AWARENESS AND PRACTICES ON PREVENTION OF HUMAN TICK-BORNE DISEASES AND THE GENETIC DIVERSITY OF *THEILERIA* AND *BABESIA* AT THE HUMAN, CATTLE AND AFRICAN BUFFALO (*SYNCERUS CAFFER*) INTERFACE IN THE OL PEJETA CONSERVANCY, LAIKIPIA COUNTY, KENYA

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Awareness and Practices on Prevention of Human Tick-Borne Diseases and the Genetic Diversity of *Theileria* and *Babesia* at the Human, Cattle and African Buffalo (*Syncerus Caffer*) Interface in the Ol Pejeta Conservancy, Laikipia County, Kenya

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DECLARATION

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

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DEDICATION

I would like to thank God for giving me the health, strength both physical and mental, to go through to complete my studies. This thesis is dedicated to my family which has stood by me through the study period. To my colleagues and friends whose words of encouragement and support has brought me this far.

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ABBREVIATIONS AND ACRONYMS

18S rRNA	18S ribosomal Ribonucleic acid
BLASTn	Basic Local Alignment Search Tool nucleotide
DNA	Deoxyribonucleic acid
DNAsp	Deoxyribonucleic acid sequence polymorphism
ECF	East Coast Fever
EDTA	Ethylenediaminetetraacetic acid
HBM	Health Behaviour Model
ILRI	International Livestock Research Institute
Kb	Kilo base
KEMRI	Kenya Medical Research Institute
KU-ERC	Kenyatta University Ethics Review Committee
KWS	Kenya Wildlife Service
MEGA	Molecular Evolutionary Genetic Analysis
MUSCLE	Multiple Sequence Comparison by Log Expectation
OPC	Ol Pejeta Conservancy
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SPSS	Statistical Package for Social Sciences
TBDs	Tick Borne Diseases
V4	Variable region 4
VNTR	Variable Number Tandem Repeat
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Awareness of tick-borne diseases: General information on what ticks are, ticks as vectors of diseases, diseases spread by ticks to humans and the common symptoms associated with tick-bites in humans.

Bootstrap support: This is the bootstrap value that shows how many times out of 100 the same branch was observed when repeating the phylogenetic construction on a re-sampled data set.

Haplotype: This is a group of genes within an organism that are inherited together on the same chromosome.

Interface: A point where human, livestock and wildlife meet and interact directly or indirectly.

Maximum likelihood method: This is a method used in construction of phylogenetic trees which provides probabilities of the sequences given a model of their evolution on a particular tree. The more probable the sequence given the tree, the more the tree is preferred.

Practice of tick-borne diseases prevention: Routine activities and actions undertaken by individuals in the prevention of tick bites and tick-borne diseases.

Sympatry: These are populations of humans, cattle and African buffalos that exist in the same geographic area and thus regularly encounter one another.

ABSTRACT

Many studies have been done on *Theileria* and *Babesia* in livestock, wildlife and humans as separate hosts. However, there is very little information on the genetic diversity of these haemoprotozoa in an ecological system where humans, cattle and African buffalos interact. In addition, few studies have investigated the level of awareness and practices associated with human tick-borne diseases among people who live in tick infested environment. The main objective of this study was to determine the level of awareness and practices on prevention of human tick borne diseases and genetic diversity of *Theileria* and *Babesia* infections in the human, cattle and African buffalos interface in the fenced Ol Pejeta Conservancy and the community adjacent to the Conservancy in Laikipia County. Semi-structured questionnaires were used to evaluate awareness and practices from 307 respondents from the community and the Conservancy. Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 23 and the test statistics utilized were Chi-square and linear logistic regression. The results showed that the majority of the respondents (44.3%) belonged to (18 - 30 years) age group. A proportion of the respondents (99.7%) positively identified a tick while 97.4% were aware that ticks spread diseases to animals; however, the number drastically decreased when asked if ticks spread diseases to humans at 67.7%. Many of the respondents (46.9%) mentioned tick fever as one of the human tick borne diseases. The most common symptom respondents associated with tick bites in humans was skin rashes at 71.7% followed by malaise (43.0%) and muscle pain at 36.2%. Significant factors associated with awareness were level of education (β 0.216, $t = 3.624$, $p < 0.001$) and gender (β -0.148, $t = -2.308$, $p = 0.022$), while the significant factor associated with uptake of prevention practices against tick bites was occupation (β -0.147, $t = -2.117$, $p = 0.035$). Polymerase Chain Reaction (PCR) was used to carry out molecular analysis targeting the hypervariable region of the 18S rRNA gene of the piroplasms, Molecular Evolutionary Genetic Analysis (MEGA) version 7 software was used to analyse the sequences obtained and finally phylogenetic analysis was done using Multiple Sequence Comparison by Log Expectation (MUSCLE) version 7 from 70 samples from humans, 98 from cattle and 92 from African buffalo. Out of 70 human samples analysed, none were positive for piroplasms. In cattle 45 (45.9%) were positive for piroplasms while 87 (94.6%) of the African buffalos were positive. The predominant piroplasm in cattle was *Babesia bigemina* (22.2%). There was a high degree of diversity within the *Theileria* species with the predominant species being *T. sp. ex. Syncerus caffer* (73.6%) in the buffalos followed by *T. parva.*

In summary, there were no *Theileria* or *Babesia* infections in humans but, higher levels of education, gender and occupation shaped the community awareness about ticks and tick borne diseases and drove specific practices of prevention to tick bites and tick borne diseases. The study recommends a public health awareness campaign on human tick borne diseases and tick bite prevention practices.

CHAPTER ONE
INTRODUCTION

1.1 Background

Ticks are considered to be the second worldwide vectors for human diseases after mosquitoes. They are however the most important vectors of diseases in domestic and wild animals. They transmit a wide variety of pathogens to both humans and animals which include viruses, bacteria, helminths and protozoa. Some of the protozoal tick-borne diseases are piroplasms from the genera *Theileria* and *Babesia.*

Cattle are affected by various *Theileria* and *Babesia* species which include *Theileria parva* which causes East Coast Fever, *Theileria mutans* which causes benign Theileriosis and *Theileria lawrenci* which causes corridor diseases. The *Babesia* species affecting cattle include *Babesia bigemina*, *Babesia bovis* and *Babesia divergens*. These tick-borne diseases immensely contributed to the stagnation in livestock industry in sub-saharan Africa by increasing cost of production due to costs of treatment and tick-control, reduced meat and milk production, abortions, high mortality (Gachohi *et al.*, 2012), and hindering the introduction of improved cattle breeds (Gitau *et al.,* 2001).

In humans, *Babesia microti*, *Babesia divergens* and *Babesia duncani* are the common parasites (Perez de Leon *et al.,* 2010). These *Babesia* species also infect a wide host range including livestock. Although human babesiosis is a neglected tropical disease, global cases suggests that it is emerging in every continent as a significant public health challenge that should be monitored and new epidemiological information obtained given that human-wildlife interface is getting more intimate (Young *et al.,* 2019). Tick-borne illnesses are associated with febrile conditions and non-specific clinical symptoms which is a diagnostic challenge coupled with the fact that many physicians are unfamiliar with many tick-borne illnesses. There is no known case of theileriosis in humans, though there is no proof that the *Theileria* cannot infect and establish in humans.

Tick-borne infections in wildlife, including *Theileria* and *Babesia* parasites, are often asymptomatic or associated with mild clinical symptoms, which entrenches the view that wildlife is a maintenance host.
In rare occasions, especially when wildlife are under nutritional or anthropogenically-induced stress, such as capture and translocation, latent infection by *Theileria* and *Babesia* progress to severe disease that sometimes become fatal (Patz *et al.*, 2003).

In addition, this study aimed to determine the awareness and practices of people towards ticks and human tick-borne diseases. This was based on the fact that awarenes and practice are some of the factors that strongly influence risk of infection and implementation of vector and disease outbreak control and prevention strategies. This study was designed to address fundamental questions on the epidemiology and public health of tick-borne parasites with particular focus on genera *Theileria* and *Babesia*. The study sought to establish the species of *Theileria* and *Babesia* that are circulating between sympatric cattle and African buffalo, the species that are dominant between the two host populations (Cattle and African buffalos) and whether the people who continually stay or share the habitat with these animals harbour *Theileria* and *Babesia* parasites. The study also sought to establish if the people knew that ticks can transmit tick-borne diseases such as *Theileria* and *Babesia* to them and their response to this knowledge.

1.2 Statement of the problem

The prevalence of tick-borne diseases in humans is increasing worldwide (Dantas-Torres *et al.,* 2013). The increase in the human population has led to building of human settlement in areas originally inhabited by wild animals. Domestic animals introduced into wildlife habitats and people who herd these animals or those who work in wildlife habitats experience a greater burden of ticks than people living in urban centres (Munderloh & Kurtti, 2011). This has increased the risk of humans living or working in wildlife habitats acquiring zoonotic tick-borne infections.

A number of diseases affecting animals are never prioritized until they impact human health. As a result, opportunities to manage such zoonotic diseases at the source are missed. By studying the role of microbes in wildlife and livestock diseases we can be able to deal with emerging zoonotic diseases before we get large outbreaks in the human population (Wiethoelter *et al.,* 2015). Most tick borne diseases such as babesiosis display symptoms that are similar to other illnesses and are often referred to as flu-like in nature (Taege, 2000). As a result a number of these cases could be misdiagnosed or go unnoticed (Demma *et al.,* 2005).

There is also a knowledge gap on the economic burden of babesiosis in humans across the world with very few studies undertaking surveillance of Babesia species in vectors and animal hosts (Young *et al.,* 2019). A few studies have determined the species of *Theileria* and *Babesia* that are shared by sympatric herds of cattle and African buffaloes (Oura *et al.,* 2011). Further, few studies have determined the genetic composition of *Theileria* and *Babesia* harboured in the African buffaloes (Chaisi *et al.,* 2011) and whether they carry any of the zoonotic piroplasms. There is also a societal knowledge gap on prevention of tick bites (Young *et al.,* 2019). It is therefore critical that the epidemiological picture of the *Babesia* and *Theileria* infections in a human-livestock-wildlife interface is established so that proper treatment, prevention and control measures are put in place for both the humans and the animals.

1.3 Justification

People living and working in and around wildlife conservancies and in farms with animals are at a higher risk for tick bites which potentially transmit various tick borne pathogens regardless of contact with animals due to the high grasses and vegetation and high density of animals (Kisomi *et al.,* 2016). Tick bite prevention and control measures including personal protective measures and public education to decrease the probability of tick contact for humans and animals is essential in prevention of tick borne diseases. However, in Kenya, there is a knowledge gap on whether this group of people understand their vulnerability to tick borne diseases and if they know the personal measures they need to take to protect themselves from tick bites and tick borne diseases. Effective public health campaigns against tick bites and tick borne

diseases requires an assessment of the community's level of awareness in tick borne diseases and prevention practices on prevention of tick bites in order to ascertain the knowledge and practice gaps that need to be addressed.

Further, the burden and prevalence of piroplasm infection in humans living or working in human-livestock-wildlife interface in Kenya is not documented. Molecular epidemiological surveillance of the piroplasms in such ecosystems where humans interact with animals is warranted because these are areas where zoonotic piroplasms circulate or are likely to emerge (Young *et al.,* 2019). Molecular characterization of the piroplasm species provides highly sensitive and specific data on the diversity of piroplasms in this ecosystems, the origin of the infections and the relationship between different piroplasm isolates (Gupte, 2016).

This study will increase the knowledge on the genetic variety of hemoprotozoa in this ecosystem which will aid in vaccine development and risk analysis of mixed grazing systems leading to an increase in livestock production and wildlife protection as well as poverty alleviation for the pastoralists. The study will also assess the awareness and practices of the people living in this community towards tick-borne zoonotic diseases especially towards theileriosis and babesiosis and this will provide information to the government and other stakeholders that will be useful in designing effective and sustainable prevention strategies that can be used in other wildlife conservancies.

1.4 Research Questions

The following were the research questions of this study.

1. What is the level of awareness of the people living or working within and around the Ol Pejeta Conservancy in Laikipia County, Kenya, on ticks, *Theileria, Babesia* and other human tick borne diseases?
2. What are the practices of people living and working within and around the Ol Pejeta Conservancy in Laikipia County, towards prevention of tick bites and human tick-borne diseases?

3. What is the proportion of *Theileria* and *Babesia* infection among sympatric humans, African buffalos and cattle in the Ol Pejeta Conservancy and in the community adjacent to the conservancy?
4. What is the genetic characteristic of *Theileria* and *Babesia* in infected humans, cattle and buffalo that share tick infested environment in Ol Pejeta Conservancy and the community owned cattle adjacent to Ol Pejeta Conservancy?

1.5 Objectives

1.5.1 Broad Objective

The broad objective of this study was to determine the level of awareness and practices on prevention of human tick-borne diseases and genetic diversity of *Theileria* and *Babesia* infections in the human, cattle and African buffalos interface in the fenced Ol Pejeta Conservancy and the community adjacent to the Conservancy in Laikipia County, Kenya.

1.5.2 Specific Objectives

The specific objectives of this study were:

1. To determine the level of awareness of the people living within and around Ol Pejeta Conservancy, Laikipia County, kenya, on ticks, *Theileria*, *Babesia* and other human tick-borne diseases
2. To determine the practices of the people living within and around Ol Pejeta Conservancy in Laikipia County, Kenya, on prevention of tick bites and infection by *Theileria*, *Babesia* and other human tick borne diseases.
3. To determine the proportion of *Theileria* and *Babesia* infections among sympatric humans, cattle and African buffalos within Ol Pejeta Conservancy in Laikipia County, Kenya, and in the community adjacent to the Conservancy.
4. To determine the genetic diversity of *Theileria* and *Babesia* in humans, African buffalos and cattle within Ol Pejeta Conservancy and in the cattle owned by the community living adjacent to Ol Pejeta Conservancy in Laikipia County, Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Theileria and Babesia

Theileria and *Babesia* are genera in the phylum *Apicomplexa.* They are in the class *Aconoidasida*, order *Piroplasmida* and are called piroplasms due to their pear shaped morphology of the multiplying parasite stage in the blood of their vertebrate host. They have been classified into the genus *Babesia* and *Theileria* based on their form of transmission and the existence or absence of schizonts (Schnittger *et al.,* 2012). The two organism's lifecycles involve mammalian and vector stages but they differ in the cells that they invade in the vertebrate hosts, the specific tick vectors that transmit them as well as the mode of transmission (Brayton *et al.,* 2007). The common species of *Theileria* include *T. parva, T. mutans, T. taurotragi, T. buffeli*, *T. equi* (Bishop *et al.,* 2004). The common species of *Babesia* include *B. bigemina* which affects cattle and buffalos, *B. gibsoni* which affects dogs (Uilenberg, 2006), *B. bovis* which affects cattle and buffalos*, B. gibsoni* which affects dogs (Criado-Fornelio *et al.,* 2003)*, B. divergens* a cattle parasite which is also zoonotic (Zintl *et al.,* 2003)*, B. canis* for dogs, *B. caballi* for horses (Schnittger *et al.,* 2000)*, B. bicornis* which affects rhinos (Nijhof *et al.*, 2003) and *B. microti* a rodent parasite which is also zoonotic (Gray and Weiss, 2008). The *Babesia* which infects humans is classified into 4 clades. The 1st clade has the *Babesia* spp which are less than 3µm in diameter such as *B. microti*. The 2nd clade is the *Babesia* spp. related to the *Babesia* of dogs and wild life. This clade contains B. *duncani.* The 3rd clade has *B. divergens* and *B. venatorum* which also parasitize cattle. The 4th clade consists of large *Babesia* that infects ungulates and includes the zoonotic KO1 strain (Gray *et al.,* 2010).

2.2 Geographic distribution of *Theileria* and *Babesia*

Theileria and *Babesia* affect animals in tropical and sub-tropical regions of the world (Uilenberg, 1995). Most species of *Theileria* and *Babesia* cause disease in their hosts; however a number of species of these parasites are carried asymptomatically by their hosts. Diseases caused by *Theileria* infection are known as theileriosis while diseases caused by *Babesia* infection are known as babesiosis. *Babesia* infections have big

impacts on domestic animals, wild life under stress and in humans. *B. bovis*, *B. divergens* and *B. bigemina* infections in cattle result in a disease known as bovine babesiosis.

