of light, ticks remain at rest and only begin to quest in response to a fall in light intensity.

It has also been hypothesized that newly molted ticks actively avoid direct light but become indifferent as they age, this may be directly linked to the increased pigment deposition in the cuticle (Belozerow, 1982). Thus most of the ixodid ticks fall under the diurnal rhythm and have peak rates of oviposition during the hours of dawn and dusk (Lane & Aderson, 2000).

Weather can affect not only tick development and mortality, but also the activity rate, a feature deeply affecting infestation risks and the intensity of tick infestation in reservoir host. Ticks quest at variable heights in the vegetation, driven by factors such as temperature and relative humidity and light intensity (Randoph, 2000).

2.4 Acaricide mode of application

The main methods employed in the control of tick borne disease include vector control, host immunization, use of chemotherapy and integrated control method which combines any of the methods. During the last 80 years the control of ticks and diseases they transmit has been largely through the application of acaricides. Introduced in 1902, the use of

acaricides was used to eradicate ECF from S. Africa by 1954 and Swaziland by 1960 (Lawerance, 1992). Methods of application employed include; dipping and spraying, with dipping being considered the most effective. In recent years , several other means of acaricide application have been developed including slow release of systemic acaricides from implants and boluses, slow release of conventional acaricides from impregnated ear tags, pour-on's (which are applied on the back and spread rapidly over the entire body surface), and spot-on's. However acaricides have developed their own disadvantages; they are expensive, resistance can be developed and are detrimental to the environment as reviewed by (George *et al.*, 2004).

Immunization concept against TBDs involves an elaborate infection and treatment strategy (ITM) whereby live parasites are inoculated into an animal while simultaneously treating the animal with a long acting tetracycline antibiotic. This procedure results in a mild controlled reaction to the parasite infection that leads to the development of immunity to subsequent infection. The immunity last up to 3 years in the absence of further tick infestation but is lifelong if infected ticks continue to challenge the immunized animal regularly (Di Giulio & Bishop, 2009). The short comings of immunization include; infection with live parasites may be lethal if misused, full protection may not be achieved in areas where several strains of the parasites are present, and finally cattle may become carriers of the parasite used in immunization.

In chemotherapy control of ECF tetracycline antibiotic was probably the first compound to be used in 1953 (Keating, 1983). As tetracycline therapeutics was more limited to only the early stages, more effective derivatives were discovered in the late 1970s. These drugs are however, expensive and reduce their efficacies in the field (George *et al.*, 2004)).

Other novel methods that have not found a wide application include pasture spelling, planting of tick repelling grasses, use of tick vaccines (Willidsen, 2004) and restriction of livestock movement.

Generally the occurrence and importance of TBDs is a reflection of a complex interaction involving the causative organism, the tick vector, the vertebrate host and the environment (Norval *et al.*, 1992). These interactions are driven and modified by a wide variety of factors ranging from climate, soil and vegetation to human activities including crop/ livestock production systems and tick control measures. The impetus to carry out this study in South rift came out after the occurrence of vector borne disease that ranked TBDs as the most important constrain to dairy production (Abate, 1992).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

This project was carried out in the Southern Rift valley of Kenya. Field sampling was conducted from the following counties/sub counties: Kericho ($0^0 22^0$E latitude, 36^0S21^0N), with an altitude of 2178m above the sea level, Bureti (0^o 36 ^{o}S 35^o 16^oE with an altitude of 2178m above the sea level (Kericho county) and Bomet (35^o21^oE, 47^o78^oS) with an elevation above the sea level of 1981m (Bomet county). Rainfall in the region ranges from 1200-1800mm monthly with long rains starting from March to September; peak periods are May and August with a mean annual temperature of 18^0c for the region. Farmers in the region practice small scale mixed farming. The grazing system is predominantly free grazing but a few farmers do practice zero grazing.

3.2 Apparatus and Consumables

The study involved both entomological and parasitological data collection. Apparatus required included: microscope, centrifuge, freezer, Elisa kit, EDTA vaccutainer tubes, VMR frosted slides, gloves, forceps, labels, venoject needles, vials, tape measure, dragging cloth material, slide box, cotton wool. Consumables/chemicals included: Geimsa stain reagents, Dulbecco's phosphate buffered saline, Tween and skimmed milk, casein, IgG monoclonal antibodies, *Theileria* and *Babesia* specific antigens, Horse Radish Peroxidase, Diammonium salt, Sodium citrate, methanol, distilled water, oil immersion.

3.3 Study design and Sampling technique

Cross-sectional study design was employed and Purposive sampling was used in site selection while the study animals were recruited from randomly selected farms. Based on the information obtained from the Veterinary Department Office, there was an estimated population of 453,634 cattle in the South Rift, Kericho (130,712), Bomet (210,855), and Bureti (112,087). Blood serum samples were collected from 48, 57 and 91 cattle from Kericho, Bureti and Bomet respectively making a total of 196 blood serum samples and a similar number of slides were also prepared. Selected cattle population composed of 70 calves (27 males, 43 females), 126 adults (9 males, 117 females). Sampling was conducted over a period of three months with the selected farms each being visited only once during the study period.

To evaluate the control strategies employed by farmers against ticks and ticks borne disease, a questionnaire was administered to investigate their knowledge on ticks and tick borne diseases, type of tick control strategies employed, method of application, and frequency of application. The questionnaire was administered in English and translated into the local kipsigis language whenever the respondents had limited knowledge of English. To maintain consistency, the questionnaire was designed in a closed format except for the introductory section. In cases where the set of expected responses was deemed not exhaustive, an option for “others: please specify” was provided (Appendix IV).

3.4 Sample size determination

Sample size was obtained by the use of the formula as described by Thrushfield (1979), with the following parameters; N = no of animals sampled (sample size), Z = statistical constant-representing the number of standard deviations on either side of the mean in a normal distribution of variables i.e the confidence limits 1.96 (95%), p = estimated percentage value of the incidence in the region (15%), q = probability of not being infected (100-p) and d= marginal error.

N = Standard deviation × Incidence rate × probability of infection/Marginal error

$$1.96\times196\times0.15\times0.85/0.05^2= 196$$

Thus the total sample size was 196.

3.5 Selection of farmers for the cross sectional study

A list of all livestock farmers was compiled with the assistance of local chiefs. Using a random number table, 10 farms were randomly selected from the 10 selected locations. Permission was obtained from farm administrators who were also notified of the intended survey and objectives of the study explained to them during the preliminary survey.

Table 3.1: Summary of selected administrative sub counties, farms and the number of cattle sampled

Administrative sites	No. of farms selected	No. of cattle sampled
Bomet Central	40	91
Bureti	30	57
Kericho West	30	48
Total	100	196

3.6 Parasitological investigations

3.6.1 Sample collection and microscopic examination

A study team comprising the investigator and a laboratory technician who assisted in handling the animals and performing the initial processing of biological samples before being dispatched to the laboratory. Every farm was visited once during the three months study period and blood smears and serum collected from at least one animal from the different farms.

To determine the prevalence of protozoan tick borne diseases, thin blood smears from marginal ear vein were prepared at the collection site and labeled based on the site, animal code, age, sex of the animal and date. They were then packed in the slide box and transported to the laboratory for processing within 48 hours. They were fixed in methyl alcohol for 10 minutes and stained with Giemsa stain for 30 minutes, washed with tap water and air dried. The stained blood smears were observed microscopically and the parasites

were identified based on their morphological characteristics as described by Souls by (1982).

3 .6 .2 Serological technique for diagnosis of tick borne protozoan diseases

Animals were bled for serum from the jugular vein into vacutainer tubes (10 ml without anticoagulant; Becton Dickinson) (Plate 3.1). Each sample was labeled with the individual animal code, sex, age, collection site and date. Efforts were made to transport the samples within 48 hours to Kericho Veterinary Investigation Laboratory where sera were separated from blood clot by centrifugation at 5000 rmp for five minutes. Collected sera were aliquoted into vials and kept at - 20^0c until dispatched to KARI Muguga for ELISA Test in December 2012.

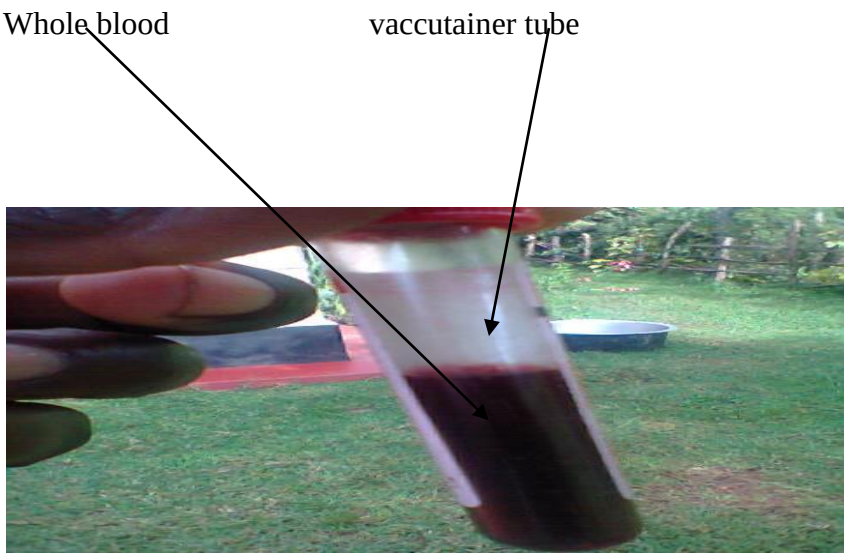

Plate 3.1: Blood sample in vaccutainer tube (Source: Author, 2013)

The ELISA tests for ECF and Babesiosis were carried out as described by Nielsen *et al.*, (1996) and Katende *et al.*, (1998). Briefly the specific antigens (the polymorphic immunodominant antigen for *Theileria parva*, and p200 kilo Dalton antigen for *Babesia bigemina* was used to coat Starwell polysorp micro-ELISA plates (Polysorp, Denmark). The specific antigens were provided by ILRI after obtaining them from infected tick sporozoites. Casein, 0.25% acted as the blocking agent. The test sera was diluted at a ratio of 1: 200 for *Theileria parva* and 1: 100 for *Babesia bigemina* in Dulbecco's phosphate buffered saline (DPBS) (pH 7.4), containing 0.1% Tween 20 and 5% skimmed milk.

The presence of antibodies to specific parasites antigens was tested by addition of test sera into wells in two replicates. The plates were incubated for 25 minutes at 25^0C to allow antibodies to bind to specific antigens and unbound antigens washed off using DPBS. To ensure that the antigen –antibody reaction had taken place, anti-bovine IgG monoclonal antibodies conjugated to Horse-Radish Peroxidase (HRP) were added.

The reaction was visualized by addition of 1% hydrogen peroxide as substrate and 40nM 2, 2'-amino-bis (3-ethylbenz-thiazoleline-6-sulphuric acid), diammonium salt (ABTS) as chromogen in sodium citrate buffer pH 4.0. The plates were then incubated in the dark for one hour for colour development.

Intensity of the colour developed (optical density; OD) was determined using an ELISA reader. OD which is reflection of the amount of antigen present in the sample increases with the level of parasitemia. Using the OD reading from highly positive reference sera (C++) included in the ELISA, the optical density value was read at 415 nm for *B. bigemina* and 450 nm for *T. parva.* The percentage positivity (PP) of the test sera was computed using the two-graph receiver operating characteristic (TG-ROC) and calculated by the formula:

(Replicate OD value of test serum/Replicate OD value of positive control) ×100.

Where by a sample was considered positive if the percent positivity value was >15 for *Babesia bigemina,* and >20 for *T. parva.*

For micro plate acceptance the strong positive standard (C++) had to fall within the upper and lower control limits established by the test protocol 0.800 –1.800 for *B. bigemina* and 0.850 –1.750 for *Theileria parva*.

3.7 Entomological survey

An estimate on the density of questing ticks was obtained by collecting questing ticks from the vegetation following the technique described by Lane& Aderson (2000). Each of selected farms (100) was visited before 11 am and sampled once over a period of three months; a piece of cloth ($1m^2$) was mounted on poles and dragged in a zig zag over homogenous patches of vegetation as described by Milne (1943).

To determine tick density (abundance per unit area) it was necessary to walk slowly over a defined distance (100m), since an area of less than 100m may adversely affect the reliability of the results. Ticks adhering to the fabric were collected at varying intervals of 20m since prolonged dragging could result in loss of some of the ticks during the exercise.

After collection, ticks were kept in corked glass tubes and later taken to the laboratory where they were preserved in 70% alcohol and processed by counting and identifying based on morphological and structural differences as described by Walker *et al.,* (2003). The following tick characteristics were examined idiosoma, punctuation pattern and shape of

posterior margin, basis capituli, festoons, lateral and post medium grooves, and spur on tronchanter. These were compared to taxonomic guide described by Hoogstraal (1979).