Theileriosis in cattle caused by *T. parva* results in the disease known as East Coast Fever and *T. annulata* infection causes tropical theileriosis. Buffalos appear not to be susceptible to *Theileria* infection. Theileriosis and Babesiosis have been reported in Turkey (Sayin, 2002), Australia (Kamau *et al.*, 2011), Japan and Korea (Chae *et al.,* 1998), United States of America (Shock *et al.,* 2014), Spain (Nagore *et al.,* 2004) and in Croatia (Beck *et al.,* 2009). In Africa, Babesiosis and Theileriosis are important diseases in Eastern, Central and Southern parts of Africa. It has been reported in 11 countries in the region: Kenya, Uganda, Tanzania, Burundi, Rwanda, Malawi, Mozambique, Southern Sudan, Democratic Republic of Congo, Tunisia, Zambia and Zimbabwe (Lawrence *et al.,* 1992; Swai *et al.,* 2007) In an extensive serological survey of tick-borne diseases in calves across five states in Southern Sudan, the prevalence of *T. parva* was 27.3% while *T. mutans* was 31.3% (Kivaria *et al.,* 2012). In Zambia, the prevalence of *T. parva* was 54.9% (Muleya *et al.,* 2012). In Tunisia, the prevalence of *T. annulata* was over 90% (Gharbi et al., 2006). Previous studies have also determined the presence of Theileriosis in Zimbabwe, South Africa (Chaisi *et al.,* 2011b; Pienaar *et al.,* 2011; Sibeko *et al.,* 2008) and Uganda (Muhanguzi e*t al.,* 2010; Oura *et al.,* 2005).

In Kenya, cases of theileriosis and babesiosis in cattle are widespread (Gitau *et al.,* 2001; Kivaria, 2006; Wesonga *et al.,* 2010; Njiiri *et al.,* 2015). Previous studies determined the prevalence of *Theileria* in the Central highlands of Kenya to be 41-55% (O'Callaghan, 1992), the Coastal lowlands at 57-79% (Maloo *et al.,* 2001) and the Central Rift Valley at 22 -33% (Gachohi *et al.,* 2012). *Theileria* has also been detected in the Western (Okuthe & Buyu, 2006), Eastern (Gachohi *et al.,* 2010) and the Lake Victoria basin regions of Kenya (Gachohi *et al.,* 2012). Theileria investigations were done on animals raised under variable livestock management systems such as small holder dairy systems (Gachohi *et al.,* 2012; Njiiri *et al.,* 2015) open grazing livestock systems and zero grazing systems (Gitau *et al.,* 1997),

traditional crop livestock systems and pastoralism (Gachohi *et al.,* 2012). The breeds studied included the indigenous short horn zebu, zebu crosses, the improved boran and the exotic breeds (Taurines). Under the small holder dairy systems and cattle kept in open yards or kraals, they found the following *Theileria* species infections *T. spp, T. parva*, *T. mutans*, *T. taurotragi*, *T. bicornis*, *T. sp (buffalo)* and *T. equi*. They also found *B. bigemina* and *B. bovis* (Gitau *et al.,* 1997; Njiiri *et al.,* 2015).

2.3 Public health importance of *Theileria* and *Babesia*

Human babesiosis is described as an emerging tick-borne disease attributed to the encroachment of humans on wildlife habitats and the increase of immunocompromised people (Jones *et al.,* 2008). The actual number of human *Babesia* infections are thought to be much higher and wide spread but many cases are undetected or underreported (Vannier & Krause, 2012). Cases of human Babesiosis have been reported in America (Persing *et al.,* 1995), Japan (Saito-ito *et al.,* 2000), China, Europe, Mexico (Gorenflot *et al.,* 1998; Hildebrandt *et al.,* 2007) Egypt (Michael *et al.,* 1987) and South Africa (Bush *et al.,* 1990).

Except for these clinical cases that were from patients' previously misdiagnosed, epidemiological surveys on human *Babesia* infection are unavailable (Young *et al.,* 2019). In Kenya, a 22% prevalence of *Babesia microti* has been identified in non-human primates (Maamun *et al.,* 2011). The first demonstrated case of human babesiosis in the world was reported in Europe in 1957 caused by *babesia divergens* a cattle parasite that also affects buffalos. Since then, other species such as *B. microti*, *B. divergens*, *B. bovis* (Hunfeld et al., 2008), *B. canis* (El-Bahnasawy *et al.,* 2011), unnamed species WAI (Quick *et al.,* 1993; Thomford *et al.,* 1994), CAI (Persing *et al.,* 1995), MOI (Herwaldt *et al.,* 1996) and *B. Venatorum* also known as the EU1 strain (Haselbarth *et al.*, 2007) have been found in humans.

Hundreds of cases of human babesiosis that have been reported had clinical manifestations that varied from asymptomatic to severe rapidly fatal disease. Most symptomatic patients became ill 1 – 4 weeks after being bitten by an infected tick. This is followed by a gradual onset of malaise and fatigue, fever, chills, sweat, headache, myalgia, anorexia, non-productive cough, arthralgia and nausea (Joseph *et al.,* 2011; Krause *et al.,* 2003). Occasional symptoms include vomiting, sore throat, abdominal pain, conjuctival injection, photophobia, weight loss, emotional lability, depression and hyperesthesia (Vannier *et al.,* 2008). On physical examination fever is the most common sign. It may be accompanied by splenomegaly, pharyngeal erythema, hepatomegaly, jaundice or retinopathy with splinter haemorrhages and retinal infarcts (Vannier *et al.,* 2008). The severity of the disease depends primarily on the immune status of the patient and the *Babesia* species causing the infection.

2.4 Livestock health importance of *Theileria* and *Babesia*

Theileria and *Babesia* infections have caused stagnation in the development of the livestock sector in the country because they hinder introduction of improved exotic cattle breeds in endemic areas (Gitau *et al.,* 2001). They cause high mortality primarily in exotic and cross bred cattle but also in indigenous calves and adults in endemically stable areas (Norval, Perry, & Young, 1992a). It is estimated that these diseases cost the livestock sector about 5.1 million US dollars annually in Kenya due to mortality, ill thrift, abortions, loss of milk and meat products and loss of draft power (Mcleod & Kristjanson, 1999).

Theileriosis in cattle has an incubation period of 1 to 3 weeks and is characterized by dullness, in appetence, fever of 39.5^{o}C and above, ocular discharge, corneal opacity and photophobia, nasal discharge, laboured breathing, petechiae on mucous membranes, generalized lymphadenopathy, diarrhoea and occasionally neurological symptoms (turning disease). East Coast Fever (ECF) and tropical theileriosis are the most severe forms of theileriosis in cattle. Babesiosis in cattle is characterized by fever, anaemia, jaundice, haemoglobinuria, respiratory distress, abortion and neurological symptoms (Aiello & Mays, 1998).

2.5 Wildlife Health Importance of *Theileria* and *Babesia*

Apart from Cattle and African buffalos, previous studies have established the presence of *Theileria, Babesia* or both in the following wild animals: Roan, Sable, greater kudu, common gray duiker, black rhinoceros, lions, buffalos, seagulls, bats, cheetahs, grant gazelles and giraffes. (Bhoora *et al.,* 2009; Fyumagwa *et al.,* 2004; Gray & Weiss, 2008; Nijhof *et al.,* 2003; Penzhorn *et al.,* 2001) Wildlife is considered a natural maintenance host for *Babesia* (Schnittger *et al.,* 2012).

The African buffalos and cattle are taxonomically related as both belong to the family Bovidae hence; they share many pathogens such as *Theileria, Babesia, Ehrlichia* and *Anaplasma* (Eygelaar *et al.,* 2015; Oura *et al.,* 2011) It is suggested that the natural reservoir host of *Theileria parva* is the African buffalo (*Syncerus caffer*), (Mans *et al.,* 2011). The buffalo has been implicated in the maintenance of *Theileria parva lawrenci* which cause the lethal corridor disease in cattle (Grootenhuis *et al.,* 1987).
Some variants of the parasite are transmitted solely from buffalo to cattle whereas others can spread from cattle to cattle. *Babesia bigemina* and *B. bovis* have also been isolated from African buffalos (Iseki et al., 2007). Buffalos therefore play a critical role in the maintenance and transmission of tick borne diseases to cattle. Rodents and other small mammals have been shown to be reservoirs of *B. microti* that affects humans (Uilenberg, 2006).

Although wildlife have been shown to harbour the piroplasms without clinical manifestation of disease, under stressful conditions such as capture, malnutrition and translocation, they have been shown to exhibit signs of the disease leading to death (Höfle *et al.,* 2004). This is because wild animals when captured become stressed and their immunity is lowered. As a result, any latent infections become clinical diseases. Translocation of animals to environments endemic with piroplasms from environments that never had piroplasms also result in deaths from diseases caused by the piroplasms because the animals find it difficult to adapt to their new environment in terms of building immunity against piroplasms (Kock *et al.,* 2010). This means

that under stressful conditions, *Theileria* and *Babesia* infections can be a threat to the survival of rare and critically endangered wildlife species.

2.6 Tick Vector

Ticks are obligate hematophagous arthropod parasites that feed on the blood of every class of vertebrates in almost all regions of the world (Jongejan & Uilenberg, 2004). Ticks belong to the subphylum Chelicerata, class Arachnida, subclass Acari and suborder Ixodida (Barker & Murrell, 2004). Ticks play an important role in transmission of a variety of diseases in livestock, wildlife and humans which include rickettsial, viral, bacteria and protozoa (Rojas *et al.,* 2014). Although there are two categories of ticks; hard (Family Ixodidae) and soft (Family Argasidae) ticks, it is the hard ticks that are central to transmission of *Theileria* and *Babesia*.

Hard ticks are characterized by the presence of a tough sclerotized plate found on the entire dorsal body surface in males and only on the anterior dorsal body region of females, nymphs and larvae (Walker *et al.,* 2003). Hard ticks belong to seven genera, *Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus* and *Boophilus*. The hard ticks have three developmental stages all of which are parasitic and feed on hosts: - the larva, nymph and adult stage.

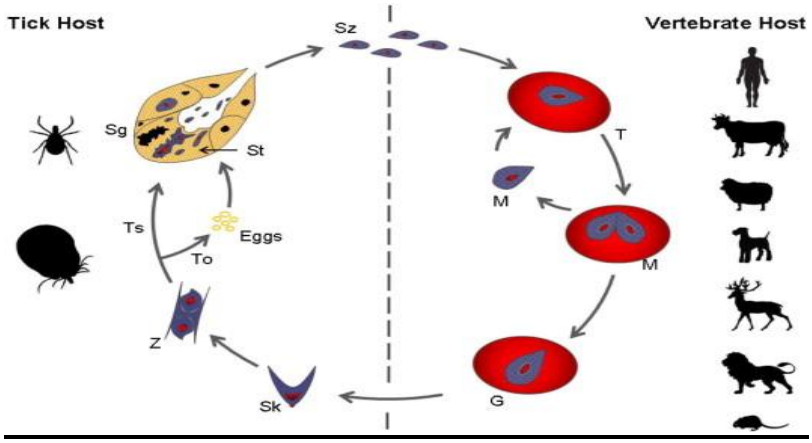
Majority of the hard ticks require three different hosts to complete their life cycle (three host life cycle) where the larva climbs on a host and feeds then drops on the ground and molts into the nymph. The nymph then attaches onto a 2nd host and feeds for about 4 – 8 days, after which it then falls to the ground and molts into the adult. The adult attaches to a 3rd host where the female feeds, mates and lays eggs on the ground (Walker *et al.,* 2003). Examples of three host ticks include *Rhipicephalus appendiculatus which transmits T. parva, T. lawrenci, T. bovis and T. taurotragi* (Norval *et al.,* 1992b; Walker *et al.,* 2003).

There are also hard ticks which require two hosts to complete their life cycle (2 host life cycle). In these cases the larva and the nymph feed on the same individual host while the adult feeds on a 2nd individual host. Such ticks include *Rhipicephalus evertsi.*

One host ticks such as *Boophilus decoloratus* that transmit *Babesiosis* have all the three life stages on one host where they feed and molt. After feeding, the female detaches from the host and lays eggs on the ground.

2.7 Life cycle of *Babesia*

The life cycle of Babesia species takes place partly in hosts and partly in ticks as shown in figure 2.1. During a blood meal an infected tick injects *Babesia* sporozoites into the vertebrate host. The sporozoites then invade red blood cells where they differentiate into trophozoites (T). Trophozoites divide asexually into merozoites (M) that exit the red blood cells and invade new red blood cells to continue with the replication process and some merozoites stop dividing to become gametocytes (G) which transform into gamonts. These gamonts are taken by ticks when feeding on a blood meal and once they reach the tick's gut they differentiate into gametes called ray bodies (Sk) that later fuse to form zygotes in the ticks mid gut. The zygotes undergo meiosis giving rise to motile haploid kinetes that move to several organs in the tick such as the salivary glands, the ovaries where transovarial transmission occurs (To). Once in the salivary glands, they differentiate and multiply to form sporozoites that will infect the vertebrate host during the tick's next blood meal a process known as transstadial transmission (Mehlhorn & Shein, 1984; Schnittger *et al.,* 2012) denoted as Ts in figure 2.1. The common species of ticks that transmit *Babesia* include *Boophilus microplus*, *Boophilus decoloratus*, *Boophilus annulatus*, *Boophilus geigyi* and *Rhipicephalus evertsi.*



Key: T - trophozoite, M - merozoite, G - gametocyte, Sk - ray bodies, To – Transovarial transmission and Ts – Transstadial transmission.

Figure 2.1: Life cycle of *Babesia* species.

Source: Schnittger et al., 2012**.**

2.8 Life cycle of *Theileria*

The infective sporozoite stage of the parasite is injected into a vertebrate host during blood meal intake by ticks the host though the tick's saliva as the tick feeds (Fig. 2.2). They invade the lymphocytes where they develop into trophozoites and multiple asexually into multinucleated schizonts. This process stimulates proliferation of the host cells leading to a further multiplication of the parasite which are disseminated throughout the host's lymphoid tissues. Once in the host's lymphoid tissue, a few schizonts differentiate into merozoites which enter red blood cells and form piroplasms that are infective to ticks when feeding. Piroplasms develop sexually inside the tick gut into gamonts which become zygotes that differentiate into mobile kinetes. These kinetes move to the salivary glands of the ticks and are transformed into sporozoites which become infective when injected into the next host by the tick (Aiello & Mays, 1998.; Bishop *et al.,* 2004). *Theileria* species are transmitted by tick species from the genera *Rhipicephalus, Amblyomma, Hyalomma* and *Boophilus* (Jongejan & Uilenberg, 2004).

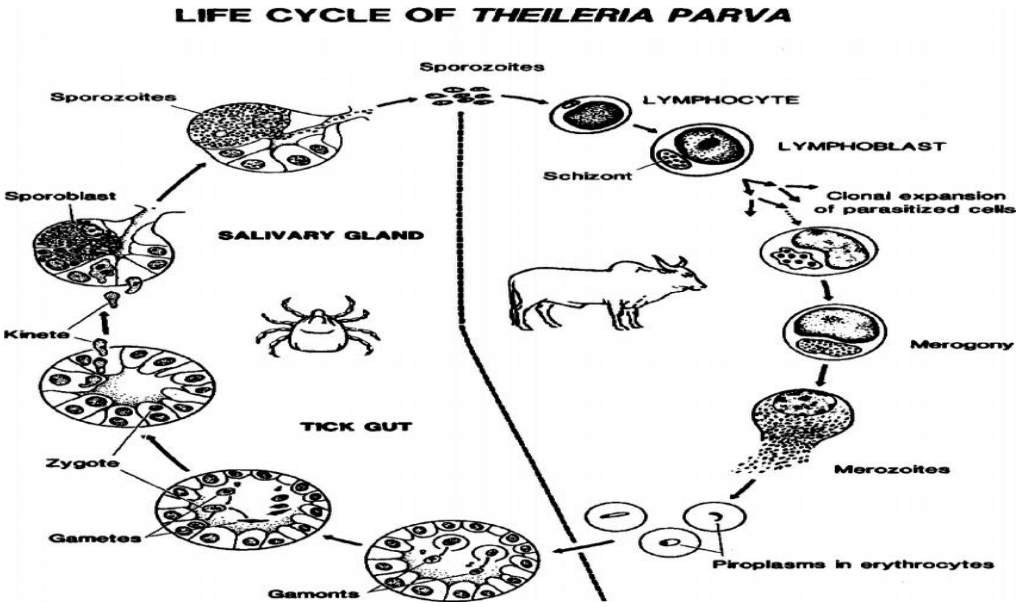


Figure 2.2: Life cycle of *Theileria.*

Source: Bishop *et al.*, 2004.

2.9 Risk factors associated with transmission of *Theileria* and *Babesia*

The geographic areas in which ticks are found are expanding and as a result tick borne diseases are also increasing. This can be attributed to factors such as climate change, socio-economic factors such as the type of housing, activities such as hunting, farming, wildlife introduction into new environments as well as wildlife translocation (Patz *et al.,* 2003). Humans who spend time in tick infested areas and outdoor workers are at risk. This includes herders, pastoralists, game wardens, foresters, people keeping livestock and people who work in areas with wildlife (www.cdc.gov/niosh/topics/tick-borne/). Most of the severe cases of human babesiosis have been reported among patients who have undergone splenectomy, those with cancer, human immune deficiency virus infection, chronic heart, lung or liver disease (Wormser *et al.,* 2010). Other groups that are at risk are the elderly (50 years and above), patients receiving treatment with immunosuppressive drugs for cancer (Haselbarth *et al.,* 2007).

2.10 Diagnosis of *Theileria* and *Babesia*

The taxonomic relationships of members of the order Piroplasmida have been controversial from discovery (Norval *et al.,* 1992a).

These species were first defined and identified according to their morphology, hosts, ticks, vectors, distribution, antigenic relationships and ability/inability to cross-protect against other organisms.

Theileria and *Babesia* organisms are often detected by microscopy using giemsa stains on thin blood smears that detect the piroplasms in the red blood cells, and microschizonts (Koch blue bodies) in lymphocytes prepared from stained lymph node smears. Although microscopy is a rapid method of diagnosis that is easy to perform and readily available, the results are affected by factors such as: - (i) the specimen quality, (ii) clinical presentation of the patient and (iii) the experience of the laboratory technician handling the microscope. It can also give a false negative when there is low parasitaemia in the patient (Misawa, 1999).

Serological and immunological tests such as enzyme linked immunosorbent assay, indirect fluorescent antibody test and compliment fixation test have also been used. However, serological tests detect past infections and these methods are difficult to use in detection of parasites in carrier host, in acute cases and in the onset of a disease when the parasite is scanty. This is because they do not measure disease or the cause of a disease directly, but the patient's immune response to the potential disease agent. In such cases they act as screening tests. Serological tests results are also difficult to interpret because a patient's immune system is dependent on other factors such as the genetic makeup or previous exposure to the disease agent. This means that the results have to be interpreted in the context of a particular clinical and epidemiological situation. There have also been reports of cross-reactions in serological tests leading to false positive results and it is impossible to use serology to differentiate species of a disease causing organism (Fierz, 2004).

Molecular tools are now being used in confirmation of the identity of the piroplasms and their distribution, their biological properties and host parasite interactions (Bhoora *et al.,* 2009). The development of DNA based tests have allowed for an increased level of sensitivity and specificity in the detection of parasites and the detection of more than one pathogen in mixed infections simultaneously. *Theileria* and *Babesi*a have a small region on chromosome 4 which encodes a highly repetitive sequence called the hypervariable V4 region (Bhoora *et al.*, 2009). The hypervariable V4 region found on the 18S r RNA gene contains a short tandem repeat sequence or polymorphic Variable Number Tandem Repeats (VNTR) whose copy numbers vary between parasite isolates and clones and have been used to differentiate between different species and sub species of *Theileria* and *Babesia* (Oura *et al.,* 2005). Such sequences are used as genome wide markers for analysis of population genetics and monitoring of some aspects of live vaccines deployed in the field. The VNTR also reveal the genetic diversity of the piroplasms (Oura *et al.,* 2005).

DNA sequencing involves determining the order of nucleotides in a DNA molecule. This can be done directly where the PCR product is purified to make sure that there is only one single amplicon in the product which is then sequenced. Alternatively the amplicon can be cloned in a suitable vector then sequenced. Direct sequencing is faster than sequencing through cloning. However, direct sequencing sometimes produces bands that do not make sense or it can give blank lanes or superimposed sequences. Another challenge when using direct sequencing is that it is affected by any residual primers and unincorporated nucleotides, the use of over concentrated sample or the use of insufficient primers. For direct sequencing to be successful only the correct fragment should be amplified. This problem can be overcome by use of nested primers to re-amplify the desired fragment and to verify the identity of the product and to eliminate any unwanted amplicons (França *et al.,* 2002; Heather & Chain, 2016)

Other PCR methods such as reverse line blotting technique (RLB) permits detection and sequencing of many amplicons from PCR products at the same time. This method uses a large number of primers to amplify the DNA template. RLB is more specific compared to direct sequencing. It allows for single nucleotide mismatch detection and

through RLB minority species can be detected in a mixed infection (Paoletta *et al.,* 2018).

The analysis of 18S rRNA gene fragments have been successfully applied in the identification and classification of several previously unknown *Theileria* and *Babesia* species (Bhoora *et al.,* 2009; Chaisi *et al.,* 2011; Schnittger *et al.*, 2000). Furthermore the phylogenetic classification of the piroplasms has provided more information on their evolutionary relationships.

2.11 Prevention and control of tick bites and human tick-borne diseases.

Tick borne diseases can be controlled or prevented in livestock by controlling vectors through regular application of acaricides in the correct concentration, clearing bushes, rotational grazing and controlled burning of bushes, Immunization using the infection and treatment method, movement control of animals, rotational grazing and introduction of cattle breeds that are resistant to Theileriosis or Babesiosis. Application of acaricides on cattle also reduces the possible transmission of the piroplasms to humans (Telford & Spielman, 2010).

Currently there are two types of East Coast Fever vaccines in Kenya. The first one is the Muguga cocktail which is a preparation of the Muguga strain, Kiambu 5 strain isolate derived from the cattle *T. parva* and buffalo derived *T. parva* isolate from Serengeti. This is a live vaccine used in areas where cattle and buffaloes graze together. The second vaccine is the Marikebuni vaccine from the Marikebuni strain in the Coastal region that is used in the dairy cattle where there is no cattle-buffalo interaction (Di Giulio *et al.,* 2009; Mutugi *et al.,* 1991).

Although the efficacy of the Muguga cocktail has been demonstrated there has been a concern that it doesn't work well in areas where the wildlife and domestic animals interact. In these interfaces it is believed that a strain of *T. parva* adapted to the buffaloes makes the vaccines less efficacious (Katzer *et al.,* 2006). Other reasons that have led to a low uptake of the vaccines include concerns from veterinary authorities

and other stakeholders on the complexity of the process of producing and transporting the vaccines. This is because of the stabilate variability and the need for extensive in vivo testing in cattle. It is therefore difficult to control the batch quality because of the multiple sources of biological variation in the *T. parva* stock (Nene & Morrison, 2016; Oura *et al.,* 2004). There is also concern about the carrier state induced by the vaccines in the cattle. This state is persistent and can result in the vaccine parasite strain being introduced into the field resulting in genetic recombination with the resident parasites in the population (Morzaria *et al.,* 2000). A study has also confirmed that local ticks and cattle acquire components of the vaccine strain from immunized cattle (Oura *et al.*, 2004). As a result of this, there has been low acceptability of the ECF vaccine because of the perceived risk of introducing foreign *Theileria* stains into new environments (Nene & Morrison, 2016).

Prevention of tick borne diseases (TBDs) in humans consists of personal, residential and community approaches. Personal approaches include avoiding habitats or minimizing exposure to areas that are tick infested, use of tick repellent containing permethrin or N, N-diethyl-meta-toluamide (DEET) on the skin and clothes when entering an area that is tick infested, thorough examination of the body after exposure, removal of ticks before they attach to the skin and even after they attach to limit the possibility of transmission of TBDs. People working in tick infested areas should wear long pants outdoors and tuck the pants legs into socks (Stafford, 2007). Residential approaches include landscape management such as keeping grass mowed, removing leaf litter and spraying areas of high tick density with acaricidal formulations (Stafford, 2007). Community approaches include public education about the risks and characteristic symptoms of tick borne diseases (Fish & Childs, 2009).

2.12 Awareness, attitude and practice studies in relation to human tick-borne diseases

The level of knowledge on a health related risk has a direct influence on how people perceive the risk associated with exposure (Leventhal *et al.,* 1973). Health related behavior can be defined as any activity that is undertaken for the purpose of preventing

or detecting a disease or for improving health and well-being (Mckenna *et al.,* 2004). Health related behaviors have many levels of influence which include individual factors, interpersonal factors, organizational factors, community factors and public policy factors (Gochman, 1997).

Health behavior refers to personal attributes such as beliefs, expectations, motives, values and perceptions which influence health maintenance, restoration and improvement (Gochman, 1997). Research has shown that health related behaviors may have a positive impact on the quality of life by directing an individual towards prevention, seeking a cure or getting well (Conner & Norman, 2005).

The Health Behavior Model (HBM) looks at the association of variables which predict behavior. It uses two aspects of individual's representations of health behavior in response to threat of illness.
Perception of threat to illnesses depends on two beliefs (1) the individuals' perceived susceptibility to the illness and (2) the individual's perceived severity of the consequence of the illnesses. These two beliefs determine the likelihood of an individual to follow a health-related action but they are also influenced by individual differences such as personality, demographic variables like gender and race, socioeconomic status such as the marital status, level of education, place of residence and occupation. These variables affect the ability of an individual to access health education (kawachi & Berkman, 2000). Studies have shown that a person's behavior strongly influences the risk of contracting vector borne infections (Townson *et al.,* 2005) and the risk of TBDs decreases with increase in educational level and increase income per household (Stefanoff *et al.,* 2012).

A knowledge, attitude and practice survey is a representative study of a specific population to collect information on what is known, believed and done in relation to a particular topic (W.H.O., 2008). They are necessary because they help to identify knowledge gaps, cultural beliefs or behavior patterns that may facilitate understanding and action as well as pose problems or create barriers to effective disease prevention or control (W.H.O., 2008). Knowledge and practice surveys may be used to identify

needs in the community as well as solutions to health problems facing a community (W.H.O., 2008).

These surveys provide quantitative data in the form of responses grouped using the Likert-type scale and coded for easy analysis. Each response has a certain value. The responses are analyzed for similarities and differences between population or subpopulation groups. The data generated can be subjected to univariate bivariate and multivariate analysis (Abdi *et al.,* 2015; Middleton, 2005)

Knowledge, attitude and practice studies done in Kenya have shown that there is traditional knowledge on tick borne diseases that affect livestock such as ECF, and Babesiosis with various communities having names for the diseases and TBD treatment including ethno pharmacology (ITDG, 1996). They have also shown that communities carry out several practices to control ticks which include the use of plants and trees such as neem trees (*Azadirachta indica*) as repellants, removing weeds and bushes from areas around the homestead and animal housing, topical application of cow dung or kerosene, hand picking ticks, burning tick infested manure, keeping animals with ticks away from healthy animals, avoiding collecting fodder from roadside where many animals graze, grazing in hot places that have fewer ticks. The communities also use acaricides on their animals and environment (ITDG, 1996; Kioko *et al.,* 2015).

There are many studies that have been undertaken in various parts of the world to determine the association between socio-demographic variables, level of knowledge, perceived susceptibility to tick-borne diseases and the use of various methods tick-bite prevention. In a study that was undertaken in Connecticut (Gould *et al.,* 2008), female gender and perceived prevalence were strongly associated with the use of pesticides. In Poland (Bartosik *et al.,* 2008), a study was done to establish wether the professions that increase the probability of tick contact as well as previous contact with ticks influenced knowledge on tick borne diseases and prevention practices. The study was able to establish that practice was associated with place of residence. In a study conducted by Bayles *et al.,* (2013), there was a disparity between the level of knowledge, perceived personal risk and the use of personal preventive measures. The

study aimed at establishing the relationship between knowledge on human tick-borne diseases, perceived susceptibility to tick borne diseases and tick bite prevention measures. In all these studies, the recommendation was for greater public health campaign to encourage education on ticks, tick-borne diseases and the use of prevention measures in tick-borne disease endemic areas.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

Field study was carried out from January 2017 to April 2017, in and around Ol Pejeta Conservancy (OPC) shown in figure 3.1. Ol Pejeta Conservancy is about 20 km west of Nanyuki town. It lies at the equator at an altitude of 1800m (0.0043^0S, 36.9637^0E) in Laikipia County. It covers 90,000 acres and practices mixed grazing of wildlife and cattle. African buffalo is one of the key herbivore species in the conservancy. Other species of wild animals include elephants, giraffes, black and white rhinos, impalas, elands, gazelles, hyenas, lions, foxes and leopards. The conservancy is fully fenced with an electric fence so no medium sized animal is able to get into or out of the conservancy. The conservancy is situated on the leeward side of Mount Kenya and has a bimodal rainfall pattern with a mean annual precipitation of 900mm. The rainfall falls in two main seasons, from April to May and from October to November. The soil is mainly black cotton soil and the vegetation is grassland-woodland mosaic dominated by *Acacia drepanolobium* and *Euclea divinorum.* However at the time of sampling due to the drought, the community area on the northern part of the conservancy did not have any ground cover.

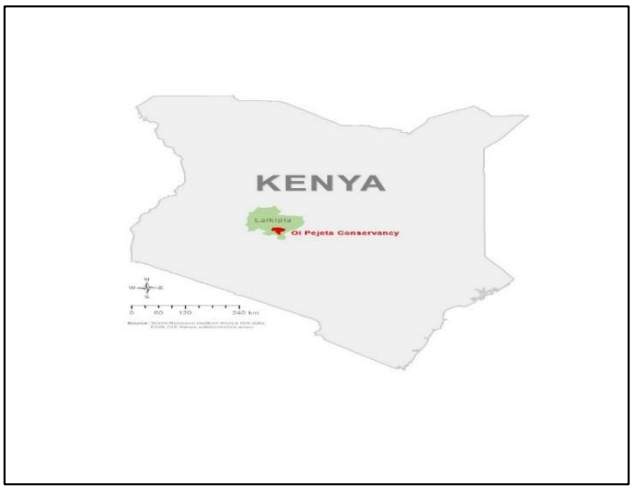


Figure 3.1: Map of Kenya showing Ol Pejeta Conservancy

Source: Internet.

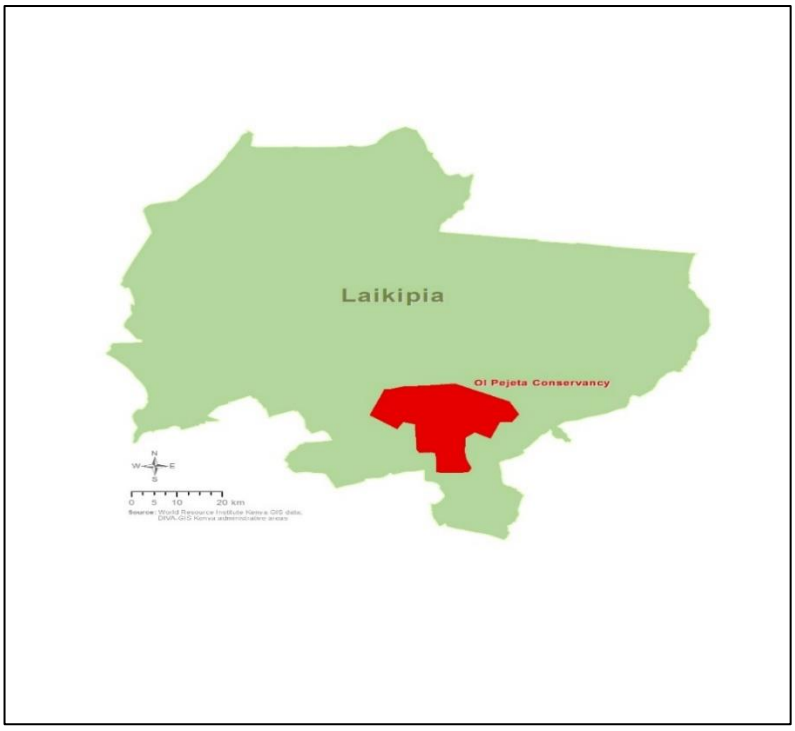


Figure 3.2: Map of Laikipia County showing Ol Pejeta Conservancy

Source: Internet

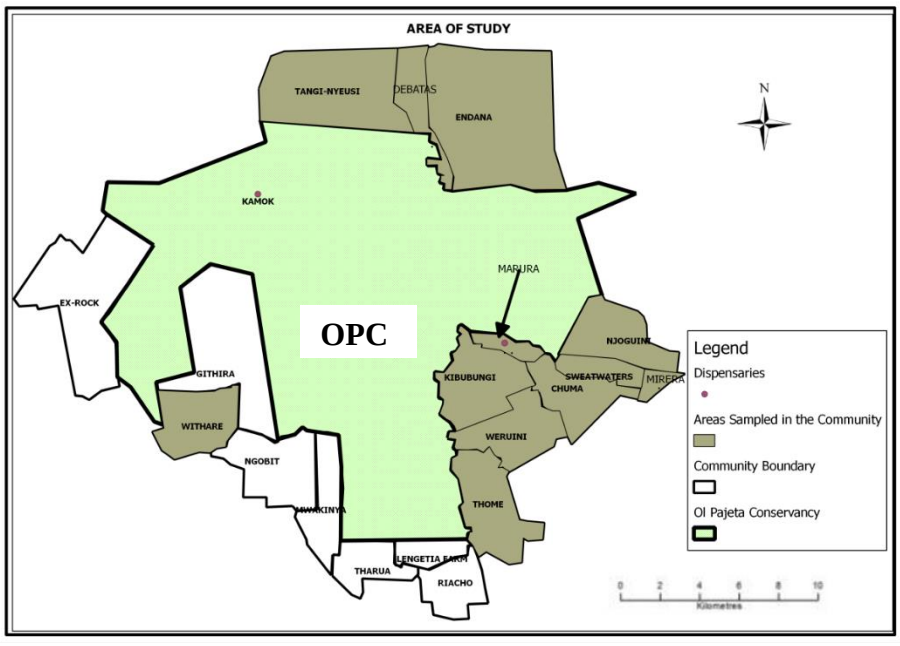


Figure 3.3: Map of Ol Pejeta conservancy and the neighbouring communities.
Source: Digitized from maps provided by Ol Pejeta Conservancy.