Weather variables measurements (temperature and relative humidity) were obtained from metrological stations in the region and they were correlated to temporal tick abundance.

3.8 Data management and Data analyses

Questionnaires were manually filled in the field while entomological and parasitological samples were only labeled. After laboratory analysis, spreadsheet (Excel) was used for data entry and storage. SPSS was used to analysis data. Statistical analysis were done using Chi square (to compare TBDs between age and sex, accaricide mode of application with the number of cattle infected), ANOVA (to analyze TBDs distribution within the study site and to compare the different developmental stages of tick collected) by use of Mini tub 14 soft ware. Correlation and Regression were also used to analyze the relationship of temperature and humidity on vector abundance as described by Terry (2002).

CHAPTER FOUR

RESULTS

The microscopic examination of 196 Geimsa stained blood films showed that Theileriosis prevalence rate was 11.2% while Babesiosis prevalence rate was 18.38 % (Table 4.1). The occurrence of Babesiosis was highest in Bomet (36.8%) while Theileriosis was highest in Kericho West (18.75%) as compared to the others. Overall prevalence rate of TBDs was 29.6%.

Table 4.1: TBDs parasite species prevalence in the study sites

Collection site	Babesiosis	Theileriosis	Total	p-values
Kericho	3 (6.25 %)	9 (18.75 %)	12 (25 %)	0.00015
Bureti	14 (15.47 %)	7 (12.3 %)	21 (27.47%)	
Bomet	19 (24.6%	6 (12.2%)	25 (36.8%)	
Total	36 (18.38 %)	22 (11.2%)	58 (29.6%)	

The difference in occurrence and distribution of these TBDs was statistically significant ($p < 0.05$)

Basing on morphological characteristics of the merozoites in infected erythrocytes by Geimsa stained blood, *Theileria parva and Babesia bigemina* were identified by light microscope under a magnification of ×100. *T. parva* appeared as small, comma shaped piroplasm in RBCs with a pale blue cytoplasm and a red chromatin granule. They were also paired at an obtuse angle to one another (Plate 4.1)

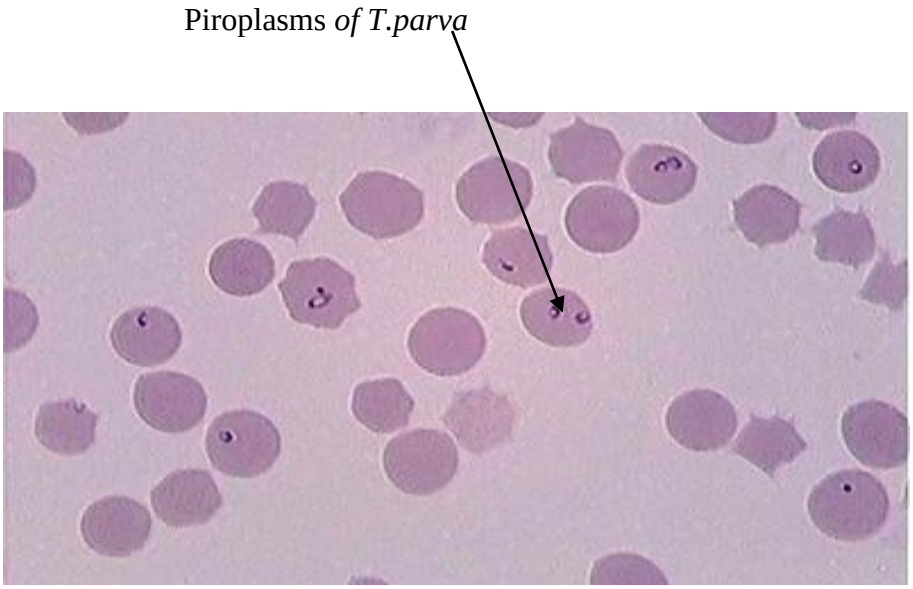

Plate 4.1 Thin blood *Theileria parva* (Source: Author, 2013*)*

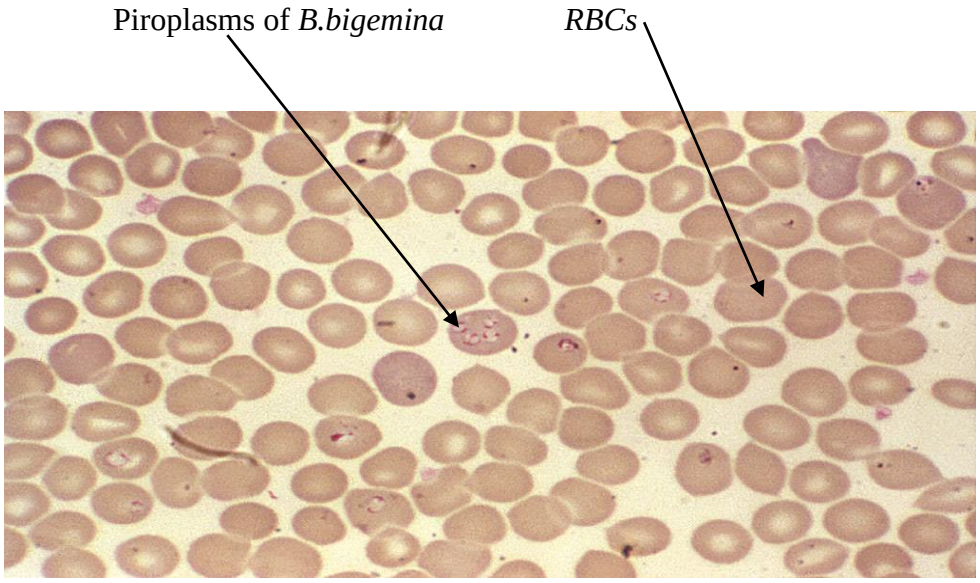

Plate 4.2 Thin blood smear of *Babesia bigemina* (Source: Author, 2013)

Serologically results indicated that TBDs occurred with an overall prevalence rate of 66.84% and females were more infected than the males (Table. 4.2). When age related prevalence was considered, it was found that bovine having less than one year were less infected than the older one's (Table 4.3).

Table 4.2 Prevalence of TBDs in relation to gender

Age	Infected	Not infected	Total	p - value
Male	15 (7.65%)	21(10.71%)	36	0.160
Female	116(59.18%)	44(22.44%)	160	
Total	131(66.84%)	65 (33.16%)	196	

The difference prevalence of TBDs in relation to gender was statistically insignificant

$\sum X^2_{(0.05)} = 12.65$, $p>0.05$.