3.2 Study design

This was a cross sectional study where humans, cattle and African buffalos were sampled. The human and cattle participants were sampled from within the Conservancy and the community around the conservancy. African buffaloes were sampled from within the Conservancy.

3.3 Study population

Ol Pejeta Conservancy had about 500 workers drawn from the communities around the conservancy and from other parts of the country, who work in livestock and wildlife conservation areas. The conservancy has approximately 5000 heads of improved boran cattle herded in groups of 100 (plate 1). The cattle are kept for beef to generate income for the conservancy. The cattle were kept in night bomas for protection from predators, however, day time they share pasture and water with diverse species of wildlife. These cattle undergo a rigorous tick control program using a spray race twice a week. The conservancy also has approximately 1500 African buffalos that occur in herds of between 50 and 200 individuals. The community on the North of the conservancy, who were mainly pastoralists, kept boran cattle through transhumance (plate 2).

Plate 1: Improved boran cattle at OPC.

Plate 2: Boran cattle at Tangi nyeusi.

The community on the southern part keep mixed breeds under zero grazing management systems (plate 3). The two communities used knap sack sprayers to control ticks in their cattle once a week. The community on the northern part of the conservancy depended on the OPC medical facility located at Kamok, within the conservancy while the community on the southern part had a dispensary at Marura which was also supported by OPC (fig. 3.2).

Plate 3:Image of cattle at Withare.

3.4 Sample size determination

3.4.1 Sample size for Awareness and Practice

The human sample size for awareness and practice study was determined using the formula

$n=Z^2P(1-P)/e^2$ (Cochran, 1977) where

n = Sample size

z = Z value corresponding to 95% level of confidence which is 1.96

p = A response distribution of 50% was assumed in this study

e = Precision. A precision of 5% was used

Thus $n = (1.96 \times 1.96 \times 0.5 (1 - 0.5)/0.05^2) = 384.16$

Since the total population of workers at OPC was 500 at the time of this study, a correction formula for the finite population was applied.

$$n = \frac{n^o}{1 + \frac{(n^o-1)}{N}}$$

Where n^o = the initial sample size (385)

n = adjusted sample size

N = population size (500)

$$n = \frac{385}{1 + (386/500)}$$

n = 223.58

n = 224.

The sample size was increased by 10 % to 246 to give room for non-response.

3.4.2 Human sample size for molecular analysis of piroplasms

The human sample size for molecular analysis of piroplasms was determined using the formula

$n =Z^2P(1-P)/d^2$ (Naing *et al.,* 2006) where

n = Sample size

z = level of confidence at 95% which is 1.96

p = Expected prevalence. A prevalence of 22% was assumed in this study for all diseases based on a *Babesia microti* prevalence study conducted on non-human primates in Kenya (Maamun *et al*., 2011)

d = Precision: A precision of 10% was used in view of the immense resources required to carry out the molecular and phylogenetic analysis

Thus n = (3.84x0.22x0.78/0.1^2) = 65.92.≈ 66.

This number was rounded off to 70. Therefore 70 participants were enrolled and sampled for this study.

3.4.3 Sample size for cattle and African buffalo for molecular analysis of piroplasms

The animal (cattle and buffalo) sample size was determined using the formula n =$Z^2P(1-P)/d^2$ (Naing *et al.,* 2006) where

n = Sample size

z = level of confidence at 95% which is 1.96

p = Expected prevalence. A prevalence of 42.1% was assumed in this study for all diseases based on a *Theileria* prevalence study conducted in Mugie, Laikipia County (Otiende *et al.,* 2014).

d = Precision: A precision of 10% was used in view of the immense resources required to sample wildlife (the immobilization drugs, reversal agents, darting accessories and transport).

Thus n = (3.84x0.421x0.579/0.1^2) = 94

3.5 Pre-sampling activities

3.5.1. Preliminary procedures

A visit was made to OPC prior to the study to obtain relevant authorization to carry out the study from the management. Ethical approval (Appendix IX) was obtained from the Kenyatta University Ethics Research Committee (KU-ERC) application number PKU/557/E52. Authorization was also obtained from the Laikipia County department of health to carry out the study in humans (Appendix X), and from the Kenya Wildlife Services (Appendix XI) for the study in animals. Approvals were also obtained from the various area Chiefs prior to the study.

3.5.2. Preparation of data collection tools

A standardized structured questionnaire with questions that sought to gain insight into respondent's awareness and practice on prevention of human tick-borne diseases like *Theileria* and *Babesia* was administered (Appendices I and II). The questionnaire had

been translated into Kiswahili and contained three major areas which included (i) Socio-demographic and economic characteristics, (ii) awareness on human tick borne diseases and (iii), human tick-borne disease prevention practices. The questionnaires were administered with the help of research assistants who were competent in understanding English, Kiswahili and vernacular depending on the community. In circumstances where the respondents did not understand English or Kiswahili, the questionnaire was administered using vernacular but responses recorded in English. The administration of the questionnaires was monitored daily.

3.5.3. Training of research assistants

During the study a total of 12 research assistants were trained for 2 days and engaged in the administration of consent forms and the questionnaires (four for the community North of Ol Pejeta Conservancy, four for Ol Pejeta Conservancy and four, for the community South of Ol Pejeta Conservancy). In addition, four nurses (two for Kamok dispensary in OPC and two for Marura dispensary) were trained as research assistants to administer the consent forms and questionnaires to the study participants before blood sampling.

3.5.4. Pre-testing of study tools

A pre-test of the questionnaires was conducted on a sample of 20 selected workers at the Ol Pejeta Conservancy. This was important because it helped to test the validity and reliability of the tool. Adjustments were done on the questionnaire based on the outcome of the pilot test.

3.6 Sampling procedure

3.6.1 Sampling for Awareness and Practices

The respondents for this study inside OPC were selected based on systematic random sampling technique in areas where the workers converged such as the cattle dips, vehicle boarding points, lunch cafeterias and as they attended the Kamok dispensary. This was due to challenges in accessing most of the areas where the workers were deployed in the conservancy because of security due to the wild animals and the impassable roads outside the main tourism circuits. The Conservancy provided the sampling frame. The questionnaire (Appendices I and II) was administered.

Consecutive sampling technique was used to select respondents in the Northern community from Tangi Nyeusi to Endana (Fig 3.2). The area had about 1470 households. However, the demographics are known to fluctuate among the pastoralist communities especially during dry seasons. Since the administration of questionnaires was during the dry season, most of the residents or part of families had relocated. Each household that had an eligible adult who willingly gave consent was recruited into the study as a respondent.

Probability proportional to size sampling method was used determine the number of households to recruit into the study in each village in the Eastern and Southern part of the conservancy. This was because the area was large and densly populated compared to the other study sites. The total number of households was about 2750 from Njoguini to Thome (Fig 3.2). The village elders provided lists of households which bordered the conservancy and these lists were used as sampling frames. Systematic random sampling technique was then used to select the households to include in the study. Only one person per household was interviewed.

Questionnaires were also administered to hospital patients at the Kamok and Marura dispensary shown in figure 3.2. The inclusion criteria for administering the questionnaires in the community, at OPC and at the dispensaries was

i. Household head or any responsible person in the household

ii. 18 years and above

iii. Willing to participate and gave consent to be interviewed.

iv. Patients at the dispensaries who had given consent to be included in the study through blood sampling and were willing to fill in the questionnaires.

The exclusion criteria included:

i. Members of the household who were below 18 years old.

ii. Household heads or patients who did not consent.

3.6.2 Blood sampling procedure

a) The human blood sampling was done through consecutive sampling whereby individuals meeting the criteria for inclusion were sampled until the required sample number was achieved. This sampling procedure was chosen because the two dispensaries did not have a heavy flow of patients and the blood sample storage facilities (liquid nitrogen tanks) did not allow for long storage hours due to evaporation. Consequently, consecutive sampling allowed the study to proceed with minimal costs. Adults above 18 years both male and female who voluntarily consented to the study were selected (Appendix VII and VIII). Blood sampling was performed by the phlebotomist at the Kamok dispensary within OPC and at the Marura dispensary located outside OPC on the Eastern edge of the conservancy (Fig. 3.2). A consent form was administered to patients at the dispensary and inclusion criteria were:

i. Adults with symptoms of fever
ii. Adults without fever who had direct contact with cattle for at least 1 year
iii. Adults without fever who are exposed to wildlife habitat for at least 1 year.

Exclusion criteria included:-

i. Patients that were too unwell to consent
ii. Patients who did not consent.

Data on the sampled patients was recorded (appendices III and IV). About 3ml of blood was collected from the cephalic vein using 23 gauge needles and transferred into EDTA tubes (4.5ml), gently swirled by hand and aliquoted into 1.8ml cyovials labelled with the patient's identity code. The samples were stored in liquid nitrogen and transported to the diagnostic laboratory in Nairobi at the Kenya Wildlife Services for storage and molecular analysis.

b) Cattle were sampled from OPC and the community adjacent to OPC. Sampling was carried out once on cattle older than 6 months of age. Ageing for cattle was based on birth date records available at OPC, while community cattle owners gave information on the age. At the OPC, sample selection was based on stratified random

sampling. Herds were stratified into male and female herds from which 3 herds were selected by simple random sampling method. From each herd of 100 heads of cattle, 10 individuals were picked through simple random sampling based on the identification numbers branded on each cattle. Another 6 cattle were sampled from a herd of 60. A total of 66 cattle were sampled from OPC. Sampling of cattle from communities adjacent to OPC was by census where all the cattle that were availed for the study were sampled. This was carried out at Tangi nyeusi in the northern part of the conservancy and Withare in the south west part of the conservancy (Fig. 3.2). A total of 32 cattle were sampled from the two sites with 15 cattle being sampled at Tangi nyeusi and 17 at Withare.

Restraint was done in the cattle crush for blood sampling. 10 ml of blood from jugular vein was drawn into EDTA tubes. The samples were then placed in a cool box and transported to the field laboratory where they were aliquoted into 1.8ml cryovials and stored in liquid nitrogen. At the Veterinary Diagnostic Laboratory of Kenya Wildlife Services in Nairobi, the samples were transferred to -80^{o}C freezer, where they remained until processing.

c) African buffalos were sampled from within OPC through convenience sampling. The sampling targeted buffalos aged above 6 months. All African buffalos that were within the range of the darting gun and could be accessed by the team were sedated and sampled. Aging was based on a combination of the body size, horn curvature structure and dental structure. At six months the passively acquired immunity from colostrum against *Theileria* and *Babesia* is expected to have worn off. The Kenya Wildlife Service veterinarians and wardens identified and tracked buffalo herds by a vehicle and target individuals were darted (Plate 4) to achieve sedation using a combination of Etorphine hydrochloride (M99®) and Azaperone (Norvatis, South Africa, PTY, Ltd). The sedation was reversed using Diprenophine (Norvatis, South Africa, PTY, Ltd) (Kock & Burroughs, 2012).

A total of 92 buffalos were sampled within a period of 2-weeks. All the animals that had been sampled were marked using oxytetracycline spray at the rump and thigh region on both sides for easy visibility to avoid re-sampling. 10 ml of blood from

jugular vein was drawn into EDTA tubes. The samples were then placed in a cool box and transported to the field laboratory where they were aliquoted into 1.8ml cryovials and stored in liquid nitrogen. At the Veterinary Diagnostic Laboratory of Kenya Wildlife Services in Nairobi, the samples were transferred to -80ºC freezer, where they remained until processing.

Plate 4: Image of African Buffalo in Ol Pejeta Conservancy having a dart on the lower neck.

3.7 Awareness and Practice survey data analysis

The study area shown in figure 3.2 was divided into 3 sections (1) The Northern community comprising of respondents from Tangi Nyeusi, Endana and Debatas (2) Ol Pejeta community and (3) The southern community which was made up of people from Njoguini, Mirera, Marura, Chuma, Sweet waters, Kibubungi, Weruini and Ngobit. All the occupations given by the respondents were amalgamated into 7 main occupations practiced in the area. These were, mixed farmers (those who kept livestock and crops), game wardens (those who were in charge of the security of the wildlife in the conservancy and included the patrol men, the security personnel, the wildlife guards, and wildlife guides), the business people, the office workers, casuals, herders (those who were employed by the conservancy to herd the livestock owned by the conservancy and they also included the dip attendants and slaughter house workers) and the pastoralists (the people who owned livestock and moved with them from place to place in search of pasture and water).

The awareness section had 13 questions which covered awareness in terms of tick identity, ticks as vectors of diseases, ticks as transmitters of zoonotic diseases and the perception on the risk of contracting human tick borne diseases. For each question a correct response was awarded two (2) points while a wrong response or 'I don't know' was awarded one (1) point.

The perception of susceptibility to human tick-borne diseases was measured using six (6) statements on a 5-point Likert scale (1= strongly disagree, 2= disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree). The awareness score for each participant was compiled by summing the scores from the correct responses and the scores from the Likert data out of 42 points (the maximum a participant could score in this section). This was then converted into a percentage for each participant. The total score of the respondents was then categorized according to Bloom's cut-off points as either poor (below 60%), fair (60% to 80%) or good (Above 80%).

The practice section was divided into four key areas of recommended behaviours that have been shown to successfully prevent tick borne diseases which were tick habitat avoidance, visual tick checks, the use of protective clothing and the application of tick repellents. The tick habitat avoidance practice indicated whether the respondent avoided areas where ticks occurred always or if they chose to walk on clear pathways. The visual tick check practice was shown by the respondent checking their body during and after visiting a tick infested area. Two more questions were added to represent the respondent's behaviour of seeking medical attention after tick bites. A total of ten (10) questions were asked. The respondents had an option to answer in any of the three (3) responses: Never, Sometimes and always. These questions were used to evaluate the personal precautionary measures that the respondents deliberately use against tick infestation and/or tick-borne diseases. For the answers; always was awarded three (3) points; sometime awarded one (2) point; and never, awarded one (1) point. A practice score against each participant was compiled by summing the number of correct responses out of 10 questions.

The practice score for each participant was compiled by summing the scores from the correct responses and the scores from the Likert data out of 30 points (the maximum a participant could score in this section). This was then converted into a percentage for each participant. The total score of the respondents was then categorized according to Bloom's cut-off points as either poor practice (below 60%), fair practice (60% to 80%) or good practice (Above 80%).

The variables in the data were coded for easy entry and analysis in Microsoft Excel 2007, cleaned to detect any missing or invalid variable. Chi-square test of association and Fisher's exact tests were used to determine the association between socio-demographic characteristic, awareness and practice using Statistical Package for Social Sciences (SPSS for windows, version 23, Chicago, USA). A P value < 0.05 was considered significant for comparison. These variables were also subjected to linear regression analysis to determine the strength of the association. Qualitative data was presented using proportions and frequencies. The conceptual framework for analysis of the variables in the study is as shown below. The Independent variables were the socio-demographic and economic variables while the dependent variable was the practice on prevention of human tick-borne diseases (Bartosik *et al.,* 2008; Bayles *et al.,* 2013; Gould *et al.*, 2008)

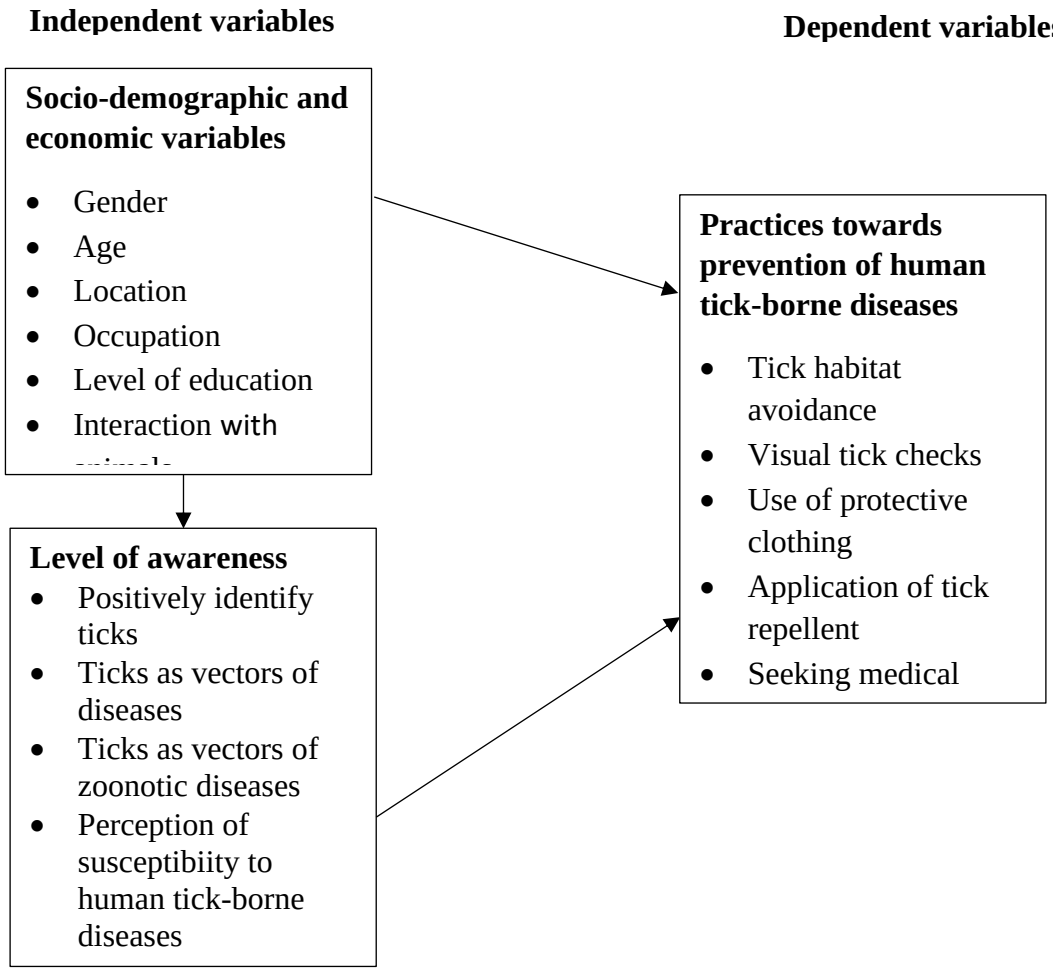


Figure 3.4: Conceptual framework for the analysis of awareness and practices on prevention of human tick-borne diseases.

3.8 Molecular analysis

3.8.1 DNA Extraction and PCR amplification

Frozen blood of humans, cattle and African buffalo was thawed and 200µl of blood used for DNA extraction using the Qiagen DNeasy blood and tissue extraction kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Two microlitres of the genomic DNA was used in the amplification that targeted the hypervariable V4 region of the 18S r RNA gene of the genera *Theileria* and *Babesia.*

The size of the target region was 450bp, which was amplified using primers RLB F_2 (5' – GAC ACA GGG AGG TAG TGA CAA G -3') and RLB R_2 (5' – CTA AGA ATT TCA CCT CTA ACA GT -3') synthesized by Inqaba biotec™, South Africa, to identify *Babesia* and *Theileria* species as described by (Gubbels *et al.,* 1999), with the following modifications according to Hooge *et al.,* (2015), Using a PCR kit (Qiagen, HotstarTaq master mix kit), PCR amplification was carried out in 25µl reactions containing approximately 2.0µl of template genomic DNA, 1.25µl of 10mmol each of the forward and reverse primer, 12.5µl of HotstarTaq master mix and 8µl of PCR water. For negative controls an equivalent of purified water was substituted for DNA. For positive control an equivalent of known DNA of *Theileria parva* was used. The PCR cycles were carried out in a thermocycler (BIORAD T100™,Singapore) under the following conditions consisting of an initial denaturation step of 4 minutes at 94°C, followed by 35 cycles of 94°C for 20seconds, 57^0C for 30 seconds and 72^0C for 30 seconds and a final extension for 10 minutes at 72^0C.

Since the amplicons obtained by RLB single step method on human samples were faint, an alternative nested PCR using ILO 9028, ILO 9030 and ILO 7782 primer was used to confirm that the amplicons obtained from RLB were either *Theileria* or *Babesia* as described by Wamuyu *et al.,* (2015).

3.8.2 Gel electrophoresis

The final PCR product was separated using gel electrophoresis on 1.5% (w/v) ultra-pure agarose gel containing ethidium bromide at 100V for 45 min. The gel was then visualized for positive amplification of the target region on a UV-trans-illuminator and photographed (UVITEC® CAMBRIDGE, France). A one Kb DNA ladder was used to identify the approximate size of the molecule run on the gel.

3.8.3 Sequencing and sequence editing

All the positive PCR products were purified and sequenced at Macrogen Inc, Europe, based on Sanger's method (ABI 3730XLs). The sequences were then cleaned and edited using the software Sequencher V 5.4.6. The raw forward and reverse traces were assembled using the software for molecular evolutionary genetics analysis (MEGA) V.7.0.20. The sequences were trimmed, gaps deleted and the consensus

nucleotide sequences aligned using the software for multiple sequence comparison by log expectation (MUSCLE V.7).

The cleaned sequences were searched on Genbank database using the basic local alignment search tool nucleotide (BLASTn) to confirm *Theileria* or *Babesia* DNA amplification and identity relatedness. The DNA sequence polymorphism software (DnaSP) was used to investigate sequence divergence and polymorphism between the haplotypes and the GenBank references.

3.8.4 Phylogenetic analysis

The program MUSCLE V.7 was used to determine the model of sequence evolution as well as the rate of heterogeneity of aligned sequences for both *Theileria* and *Babesia.* The Phylogeny was inferred using the maximum likelihood method based on Kimura two parameter model (Kimura, 1980).

3.9 Ethical Considerations

Ethical approval (Appendix IX) was obtained from the Kenyatta University Ethics Research Committee (KU-ERC) application number PKU/557/E52. All the human participants provided written consent voluntarily before participating in the study (Appendices V, VI, VII and VIII). Those who could not write gave their consent by a fingerprint. The participants were also informed about their right to withdraw from the study at any time without any consequence. The protocol and procedures for animal handling and care during the study followed the Kenya Wildlife Veterinary Guidelines and ethical practice 2006 and the Veterinary Surgeons Act, 2011 (CAP 366) of the laws of Kenya. The subjects' privacy and confidentiality was protected through secure storage of the data by keeping the data in lockable cabinets and password protected computers and files. All the research assistants signed secrecy documents to ascertain confidentiality. The participant's identification was also kept private by coding the blood samples and questionnaires.

3.10 Limitations of the study

This study could not rule out information bias as a result of translation of the questionnaires by the research assistants into the local dialect for the respondents who were illiterate. The semi-structured questionnaire was limiting in obtaining the deep knowledge the respondents had on human tick borne diseases or prevention strategies that they used routinely.

The study took place at a time when there was a security operation in the area because of drought and invasion of conservancies by pastoralists. Ol Pejeta Conservancy management was reluctant in giving permission for the pastoralists from the North to be sampled from within their health facility. As a result none of the pastoralists, who were an important segment of the study population, were sampled for the molecular part of the study. Most of the males in the north had moved as a result of the security operation and also in search of pasture and water. The study could therefore have missed out on important information from this segment of the population since they spend most of their time in tick infested habitats.

The cross-sectional study design also had limitations because it was not possible to establish whether the associations observed in the study between various variables had any aetiological relationship. Some of the associations were difficult to interpret.

CHAPTER FOUR
RESULTS

The study took place between January and May 2017. A total of 307 questionnaires were administered with 55 questionnaires being administred in the community north of OPC, 152 being administered within OPC and 100 being administred in the community south of OPC. The response rate was 97.2%. In summary the study established that there was an association between some socio-demographic and economic variables with the level of awareness and practices towards the prevention of human tick-borne diseases. It was also able to establish the presence of piroplasms in the sympatry cattle and African buffalos but not in the humans.

4.1. Socio-demographic and economic characteristics of the study population

The total sample size was 307. About 70% of the respondents were male and the rest were female (n=214 males and n = 93 females). All the respondents were 18 years and above. The minimum age was 18 and the maximum was 83 with a range of 65. The mean age was 36.5 years. Majority of the respondents (44%) were between the ages of 18 to 30 (fig.4.1). The median age was 32 years and the Interquartile range was 18.5.

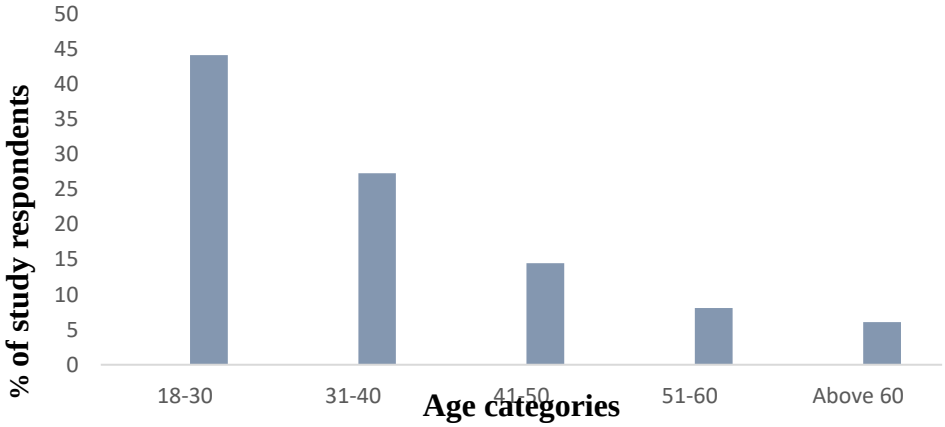


Figure 4.1: Age distribution of the study respondents at Ol Pejeta Conservancy, Kenya.

A large percentage of the respondents had no formal schooling (26.7%). The main occupation in the community was herding at 25.4% followed by mixed farming

where the farmers kept livestock and grew crops for substistence and as a source of income (23.5%). Most of the respondents (57.0%) interacted with both cattle and wildlife in their daily routine (Table 4.1).

Table 4.1 Socio-demographic characteristics of the respondents to the questionnaires administered to the communities living in and around Ol Pejeta Conservancy, Kenya

Variable	Category	Number	Proportion (%)
Gender	Male	214	69.7
	Female	93	30.3
Location	North of OPC	55	17.9
	OPC	152	49.5
	South of OPC	100	32.6
Age	18-30	131	42.7
	31-40	81	26.4
	41-50	43	14.0
	51-60	24	7.8
	Above 60	18	5.9
	Non-response	10	3.3
Level of Education	No formal schooling	82	26.7
	Complete primary school	48	15.6
	Complete secondary school	80	26.1
	Incomplete primary school	22	7.2
	Incomplete secondary school	62	20.2
	Post-secondary certificate or diploma	8	2.6
	Degree and above	2	0.7
	Non-response	3	1.0
Occupation	Employed herders	78	25.4
	Mixed farmers	72	23.5
	Game wardens	55	17.9
	Pastoralists	49	16.0
	Office workers	22	7.2
	Business men	22	7.2
	Casual laborers	9	2.9
Interaction with animals	Cattle and wildlife	175	57.0
	Cattle only	125	40.7
	Wildlife only	4	1.3
	None	3	1.0
	Total	**307**	**100.0**

Livestock ownership was a key economic resource for this community who mainly dwelt in semi-permanent houses (73.3%) and depended on firewood (80.5%) and kerosene (47.9%) for cooking and lighting, respectively (Table 4.2)

Table 4.2 Socio-economic characteristics of the respondents at Ol Pejeta Conservancy, Kenya.

Variable	Category	Number	Proportion (%)
Livestock ownership	Own livestock	258	84.0
	Do not own livestock	49	16.0
Where livestock are housed	Livestock enclosure	256	99.2
	In the house	2	0.8
Type of house	Semi-permanent	225	73.3
	Permanent	47	15.3
	Temporary	35	11.4
House wall material	Wood	106	34.5
	Iron sheets	82	26.7
	Mud	67	21.8
	Stone	31	10.1
	Brick	17	5.5
	Polythene paper	4	1.3
House floor material	Earth	204	66.4
	Cement	100	32.6
	Tiles	2	0.7
	Wood	1	0.3
House roofing material	Iron sheets	249	81.1
	Grass	29	9.5
	Polythene paper	29	9.5
Domestic water source	Piped water	134	43.7
	Open source	89	29.0
	Bore-hole	77	25.1
	Supplied by vehicles	7	2.3
Toilet facilities	Pit-latrine	235	76.5
	No toilet facilities	66	21.5
	Flush toilet	6	2.0
Source of cooking energy	Firewood	247	80.5
	Gas	27	8.8
	Kerosene	16	5.2
	Charcoal	12	3.9
	Electricity	5	1.6
Source of lighting	Kerosene lamp	147	47.9
	Electricity	84	27.4
	Solar	60	19.5
	Rechargeable lamp	12	3.9
	None	2	0.7
	Firewood	1	0.3
	Wax candle	1	0.3

4.2 Awareness on *Theileria, Babesia* and other human tick-borne diseases

Analysis of the scores of the respondents on their level of awarenesson ticks and human tick-borne diseases showed that they were aware about ticks and human tick-borne diseases existing in their community. Out of the 307 respondents, 55.4% scored above 80% on the questions related to awareness. 43.7% had fair knowledge while 0.98% scored poorly on awareness.

4.2.1. Awareness on ticks and their role as disease vectors

Majority of the respondents positively identified a tick (99.7%) and those that were aware that ticks could spread diseases to livestock were (97.4%). However, the number of respondents decreased when asked about ticks transmitting diseases to humans or whether there were diseases shared between wildlife and livestock. Most of the respondents (69.0%) experienced heavy tick bites during the dry season (Table 4.3).

Table 4.3 Level of awareness on ticks and their role as vectors of diseases in Ol Pejeta Conservancy, Kenya.

	Variable	Response	N	Proportion (%)
1	Positively identify a tick	**Yes**	**306**	**99.7**
		No	1	0.3
2	Ticks transmit diseases to livestock	**Yes**	**299**	**97.4**
		Don't know	6	2.0
		No	2	0.6
3	Ticks transmit diseases to wild animals	**Yes**	**280**	**91.2**
		Don't know	22	7.2
		No	5	1.6
4	Ticks transmit diseases to humans	**Yes**	**208**	**67.7**
		Don't know	73	23.8
		No	26	8.5
5	There are diseases shared between livestock and wildlife	**Yes**	**199**	**64.8**
		Don't know	80	26.1
		No	28	12.1
6	Season of the most tick bites	**Dry season**	**212**	**69.1**
		Wet season	51	16.6
		All year round	34	11.1
		Don't know	10	3.3

4.2.2 Awareness on human tick-borne diseases

Majority of the respondents (46.9%), picked Tick fever as a human tick borne disease followed by East Coast Fever (ECF) as a human tick borne disease (27.7%). This is a tick borne disease known to affect cattle and not man (Fig. 4.2). The responses pointed towards a general lack of awareness on human tick-borne diseases among the respondents.