Table 4.3: Comparison of TBDs infection rate in relation to age groups

Age group	Infected	Not infected	Total	p - value
<1year	21 (10.72%)	49 (25%)	70	0.340
>1year	110 (56.12%)	16 (8.16%)	126	
Total	131 (66.84%)	65 (33.16%)	196	

The difference in prevalence of TBDs in relation to age was statistically insignificant

$X^2_{(0.05)} = 66.66$, $P > 0.05$.

From the entomological survey, a total of 195 ticks were collected and most of them were in their larval stage (Figure 4.1)

Plate 4.4: Adult *Boophilus microplus* (Source: Author, 2013)

Plate 4.5: Nymph of *Rhipicephalus appendiculatus* (Source: Author, 2013)

Table 4.4: **Number of different developmental stages of questing ticks collected in sub counties**

Sub county	Larva	Nymphs	Adults	Total	p-value
Kericho	41	13	7	61	0.0257
Bureti	36	22	6	64	
Bomet	46	10	14	70	
Total	123	45	27	195	

Larva occurred in the highest number (123), followed by nymphs (45) and finally adults (27) with the highest collection site being Bomet, Bureti and Kericho respectively. The difference in the occurrence and distribution of these developmental stages of ticks was statistically significant, ($P < 0.05$).

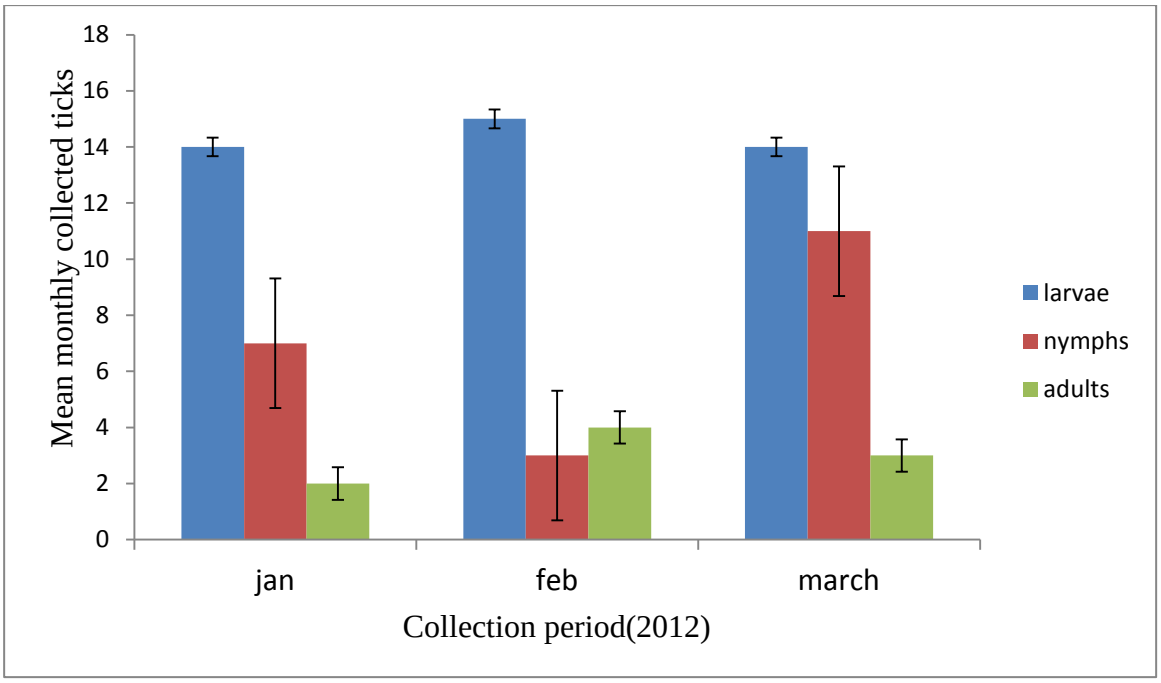

Figure 4.1: A multiple bar chart showing the mean monthly collected ticks in the study region.

The graph shows that a mean total of $14\pm0.14_{S.E}$ (larva), $7\pm 2.7_{S.E}$ (nymphs) and $2\pm0.21_{S.E}$ (adults) in January were collected. February, a mean total of $15\pm0.93_{S.E}$ (larva), $3\pm1_{S.E}$ (nymphs) and $4\pm0.57_{S.E}$ (adults). March a mean total of $14\pm0.7_{S.E}$ (larva), $11\pm2_{S.E}$ (nymphs) and $3\pm0.5_{S.E}$ (adults) were collected. High larval count was observed throughout the three months with the highest being in February.