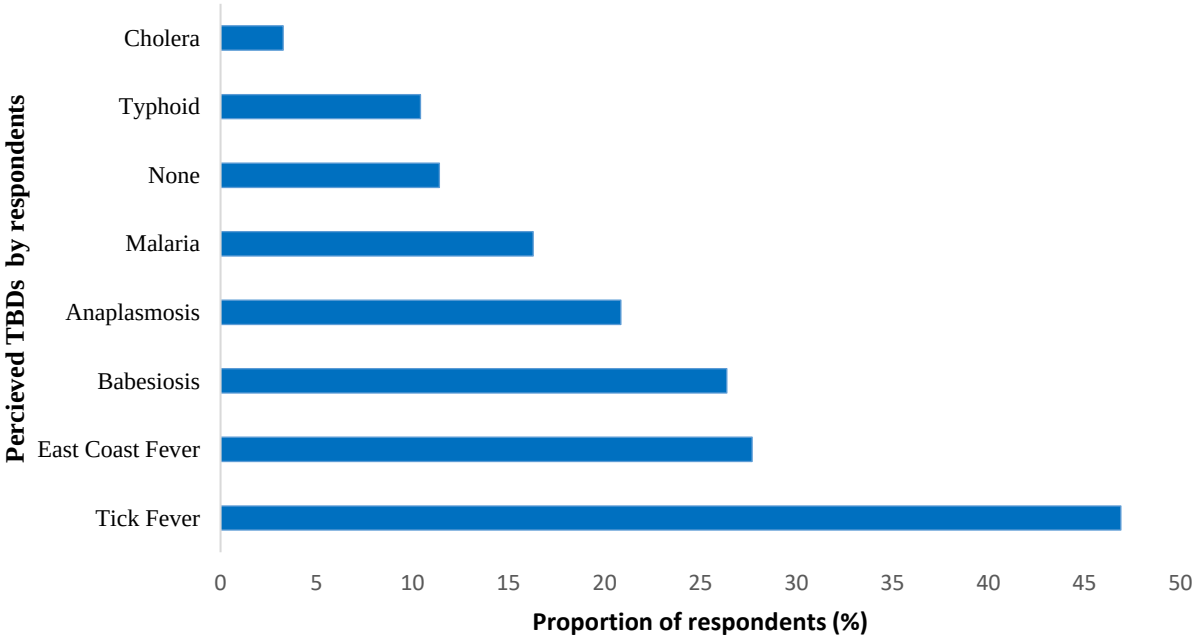


Figure 4.2: Awareness on human tick-borne diseases at Ol Pejeta Conservancy, Kenya.

When asked to identify common signs and symptoms associated with tick bites in humans, 71.7% of the respondents mentioned skin rash followed by general weakness (43.0%) as the main symptoms (Fig. 4.3).

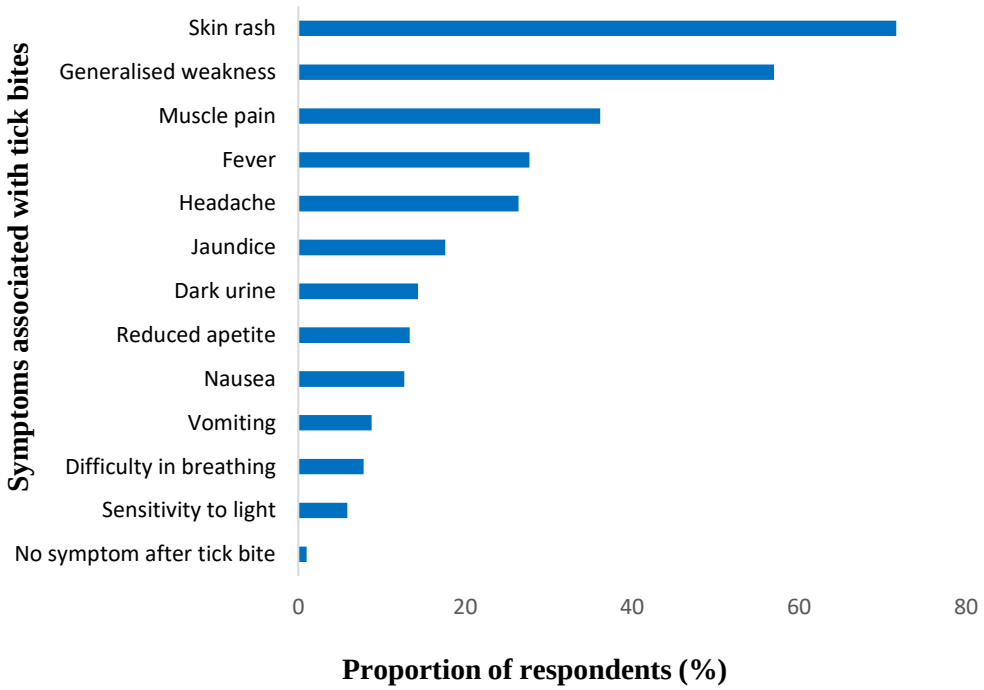


Figure 4.3: Signs and symptoms that respondents associated with tick bites in humans at Ol Pejeta Conservancy, Kenya.

4.2.3 Perception of susceptibility to human tick-borne diseases

A proportion of 60.9% of the respondents thought tick borne diseases occurred in the area while 63.5% of the respondents thought that they were at risk of infection with tick-borne diseases. The respondents strongly agreed that use of proper prevention strategies such as use of protective clothing and avoiding tick habitats was important (73.0%). However only 49.2% believed that use of tick repellents was an effective prevention strategy (Table 4.4). Overall, there was a strong agreement for need of community education about tick borne diseases (72.3%).

Table 4.4: The perception of susceptibility to human tick-borne diseases at Ol Pejeta Conservancy, Kenya.

Assessment variables	I Strongly disagree		I disagree		I neither agree nor disagree		I agree		I strongly agree	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Diseases spread by ticks occur in my area	11	3.6	37	12.1	72	23.4	117	**38.1**	70	22.8
A tick can spread more than one disease	10	3.3	31	10.1	83	27.0	103	**33.5**	80	26.1
I am at risk of getting a disease spread by ticks	19	6.2	42	13.7	51	16.6	129	**42.0**	66	21.5
By use of proper prevention strategies, I can prevent diseases spread by ticks	13	4.2	37	12.0	33	10.8	105	34.2	119	**38.8**
I believe tick repellents are effective	56	18.2	45	14.7	55	17.9	113	**36.8**	38	12.4
There should be more education about diseases spread by ticks	10	3.3	6	2.0	1	0.3	68	22.1	222	**72.3**

Key: The responses with the highest proportion in each category are in bold.

4.3 Association between socio-demographic and economic variables and the level of awareness of the respondents.

Analysis of the association between socio-demographic factors on awareness on ticks and tick-borne diseases showed that location of residence of the respondents, gender, level of education and the occupation was statistically significant or influenced the level of awareness on ticks and human tick-borne diseases. Location was statistically significant at (χ^2 12.699, df = 4, P = 0.013). Gender at (χ^2 8.295, df = 2, P = 0.016),

level of education at (χ^2 29.139, df = 12, P = 0.002) and occupation at (χ^2 22.592, df = 12, P =0.031). Age and livestock ownership did not have any association with awareness (Table 4.5).

Table 4.5: Association between socio-demographic and economic variables and the composite level of awareness at Ol Pejeta Conservancy, Kenya.

Variable	Level of Awareness								Chi-Square	df	P-value
	Poor		Fair		Good		Total				
	n	%	n	%	n	%	n	%			
Gender											
Male	2	0.7	82	26.7	130	42.4	214	69.7			
Female	1	0.3	52	16.9	40	13.0	93	30.3	**8.477**	**2**	**0.016***
Total	**3**	**1.0**	**134**	**43.7**	**170**	**55.4**	**307**	**100.0**			
Location											
North	2	0.7	32	10.4	21	6.8	55	17.9			
OPC	1	0.3	58	18.9	93	30.3	152	49.5	**12.699**	**4**	**0.013***
South	0	0.0	44	14.3	56	18.2	100	32.6			
Total	**3**	**1.0**	**134**	**43.7**	**170**	**55.4**	**307**	**100.0**			
Age											
18-30	1	0.3	55	18.5	75	25.3	131	44.1			
31-40	1	0.3	38	12.8	42	14.1	81	27.3			
41-50	1	0.3	21	7.01	21	7.1	43	14.5			
51-60	0	0.0	8	2.7	16	5.4	24	8.1	5.3	8	0.815
Above 60	0	0.0	10	3.34	8	2.7	18	6.1			
Total	**3**	**1.0**	**132**	**44.4**	**162**	**54.6**	**297**	**100.0**			
Level of Education											
No formal schooling	2	0.7	53	17.4	27	8.9	82	27.0			
Complete primary	1	0.3	18	5.9	29	9.5	48	15.8			
Complete secondary	0	0.0	24	7.9	56	18.4	80	26.3			
Incomplete primary	0	0.0	9	3.0	13	4.3	22	7.2			
Incomplete secondary	0	0.0	25	8.2	37	12.2	62	20.4	**31.337**	**12**	**0.0001***
Post-secondary certificate or diploma	0	0.0	3	1.0	5	1.6	8	2.6			
Degree and above	0	0.0	0	0.0	2	0.7	2	0.7			
Total	**3**	**1.0**	**132**	**43.4**	**169**	**55.6**	**304**	**100**			

Key: * Statistically significant

Table 4.5 (continued)

Variable	Level of Awareness								Chi-Square	df	P-value
	Poor		Fair		Good		Total				
	n	%	n	%	n	%	n	%			
Occupation											
Employed herders	1	0.3	31	10.1	46	15.0	78	25.4			
Mixed farmers	0	0.0	34	11.1	38	12.4	72	23.5			
Game wardens	0	0.0	22	7.2	33	10.8	55	17.9			
Pastoralists	2	0.7	31	10.1	16	5.2	49	16.0	**22.592**	**12**	**0.031***
Office workers	0	0.0	8	2.6	14	4.6	22	7.2			
Business men	0	0.0	7	2.3	15	4.9	22	7.2			
Casual laborers	0	0.0	1	0.3	8	2.6	9	2.9			
Total	**3**	**1.0**	**134**	**43.7**	**170**	**55.4**	**307**	**100**			
Interaction with animals											
Cattle and wildlife	3	1.0	71	23.1	101	32.9	175	57.0			
Cattle only	0	0.0	60	19.5	65	21.2	125	40.7			
Wildlife only	0	0.0	2	0.7	2	0.7	4	1.3	3.815	6	0.702
None	0	0.0	1	0.3	2	0.7	3	1.0			
Total	**3**	**1.0**	**134**	**43.7**	**170**	**55.4**	**307**	**100**			
Livestock ownership											
Own livestock	3	1.0	112	36.5	143	46.6	258	84.0			
Do not own livestock	0	0.0	22	7.2	27	8.8	49	16.0	8.477	2	0.744
Total	**3**	**1.0**	**134**	**43.7**	**170**	**55.4**	**307**	**100**			

Key: * Statistically significant

4.4 Practices on prevention of tick bites and human tick-borne diseases.

Responses to questions that assessed the respondents' practices towards prevention of tick bites and tick-borne diseases are summarized in table 4.5. Overall, the respondents had poor practices against prevention of tick bites (46.6%). It was noted that 45.9% of the respondents did not take any preventive measures against tick exposure. The most popular protection measure with the respondents was visual tick checks on their clothes after exposure to ticks (34.5%) followed by visual tick checks on the body after exposure (34.2%) while the least used method of protection was application of tick repellents (7.2%). Many respondents (77.2%) sought medical intervention when they developed any fever (Table 4.6).

Table 4.6: Respondents practices on prevention of tick-bites and tick-borne diseases at Ol Pejeta Conservancy, Kenya.

Assessment Variable	Proportion of respondents					
	Always		Sometimes		Never	
	n	%	n	%	n	%
Use of tick repellents	22	7.2	37	12.0	248	**80.8**
Wearing long sleeved shirt and trouser	59	19.2	94	30.6	154	**50.2**
Tucking in the trouser into socks or boots	61	19.9	90	29.3	156	**50.8**
Removing clothes and washing after tick exposure	106	34.5	109	**35.5**	92	30.0
Avoiding areas infested with ticks whenever possible	60	19.5	70	22.8	177	**57.7**
Sticking to clear pathways when walking	46	15.0	74	24.1	187	**60.9**
Checking the body for ticks when walking	100	32.6	114	**37.1**	93	30.3
Thoroughly check the body for ticks after exposure	105	34.2	113	**36.8**	89	29.0
Removal of ticks and application of a disinfectant on the skin when bitten by a tick	48	15.6	64	20.9	195	**63.5**
Seeking medical care when fever develops	237	**77.2**	53	17.3	17	5.5

Key: The responses with the highest proportion in each category are in bold.

4.5 Association between level of awareness and practices on prevention of tick bites and human tick-borne diseases

Generally, there was an association between the level of awareness and the practices of the study respondents towards prevention of tick bites and tick borne diseases (table 4.7). The higher the level of awareness the better the practice towards prevention of tick borne diseases.

Table 4.7 Association between level of awareness and practice using Chi-square at Ol Pejeta Conservancy, Kenya.

Awareness level	Practice			Totals	Test statistic
	Poor	Fair	Good		Chi square
Poor	1	1	1	3	$\chi^2 = 9.734$
Fair	58	70	6	134	P-value: **0.025***
Good	84	67	19	170	df = 4
Totals	143	138	26	307	

Key: * Statistically significant.

4.6 Association between socio-demographic and economic variables and practices on prevention of tick bites and human tick-borne diseases

Out of all the independent variables cross tabulated with practice in general (the total score for practice for the respondents), only occupation influenced the respondents general practices towards prevention of tick bites and tick-borne diseases occupation (χ^2 = 22.964, df =12, P =0.028) as shown in table 4.8. There was no statistically significant association between the general practices of the respondents and their location, gender, age, interaction with animals, livestock ownership or their level of education.

Table 4.8: Association between practice and the socio-demographic and economic Variables at Ol Pejeta Conservancy, Kenya.

Independent variables	Chi- Square test of statistics		
	χ^2 Value	df	P -Value
Location	9.165	4	0.061
Gender	1.225	2	0.542
Age	1.615	8	0.993
Interaction with animals	4.903	6	0.556
Livestock ownership	0.501	2	0.778
Level of education	8.363	12	0.756
Occupation	22.964	12	**0.028***

Key: * statistically significant

The independent variables were then cross tabulated with specific practices geared towards prevention of tick bites and human tick-borne diseases. The results showed that the specific practises were influenced by certain socio-demographic and economic variables as shown below in table 4.9.

Table 4.9: Socio-demographic and economic variables that had significant association with various practices towards prevention of tick bites and human tick-borne diseases at Ol Pejeta Conservancy, Kenya.

Practice	Associated variable	χ^2 Value	df	P-value
Use of tick repellent	Location	29.976	4	**0.0001***
	Gender	14.9	2	**0.0001***
	Occupation	25.288	12	**0.007***
Use of protective clothing	Location	25.196	4	**0.0001***
	Gender	12.431	2	**0.002***
	Occupation	34.441	12	**0.001***
Visual checks for ticks	Level of education	20.505	12	**0.039***
Tick habitat avoidance	Location	36.240	4	**0.0001***
	Gender	14.516	2	**0.001***
	Interaction with animals	20.997	6	**0.002***
	Occupation	58.674	12	**0.0001***
Seeking medical attention	Location	44.105	4	**0.0001***
	Gender	14.333	2	**0.001***
	Age	15.481	8	**0.042***
	Interaction with animals	21.474	6	**0.001***
	Livestock ownership	13.265	2	**0.001***
	Level of education	33.410	12	**0.0001***
	Occupation	49.819	12	**0.0001***

Key: * Statistically significant

4.7 Regression analysis for the association between socio-demographic and economic variables, awareness and practices on prevention of human tick-borne diseases

Logistic regression analysis established that the key predictors of level of awareness was gender (β = -0.148, t=-2.388, p = 0.022) and level of education (β =1.097, t =3.64, p <0.001) as shown in table 4.10.

Table 4.10: Regression analysis for the association between socio-demographic and economic variables, awareness and practices on prevention of human tick-borne diseases at Ol Pejeta Conservancy, Kenya.

Dependent Variable	Independent Variable	B	Std Error	β Coeff.	t	P value
Level of knowledge	Location	-0.072	0.638	-0.008	-0.113	0.910
	Gender	**-2.554**	**1.106**	**-0.148**	**-2.308**	**0.022***
	Level of education	**1.097**	**0.303**	**0.216**	**3.624**	**0.000***
	Occupation	-0.153	0.231	-0.044	-0.662	0.508
Practices on prevention of human tick-borne diseases	Location	0.299	1.392	0.19	0.215	0.803
	Gender	-0.952	2.023	-0.032	-0.470	0.638
	Level of education	0.208	0.557	0.024	0.373	0.709
	Occupation	**-0.883**	**0.417**	**-0.147**	**-2.117**	**0.035***
	Interaction with animals	-2.325	1.934	-0.095	-1.202	0.230
	Livestock ownership	-0735	2.440	-0.019	-0.301	0.764
Use of tick repellent	**Location**	**-6.346**	**1.885**	**-0.288**	**-3.367**	**0.01***
	Gender	**6.231**	**2.741**	**0.146**	**2.274**	**0.024***
	Level of education	0.678	0.755	0.054	0.897	0.370
	Occupation	-0.109	0.565	-0.013	-0.193	0.847
	Interaction with animals	3.956	2.620	0.114	1.510	0.132
	Livestock ownership	-1.201	3.305	-0.023	-0.363	0.717
Use of protective clothing	Location	3.113	1.978	0.138	1.574	0.117
	Gender	**-6.241**	**2.876**	**-0.143**	**-2.170**	**0.031***
	Level of education	0.395	0.792	0.031	0.498	0.619
	Occupation	**-1.745**	**0.593**	**-0.201**	**-2.944**	**0.003***
	Interaction with animals	-0.844	2.749	-0.024	-0.307	0.759
	Livestock ownership	-0.553	3.468	-0.010	-0.159	0.874
Tick visual checks	Location	2.949	2.400	0.110	1.229	0.220
	Gender	-1.420	3.490	-0.027	-0.407	0.684
	Level of education	-0.371	0.961	-0.024	-0.386	0.700
	Occupation	-1.378	0.719	-0.134	-1.916	0.056
	Interaction with animals	-4.228	3.336	-0.100	-1.267	0.206
	Livestock ownership	-6.332	4.208	-0.098	-1.505	0.133
Tick habitat avoidance	**Location**	**-4.724**	**2.100**	**-0.197**	**-2.249**	**0.025***
	Gender	4.317	3.054	0.093	1.414	0.159
	Level of education	-1.158	0.841	-0.085	-1.377	0.170
	Occupation	0.176	0.629	0.019	0.280	0.780
	Interaction with animals	-1.823	2.919	-0.048	-0.625	0.533
	Livestock ownership	4.506	3.683	0.078	1.223	0.222
Seeking medical attention	Location	1.707	1.564	0.096	1.092	0.276
	Gender	-1.336	2.274	-0.039	-0.588	0.557
	Level of education	**1.644**	**0.626**	**0.162**	**2.624**	**0.009***
	Occupation	-0.544	0.468	-0.079	-1.161	0.247
	Interaction with animals	**-6.737**	**2.173**	**-0.239**	**-3.100**	**0.002***
	Livestock ownership	-0.291	2.742	-0.007	-0.106	0.916

Key: * statistically significant. All variables that are statistically significant are in bold.

Practice in general was predicted by occupation (β = -0.147, t = -2.117, p = 0.035). However, specific practices were influences by specific variables. The use of tick repellent was strongly associated with location (β = -0.288, t = -3.367, p = 0.01) and gender (β = 0.146, t = 2.274, p = 0.024).

The use of protective clothing while in the tick infested habitats had a strong relationship with gender (β = -0.143, t = -2.170, p = 0.031) and occupation (β = -0.201, t = -2.944, p = 0.003). The practice of carrying out tick visual checks either when walking through a tick infested area or after exposure did not have any association with any of the variables. Tick habitat avoidance had a strong association with location (β = -0.197, t = -2.249, p = 0.025). Although Chi-square test of association showed that seeking medical attention had an association with all the variables, linear regression was only able to establish a strong association with the level of education of the respondent (β = 0.162, t = 2.624, p = 0.009) and interaction with animals (β = -0.239, t = -3.100, p = 0.002).

4.8 Proportion and genetic diversity of *Theileria* and *Babesia* infection in humans

A total of 70 human samples were collected from the Kamok dispensary and the Marura dispensaries. Out of the 70 participants, 48 males (68.6%) and 22 females (31.4%). Their ages ranged from 20 to 87 years with a mean of 41.48 years. 44.3% of the patients were mixed farmers (crop farming and livestock husbandry) followed by 27.1% herders and 24.3% game wardens.

From the samples, 11 (15.7%) were positive for piroplasms on PCR using the RLB primer. Marura dispensary had 6 positive samples while OPC had 5 positive samples. The common symptoms reported at the dispensaries by the patients whose blood samples turned out positive on PCR was malaise (82.9%) followed by fever (68.6%) as illustrated in figure 4.4.

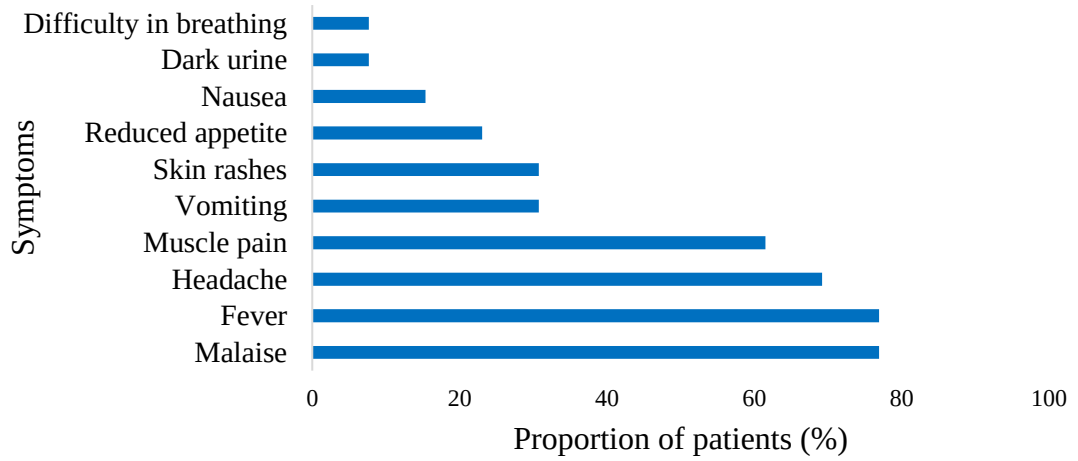
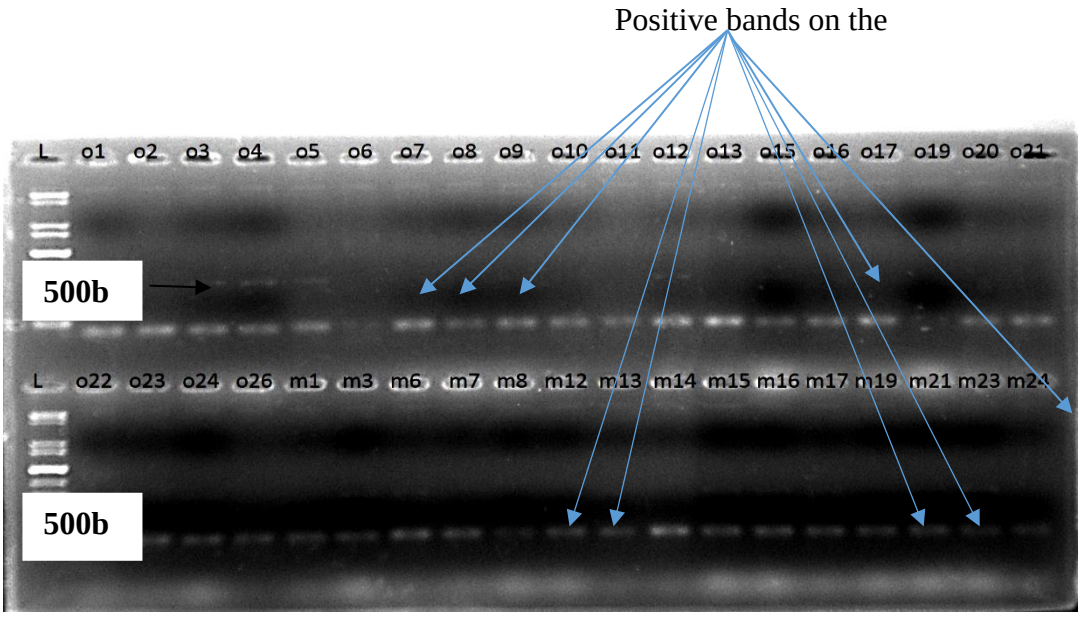


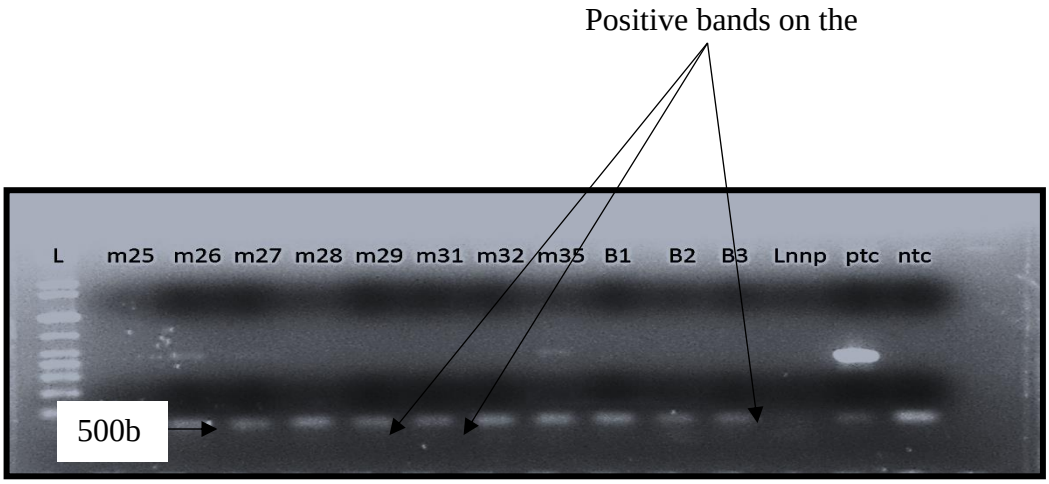
Figure 4.4: Symptoms exhibited by the patients whose samples turned out positive on PCR at Ol Pejeta Conservancy, Kenya.

The bands on the electrophoresis gel obtained from the PCR using RLB primers are shown in Plate 5 and 6.



Key: ptc - positive test control, ntc - negative test control, L – 1 Kb DNA ladder, m – human samples from Marura dispensary, o – human samples from Ol Pejeta Conservancy

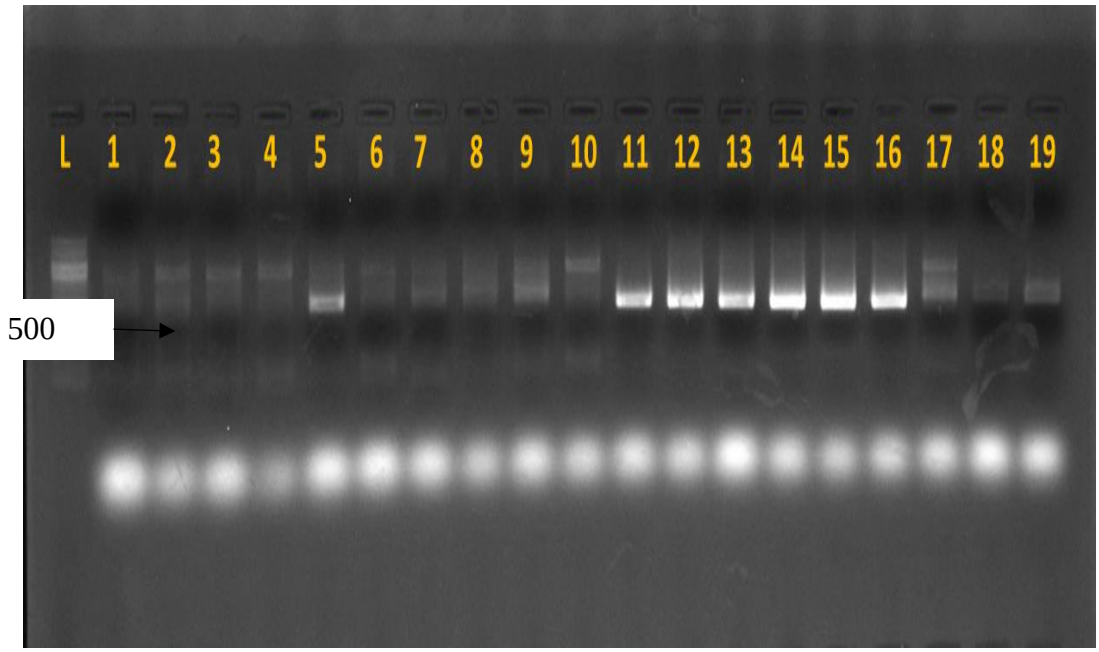
Plate 5. Agarose gel electrophoresis separation of PCR products by PCR detection method for the detection of Theileria and Babesia piroplasms using genomic DNA extracted from human samples and RLB primer. Amplicons of interest are marked with arrows which could be either Theileria or Babesia.



Key: ptc - positive test control, ntc - negative test control, L – 1 Kb DNA ladder, m – human samples from Marura dispensary, B- human samples from Marura dispensary, Lnnp – rhino sample from Lake Nakuru National Park. Amplicons of interest are marked with arrows which could be either *Theileria* or *Babesia*.

Plate 6. Agarose gel electrophoresis separation of PCR products by PCR detection method for the detection of *Theileria* and *Babesia* piroplasms using genomic DNA extracted from human samples and RLB primer.

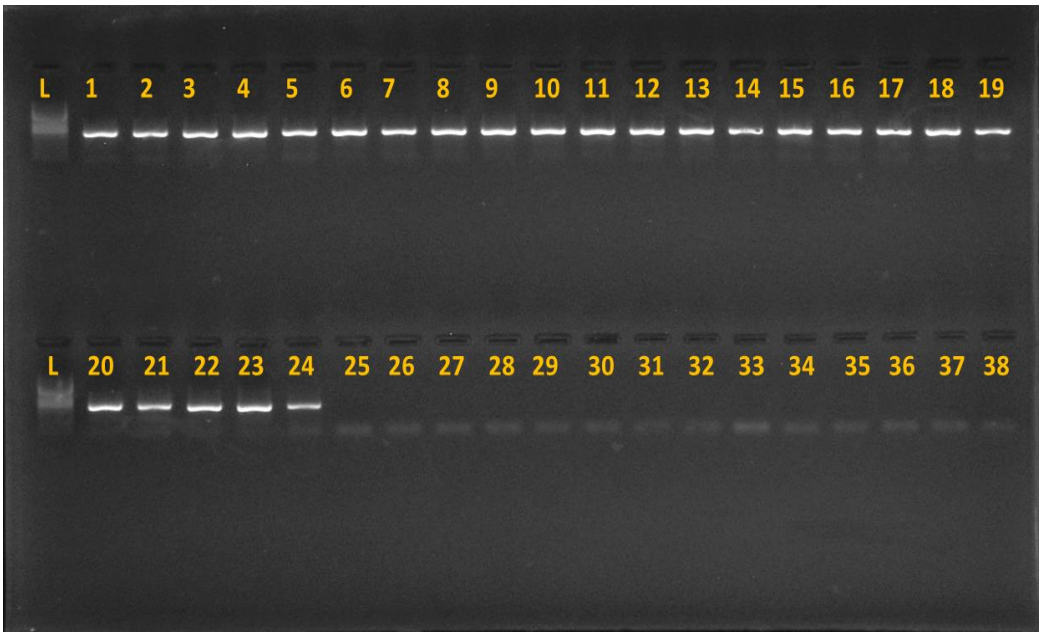
Since the first PCR run (Plate 5 and 6) showed 11 very faint positive amplicons on human samples for either *Theileria* or *Babesia*, a second PCR run was repeated for only those (No 1-10 and 17) samples (Plate 7) together with wildlife samples (No 11 -16) known to be positive for either *Theileria* or *Babesia.* This repeat PCR analysis had 2 human samples with positive amplifications (No 5 and No 17) as shown in plate 7.