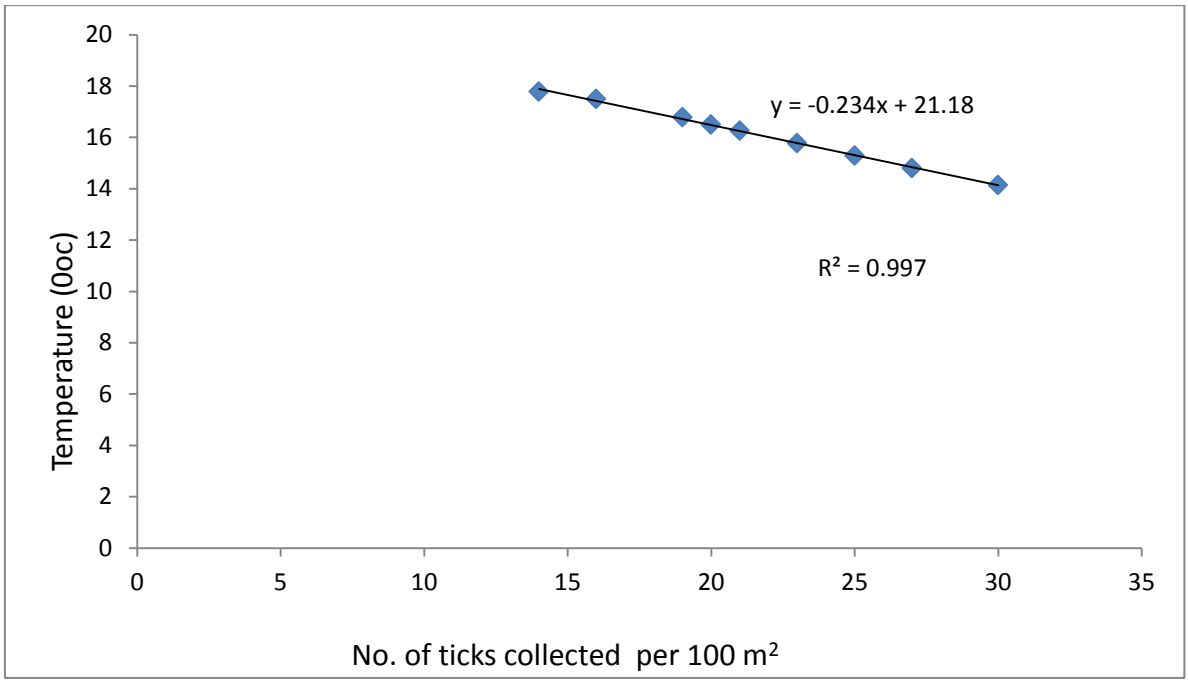

Figure 4.2: Relationship of ticks collected with the prevailing Temperature (0^{o}c)

The equation y = -0.234x+21.18 represents the regression equation obtained from the least square regression analysis. The slope or the regression coefficient (b) was found to be negative (- 0.235) and the intercept (a) was found to be 21.19. R^2 (0.996), represents the coefficient of determination and indicates that the regression equation accounts for 99.6% of the total variation in vector abundance during the collection period. Statistically the regression coefficient was found to be significant ($p<0.05$).

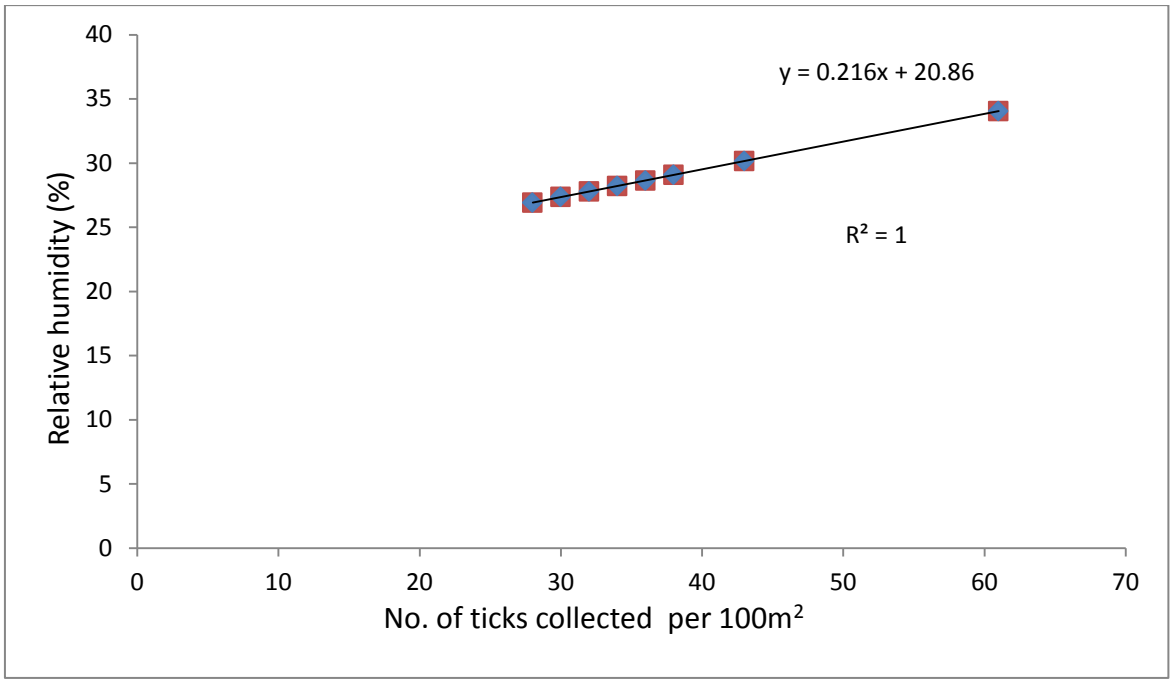

Figure 4.3: **Relationship of ticks collected and the prevailing Relative Humidity (%)**

The equation y = 0.216x + 20.86 represents the regression equation obtained from the least square regression analysis. The slope or the regression coefficient (b) was found to be positive (0.216) and the intercept (a) was found to be 20.86. R^2 (1) represents the coefficient of determination and indicates that the regression equation accounts for 100% of the total variation in vector abundance during the collection period. Statistically, the regression coefficient was found to be significant ($p<0.05$).

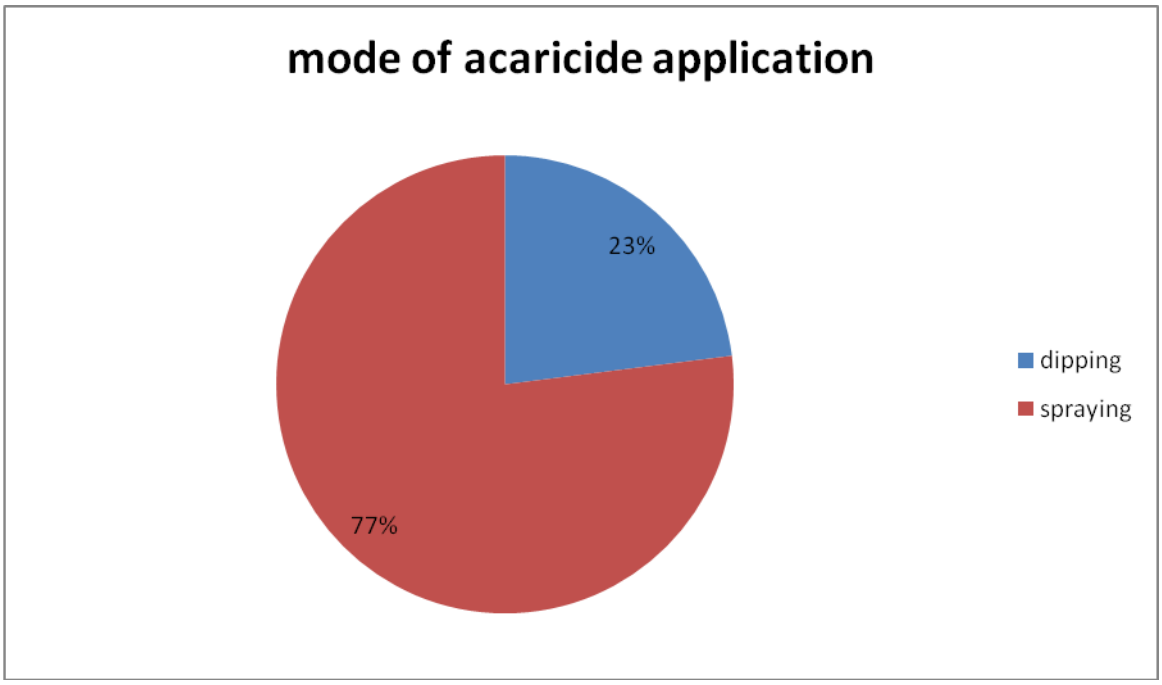

Figure 4.4 Pie graph on accaracide mode of application

An investigation on methods of accaricide application the residents used in tick control showed that 23% of the farmers used dipping while77% used hand spraying (Figure 4.4).

Table 4.5: Number of cattle infected with TBDs distribution according to the method of tick control applied in the study region

Method of application	Infected	Not affected	Total	p-value
Dipping	22 (11.22%)	40 (20.4%)	62	0.065
Hand spraying	109 (55.61)	25 (12.7%)	134	
Total	131(66.83%)	65 (33.1%)	196	

Hand sprayed cattle were more infected 109(55%) than dipped cattle. The difference in the tick control strategies applied was statistically insignificant, $\sum X^2_{(0.05)} = 40.2$, $p > 0.05$.

CHAPTER FIVE

DISCUSSION

The diagnosis of bovine piroplasmosis in Kenya in earlier studies was mainly based on microscopic examination. Up to date the diagnosis of tick borne diseases still depends on observing the parasites in the infected RBCs when the parasitemia rates are indicative. Even though this diagnostic method can be easily applied in the field, the sensitivity of the method and its failure to detect piroplasmosis, if the number of parasite in the peripheral blood is low, illustrate the limitation of the parasitological diagnosis by Geimsa staining (Katende *et al,*. 1998). In this study serology was seen to be more effective than microscopic. Morphologically *T. parva* appeared comma shaped (Plate 4.1) while *B. bigemina* appeared pear shaped (Plate 4.2), this conforms with Souls by (1986) who found that piroplasms could either be oval, round, comma shaped, ring shaped, piriform or amoeboid. Statistical analysis on distribution and occurrence of these TBDs was significant (Table 4.1 & Appendix: VII).

More females were infected than males and bovids of less than one year of age were also less infected as compared to those of more than one year of age (Table.4.2). Statistically the results were insignificant. This agreed with Morzaria *et al.*,(1999) who found that age , sex and breed influence the prevalence of tick borne protozoan diseases and that age of the host influence it's susceptibility to infection. Also increasing age was associated with TBDs prevalence and this was particularly noted also in studies conducted at the Kenya Coast and in Rusinga highlands (Morzaria, *et al.*, 1988, Muraguri, 2000). These results may be expected since age was a proxy for time exposure and antibodies persist in circulation for as long as six months only (Katende, *et al*., 1998). Radostits, *et al*., (2007)

found that age of the host influences its susceptibility to infection and therefore affects the incidence of a disease from the age of six months. However this might be the role of passive immunity protection which can be induced via colostrums in first age groups and recovery from acute phase of infection resulting to preimmunity thus preventing challenge infection.

Babesiosis had a high infection rate as compared to Theileriosis (Table: 4.1). Statistically there were no significant difference. These results concurred with those of previous studies conducted in countries from the tropical regions (Johsson *et al.*, 2008). In regions in Eastern and Southern Africa, studies indicate that bovine babesiosis presents sero positivity ranging from 19.5-94% (Lawrence *et al*., 1995), also in a study in Kenya the prevalence of babesiosis in cattle was 37.1% (Okutu & Buyu, 2006). The presence of TBDs could be attributed to the presence of their vectors in the study sites and this indicates the presence of a positive correlation between the prevalence of disease and the distribution of the vectors.

The study also aimed at correlating temperature and relative humidity with vector abundance, elucidating which factors are essential for predicting 'hot spot' areas. The study revealed that ticks were abundant in Bomet. These can largely be attributed to the warm weather conditions, availability of the host and break down in tick control services formerly supported by the government not only in the county but in the rest of the country. The highest temperature recorded was associated with low number of ticks collected. This was not surprising as ticks are more likely to be found hiding to prevent desiccation. From the regression equation temperature was found to have a negative slope, (Figure 4.2) with tick abundance, implying that as the temperature increase the number of questing ticks decrease in order to avoid desiccation and the variation in the questing activity of ticks is brought about by temperature changes. Statistically the regression coefficient was found to be

significant and this assumes that it is a reasonable hypothesis that tick abundance and questing activity is influenced by temperature**.**

Another weather variable studied was relative humidity, with a large number of ticks being collected at the highest relative humidity recorded (figure4.3) a value slightly lower than 80% RH required for survival as stated by Macleod, (1939). Arthur (1962) suggested that between 86-96% RH the water balance is in equilibrium with the atmosphere, allowing the tick to gain moisture from the air and conversely loose water when humidity is low. But still at higher humidities, studies have shown that it is not favorable for tick presence as they become prone to over saturation thus limiting their activities.

From the regression equation (Figure 4.3) relative humidity was found to have a positive slope meaning as the relative humidity increase, the number of questing tick increase since they rehydrate at high relative humidity. The regression coefficient showed that the variation in the questing activity and abundance of ticks is influenced by changes in relative humidity. As an assumption it is a reasonable hypothesis that tick abundance and questing activity is influenced by relative humidity.

Two tick genera (*Boophilus* and *Rhipicephalus*) were identified in the study area with varying abundance (Plate 4.3&4.4). There were a high number of larvae collected than nymphs **(**Figure 4. 1). This could be construed as a consequence of the greater number of adults on these animals, also because larvae tend to show aggregated distribution with large population usually arising from one egg batch. *Boophilus* adults were also found and theoretically only the larvae should quest for the host from vegetation, implying that they must have detached shortly before or after molting and were now questing for the second host. These shows that different developmental stages of ticks quest at different rates due to the prevailing weather conditions. This agrees with Dobson, *et al*., (2011) who observed

that the onset of questing occurs when daily maximum temperature reaches above 7^0c for nymphs and adults and 10^0c for larvae, but this may vary between regions (Gilbert, *et al.*, 2014).

Many modes of accaricide applications have been employed in Kenya but the Enactment of the Cattle Cleansing Act (GOK, 1976) led to the initiation of a national tick control programme. However, adequate control of ticks and TBDs is still far from being achieved. The escalating costs of acaricides, relevant infrastructure and monitoring services for the intensive tick control strategies advocated by the Cattle Cleansing Act led to the inability of the government services to sustain the programme. Just like in other parts of the country, poor management of dips by local committees in the counties such as in Kericho virtually led to the collapse of the tick control facilities (dipping). As a result virtually almost all the farmers depend on hand sprayings as a vector control strategy than dipping **(**Figure 4.4**).** Dipped cattle were less infected than hand sprayed cattle. This shows that dipping is more effective but was not much used in the study area (Table 4.5). It was evident from the questionnaires administered that although most of the farmers applied conventional acaricides (Organophosphates) on regular basis still ticks were found on their farms**.** This was so even in instances where farmers had indicated that they had applied acaricides on the animals a few days prior to the visit. This showed that probably farmers applied under strength concentrations of acaricides either due to inability to follow instructions or due to financial difficulties or ticks might have developed resistance to them. The problem was clearly demonstrated in Bomet central sub county which had a significantly higher prevalence of TBDs compared to most of the others. This was probably contributed by the grazing system (communal grazing), cross breed cattle grazing together with goats, warm weather conditions which accelerate tick development cycle and thus increased egg

production. Recently, goats have been implicated as alternate hosts for *Boophilus* species, but in the presence of cattle in order to maintain populations (Nyangiwe and Horak, 2007).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

As per the research, it can be concluded that tick borne protozoan diseases are still a problem in Southern Rift Valley and the presence of the parasites serves as indicators of being the causative agents for the past endemic diseases encountered in the region. However we reject the null hypothesis that infection is independent of age and gender.

Vector abundance is correlated to weather variables and the regression equation obtained can be used as a basis of prediction.

Though hand sprayed cattle showed high infection, but still vector control measures employed were not effective.

6.2 Recommendation

Kericho West Sub County which had the highest prevalence of Theileriosis infection should be given the first priority in the upcoming planned ECF vaccination.

Further studies should be conducted on how other weather variables affect tick activity. This will assist in predicting the areas of hotspot activity of ticks and thus acting as a warning indicator to reduce the risk of exposure.

Farmers should be encouraged to utilize dipping method and other integrated control package should be initiated. It was also suggested that dip analysis should be conducted and monitored for correct use.

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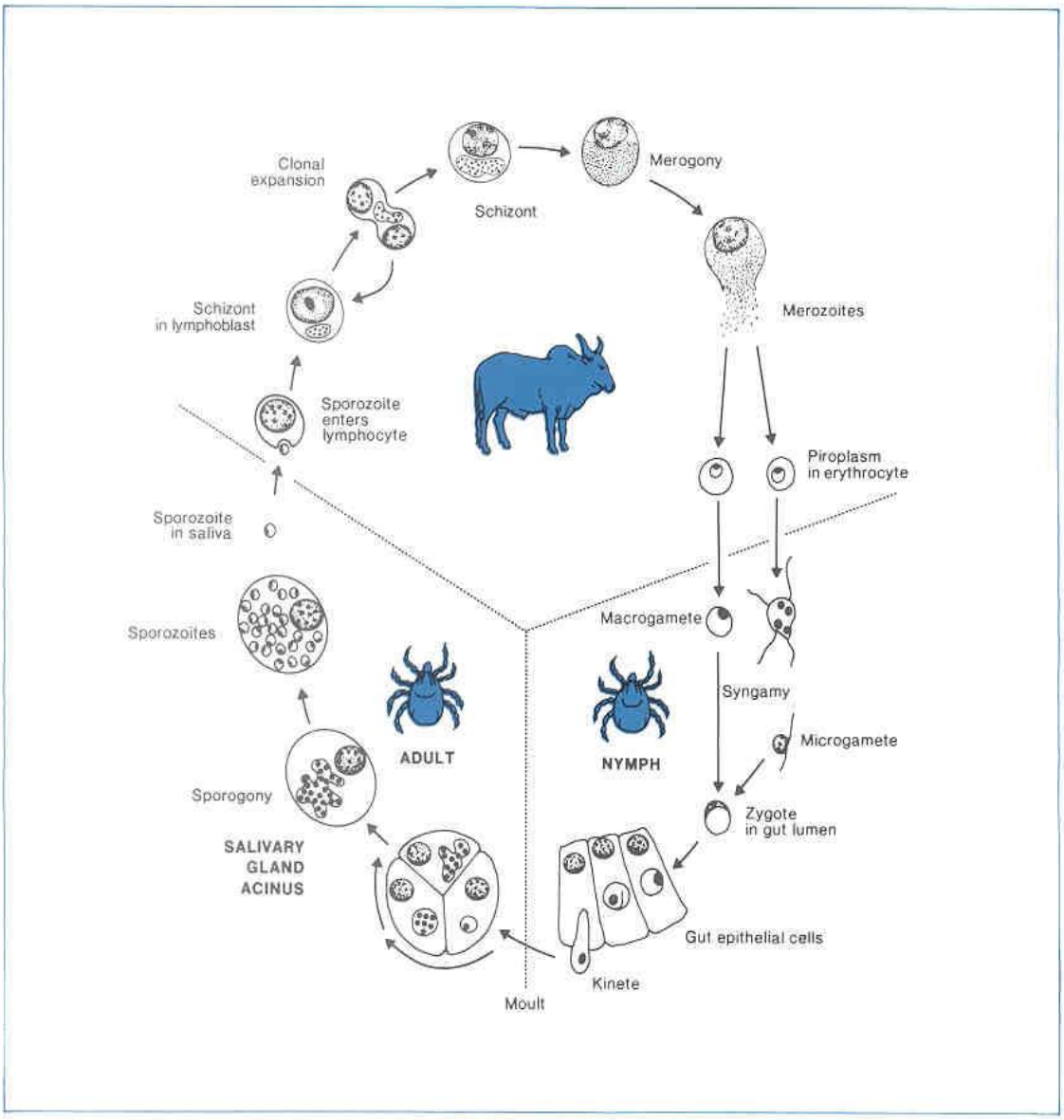
APPENDICES

Appendix I: Important species of *Theileria* (Source: Menlhorn and schein, 1984)

Species	Vector	Vertebrate host	Disease	Geographical
Theileria parva parva	*Rhiphicephalus appendiculatus*	cattle, African buffalo	ECF	Africa
T. p . lawrencei	*Rhiphicephalus appendiculatus*	cattle, African buffalo	Corridor disease	Africa
T. annulatu	*Hyaloma* species	cattle, Buffalo	Mediterranean theileriosis	Africa, Asia,
T.muttans	*Amblyoma* species	cattle	Benign Africa theileriosis I	Africa
T. taurotragi	*Rhipicephalus appendiculatus*	cattle, *T. oryx*	Africa Benign theileriosis II	Africa
T. orientalis	*Haemaphysalis* species	cattle	Oriental theileriosis	Asia Europe,
T. hirci	*Hyaloma*	sheep goats	Malignant, Ovine, Caprine theileriosis	Europe, Asia

Appendix II: Life cycle of *T. parva* in the cattle & the ixodid tick (Source: Moll, 1986)



Appendix III: Important species of *Babesia* (source: Mehlhorn and Schein, 1984)

Species	Vector	Infective stage	Veterbrate host	Geographical distribution	size (µm)
B. bigemina	*Boophilus* Species	Adults, nymphs	cattle, water buffalo	America, Asia, Africa, Europe	5×2
B. bovis	*Boophilus* Species	larvae	cattle, water buffalo	S. Europe Asia, Africa	2.5×1.5
B.divergens	*I. ricinus*	larva	cattle,	Europe	1.5×0.5
B.major	*H.puctata*	Adults	cattle	Britain	3×1.5
B. motasi	*Haemaphysalis*	Adults	sheep, goats	USSR,	4×2.5
B.ovis	*Rhipicephalus Bursa*	Adults	sheep goats	Europe Asia, Africa	2×1
B. cabali	*Hyalomma*	Adults	Horses	Europe Africa,	4×2.5

Appendix IV: Questionnaire Format

Section one: Introduction

Good morning, I am Irene chepkoech an Msc student from University of Eldoret and currently I am doing a research on the prevalence of tick borne protozoan diseases (ECF and Babesiosis) in southern Rift valley of Kenya. I kindly request for your assistances and permission to use your bovids, your responses will be confidential. Thank you.

Section two: Respondent records.

Name of the farm administrator:

Date of the interview:

District:

Village:

Educational level of the respondent (std), (form)

Section three: Diagnosis of TBDs

Have you heard of TBDs? for example ECF (Cheptikonit) and Babesiosis (Sasioto).

Yes (1)

No (0)

Have your cattle been affected? If so, to whom do you go for assistance / treatment?

Veterinary Investigation labs (1)

Private veterinary practitioners (2)

Traditional herbalist (3)

Do you have any knowledge on their vectors? If so what control strategies do you employee in their control?

Conventional acaricides (1)

Botanicals (please specify) (0)

What methods do you employee?

Dipping (4)

Hand spraying (3)

Hand dressing (2)

None (1)

How frequent do you apply?

Regular - As per the manufactures instructions (3)

Irregular - In cases of tick challenge (2)

None-No tick control measures applied (1)

What grazing systems do you employee in your farm?

Zero grazing (1)

Semi zero (2)

Tethering (3)

Communal (4)

Appendix V: Letter from KVIL

REPUBLIC OF KENYA

Ministry of Agriculture, Livestock and Fisheries

Telephone: 020-2168147
When replying please quote

Ref. No. **VIL/TRAIN/VOL.I/161**
and date

VETERINARY SERVICES DEPARTMENT
VETERINARY INVESTIGATION LABORATORY
P. O. BOX 191
KERICHO 20200
Email: vilkericho@yahoo.com

15th July 2013

TO WHOM IT MAY CONCERN/Ms IRENE CHEPKOECH

Kericho Regional Veterinary Investigations Laboratory is a Public Institution which was established to combat animal diseases through application of veterinary laboratory technology. The facility is a regional resource whose jurisdiction covers 8 counties in total: 6 in the former Nyanza Province and 2 in southern Rift Valley – Siaya, Kisumu, Homabay, Migori, Kisii, Nyamira, Kericho, Bomet and parts of Narok (Transmara).

In broad terms the laboratory provides the following services:

1. Disease diagnosis
2. Disease investigations
3. Epidemiology and surveillance of trade sensitive diseases for regional and national strategic planning
4. Research-extension-farmer linkage
5. Research liaison
6. Quality control

In the new constitutional dispensation we will be undertaking food safety testing as well.

Ms Irene Chepkoech worked at our laboratory facility for her research project between January 22nd 2012 and 19th March 2012, processing a total of 197 thin blood smears as well as harvesting 196 sera.

I admitted her on conviction that her work would greatly enhance our understanding of the salient factors critical to the control of East Coast Fever in the region, a disease that has a serious economic impact on dairying. Ms Irene was a keen listener, who managed her time well, as protocols for collecting and submitting samples to the laboratory were very tight; she is no doubt a team player, with effective people/communication skills that

enabled her to access the sampling sites without difficulties. She would not hesitate to ask to be guided on what she did not know.

Ms Irene is a budding researcher exhibiting focus and zeal whose self-drive produces positive outcomes. Any assistance accorded to her will be greatly appreciated as we nurture the great research potential in her.

MINISTRY OF LIVESTOCK & FISHERIES DEVELOPMENT,
VETERINARY INVESTIGATION LABORATORY,
P. O. BOX 191,
KERICHO-20200.

Dr E W Wamalwa
Officer-in-Charge
REGIONAL VETERINARY INVESTIGATION LABORATORY
KERICHO.

Appendix VI: ANOVA table on TBDs parasite species prevalence in the study sites

Source of variation	Df	SS	MS	F ratio	p- value
Variance among	1	1.778	1.778	0.02563	0.00015
Variance within	2	34.675	17.3375		
Residual	2	138.7	69.35		
Total	5	175.153			

Appendix VII: ANOVA TABLE on the No. of different developmental stages of questing tick in the study sites

Source of variation	Df	SS	MS	F ratio	p-value
Variance among	2	868.05	434.025	10.458	0.0257
Variance within	2	27.67	4.611		
Residual	4	166	41.5		
Total	8	1061.72			

Appendix VIII: ANOVA Table on the relationship of tick collected with the Prevailing temperature

Source of variation	Df	SS	MS	F ratio	p- value
Correlation explained	1	0.058564	0.058564	0.4354	0.0065
Correlation unexplained	7	0.941436	0.1344901		
Total	8				

Appendix IX: ANOVA Table on the relationship of ticks collected with the prevailing Relative humidity

Source of variation	Df	SS	MS	F ratio	P -value
Correlation explained	1	0.0462	0.0462	0.3393	0.0034
Correlation unexplained	7	0.9533	0 .1362		
Total	8				