Key: L – 1 Kb DNA ladder, N0 1-10 human samples, No 11-16 (samples from wildlife species), No 17 human sample, No 18 and 19 negative controls.

Plate 7. Agarose gel electrophoresis separation of PCR products by PCR detection method for the detection of *Theileria* and *Babesia* piroplasms using genomic DNA extracted from human samples that were positive in the previous PCR test and RLB primer.

The previously positive samples were then subjected to a nested PCR analysis using ILO primers which is a more sensitive two-step method in the amplification of *Theileria* and *Babesia* piroplasms and from the results all the samples that were previously positive turned negative (plate 8). This confirmed that none of the human samples collected during the study were positive for either *Theileria* or *Babesia* piroplams.



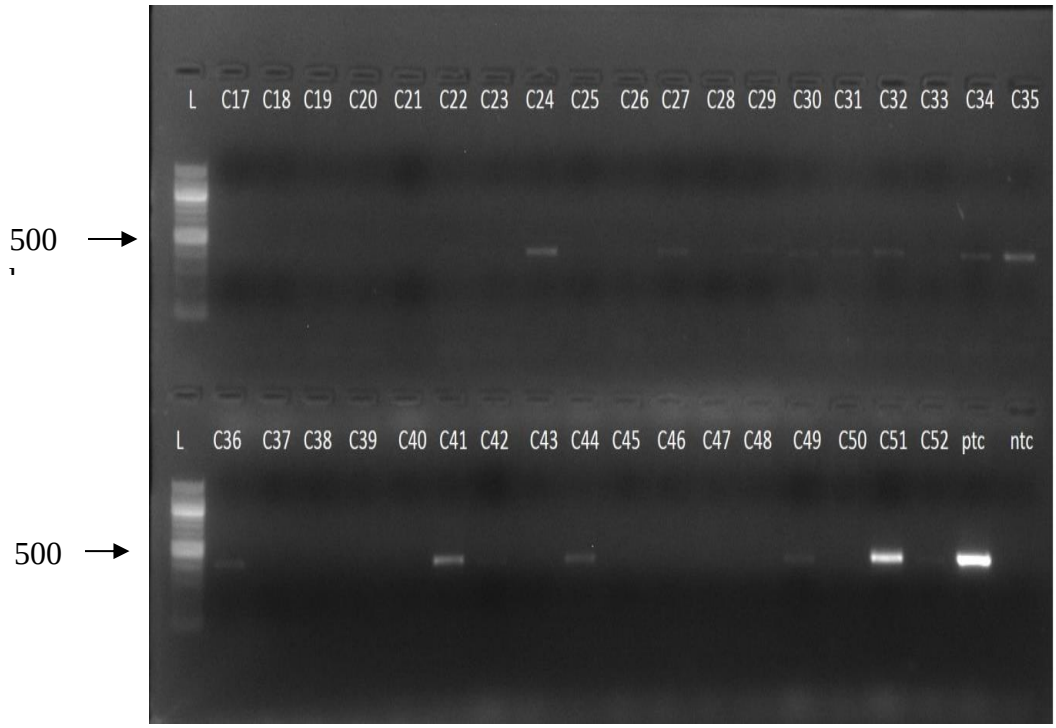
Key: L – 1 Kb DNA ladder, No 1-24 (positive samples from wildlife species), No 25 – 38 human samples

Plate 8. Agarose gel electrophoresis separation of PCR products by PCR detection method for the detection of Theileria and Babesia piroplasms using genomic DNA extracted from human samples and ILO nested primer.

4.9 Proportion and genetic characteristic of *Theileria* and *Babesia* in cattle within OPC and the community adjacent to OPC

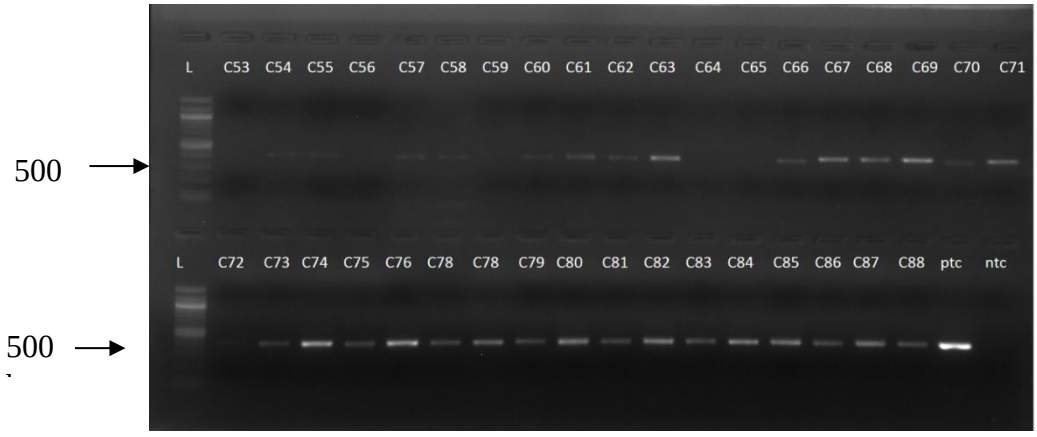
A total of 98 cattle were sampled for blood from OPC and two locations outside OPC: Withare and Tangi nyeusi. The cattle were aged 2 years and above with a mean age of 4 years. In OPC, all the cattle sampled were improved boran breed managed under the mixed grazing system where they co-grazed with the wildlife. Out of the 66 cattle sampled in OPC, 14 (21.2%) turned out positive for piroplasms on PCR.

In Tangi nyeusi, all the 15 cattle sampled were positive for piroplasms on PCR (100%). In Withare, out of the 17 cattle sampled 16 were positive for piroplasms (94.1%). In total, out of the 98 cattle blood samples analysed by PCR, 45 (45.9%) were positive for piroplasms (*Theileria*/*Babesia* species). Some of the positive results are shown by the agarose gel images (Plate 9 and 10).



Key: ptc – positive test control; ntc – negative test control; L- 1 Kb DNA ladder**.**

Plate 9. Agarose gel electrophoresis image showing separation of PCR products of piroplasms by PCR detection method for the detection of *Theileria* and *Babesia* piroplasms using genomic DNA extracted from cattle from OPC (C17-C52).



Key: ptc – positive test control; ntc – negative test control; L- 1 Kb DNA ladder.

Plate 10. Agarose gel electrophoresis image showing separation of PCR products of piroplasms by PCR detection method for the detection of *Theileria* and *Babesia* piroplasms using genomic DNA extracted from cattle from OPC cattle (C53- C66) and Community cattle (C67-88).

On sequencing the 45 positive samples, 22 were high quality sequences while 23 were very low quality and could not be edited, hence discarded. The results show that except for *T. mutans* that occurred in Tangi nyeusi *T. parva, T. taurotragi* and *B. bigemina* was present in all the three locations (within the conservancy and outside at Withare and Tangi nyeusi). *B. bigemina* was more pronounced compared to *Theileria* species. (Table 4.11).

Table 4.11: Piroplasms identified from the positive PCR samples obtained from cattle at Ol Pejeta Conservancy, Kenya.

Location	**Sex**	**n**	**PCR +**	**Species identified**			
				B. bigemina	***T. parva***	***T. taurotragi***	***T. mutans***
OPC	M	31	7	3	1	1	0
	F	35	7	6	1	0	0
Withare	M	2	1	0	0	1	0
	F	15	15	1	1	3	0
Tangi nyeusi	M	0	0	0	0	0	0
	F	15	15	0	1	2	1
Total		**98**	**45**	**10**	**4**	**7**	**1**

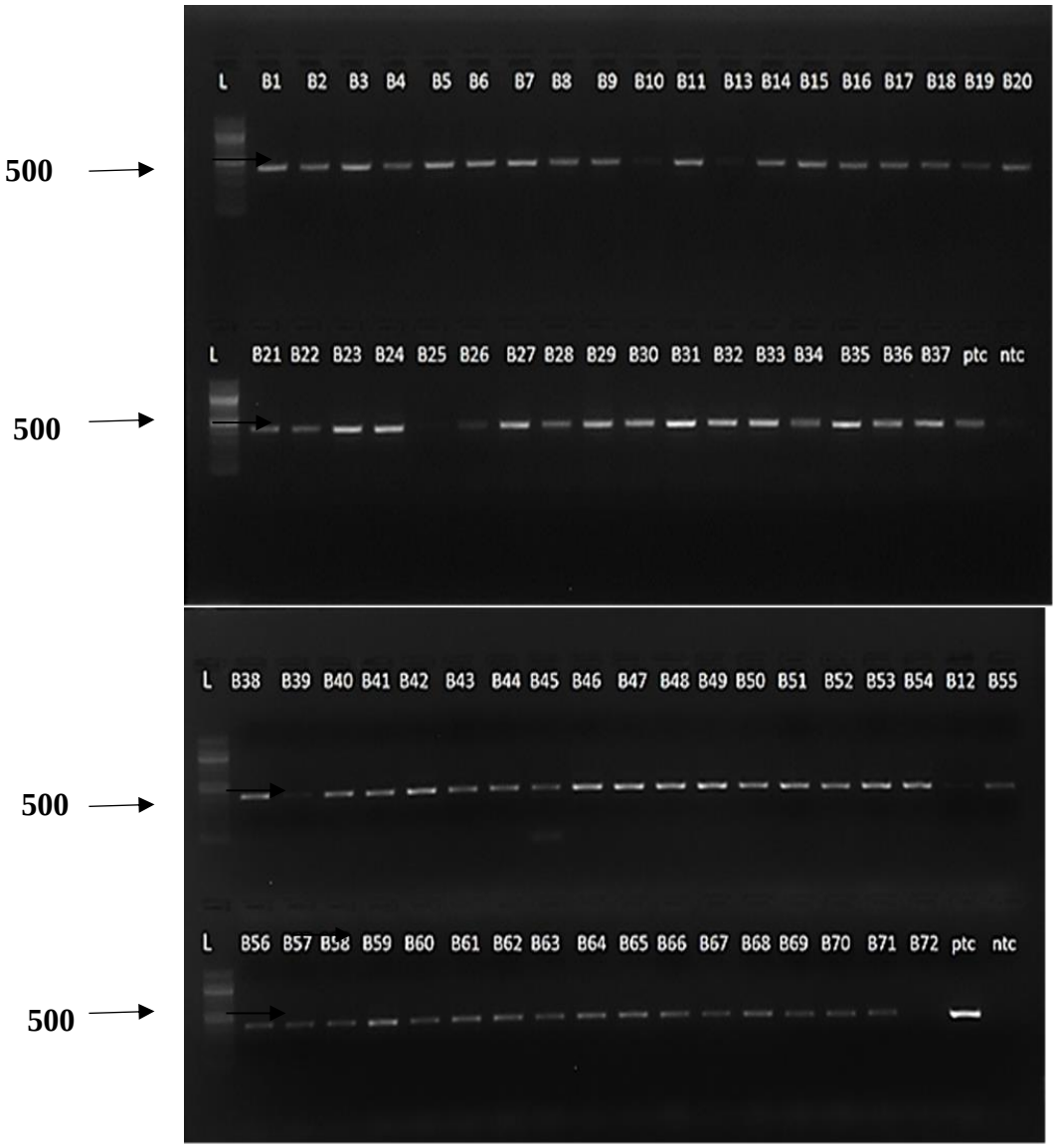
The cleaned sequences were submitted to basic local alignment search tool (BLAST) in Genbank to identify homologous sequences. The search results showed that the sequences closely matched with *Babesia bigemina*, *Theileria taurotragi*, *Theileria parva,* and *Theileria mutans.* The homology parameters associated with the BLAST results are summarized in table 4.12.

Table 4.12 Genbank references for *B. bigemina* identified from samples obtained from cattle at the Ol Pejeta Conservancy, Kenya.

Haplotype number	Organism	Max	Total	Query cover %	Identity %	Accession no	Freq
1	*Babesia bigemina*	715	715	100	100	KY038944.1	1
2	*Babesia bigemina*	721	721	100	99	KY038944.1	4
3	*Babesia bigemina*	710	710	100	99	KF606864.1	1
4	*Babesia bigemina*	721	721	100	99	EF458206.1	2
5	*Babesia bigemina*	712	712	100	99	HQ688686.1	1
6	*Babesia bigemina*	713	713	100	99	KY038944.1	1

4.10 Proportion and genetic characteristic of *Theileria* and *Babesia* infection in African buffalos

A total of 92 African buffalos were sampled for blood from OPC. The buffalos were 6 months old and above with mean age of 3.63 years. Out of the 92 blood samples analysed by PCR, 87 (94.6%) were positive for piroplasms (*Theileria* species) as shown by gel images (Plate 11).



Key: ptc – positive test control; ntc – negative test control; L-1 Kp DNA ladder.

Plate 11: Agarose gel electrophoresis image showing separation of PCR products of piroplasms by PCR detection method for the detection of *Theileria* and *Babesia* piroplasms using genomic DNA extracted from African buffalos (B1 – B72).

On sequencing the 87 positive samples, 80 were high quality sequences while seven were very low quality and could not be edited, hence discarded. The cleaned sequences were submitted to BLAST in Genbank to identify homologous sequences. The search results showed that the sequences closely matched with *Theileria sp. Ex. Syncerus caffer*, *Theileria parva*, and *Theileria cf. sinesis*. The homology parameters

associated with the BLAST results are summarized in Table 4.13. The results showed that the African buffalo were dominated by *Theileria* species especially *Theileria sp. Ex. Syncerus caffer.* No *Babesia* species were recovered from the buffalo samples.

Table 4.13: Piroplasms identified from the positive PCR samples from the African buffalos at the Ol Pejeta Conservancy, Kenya.

Location	**Sex**	**n**	**PCR +**	**Species identified**		
				T. parva	***T. cf sinensis***	***T. sp ex Syncerus caffer***
OPC	M	37	35	7	3	23
	F	55	52	6	0	41
Total		**92**	**87**	**13**	**3**	**64**

The DNA sequence polymorphism software version 5 (DnaSP) was used to investigate sequence divergence and polymorphism between the haplotypes and the GenBank references for both the cattle and African buffalos yielded the results shown in table 4.14.

Table 4.13: Genbank references for *Theileria* species identified from samples obtained from cattle and African buffalos at the Ol Pejeta Conservancy, Kenya.

Haplotype No	Organism	Max	Total	Query cover %	Identity %	Accession no	Freq
1	*T. sp. ex. Syncerus caffer*	730	730	99	100	HQ895982.1	41
2	*T. sp. ex. Syncerus caffer*	725	725	99	99	HQ895982.1	4
3	*T. parva*	719	719	99	99	KM211712.1	2
4	*T. sp. ex. Syncerus caffer*	719	719	99	99	HQ895982.1	2
5	*T. sp. ex. Syncerus caffer*	719	719	99	99	HQ895982.1	1
6	*T. sp. ex. Syncerus caffer*	713	713	99	99	HQ895982.1	1
7	*T. sp. ex. Syncerus caffer*	713	713	99	99	HQ895982.1	2
8	*T. sp. ex. Syncerus caffer*	728	728	99	100	HQ895982.1	1
9	*T. parva*	719	719	99	99	KM211712.1	1
10	*T. parva*	730	730	99	100	KM211712.1	3
11	*T. parva*	708	708	99	99	KM211712.1	1
12	*T. sp. ex. Syncerus caffer*	719	719	99	99	HQ895982.1	1
13	*T. parva*	726	726	99	99	AF013418.1	1
14	*T. parva*	730	730	99	100	HQ684067.1	4
15	*T. cf. sinensis*	719	719	99	99	JQ037790	2
16	*T. parva*	665	665	99	97	KM211712.1	1
17	*T. parva*	697	697	99	98	KM211712.1	1
18	*T. parva*	719	719	99	99	KM211712.1	1
19	*T. parva*	641	641	99	96	KM211712.1	1
20	*T. parva*	708	708	99	99	KM211712.1	1
21	*T. cf. sinensis*	691	691	99	98	JQ037790.1	1
22	*T. taurotragi*	723	723	99	100	L19082.1	7
23	*T. mutans*	723	723	99	100	KU206320.1	1

4.11 Phylogenetic analysis

The evolutionary history of *Babesia* was inferred using the maximum likelihood method based on Kimura-2 Parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3513)). The analysis involved 20 nucleotide sequences. There was a total of 466 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

The phylogenetic analysis result showed that the six haplotypes all had very strong bootstrap support. They have 94% support for clustering within the *B. bigemina* clade (fig. 4.5)

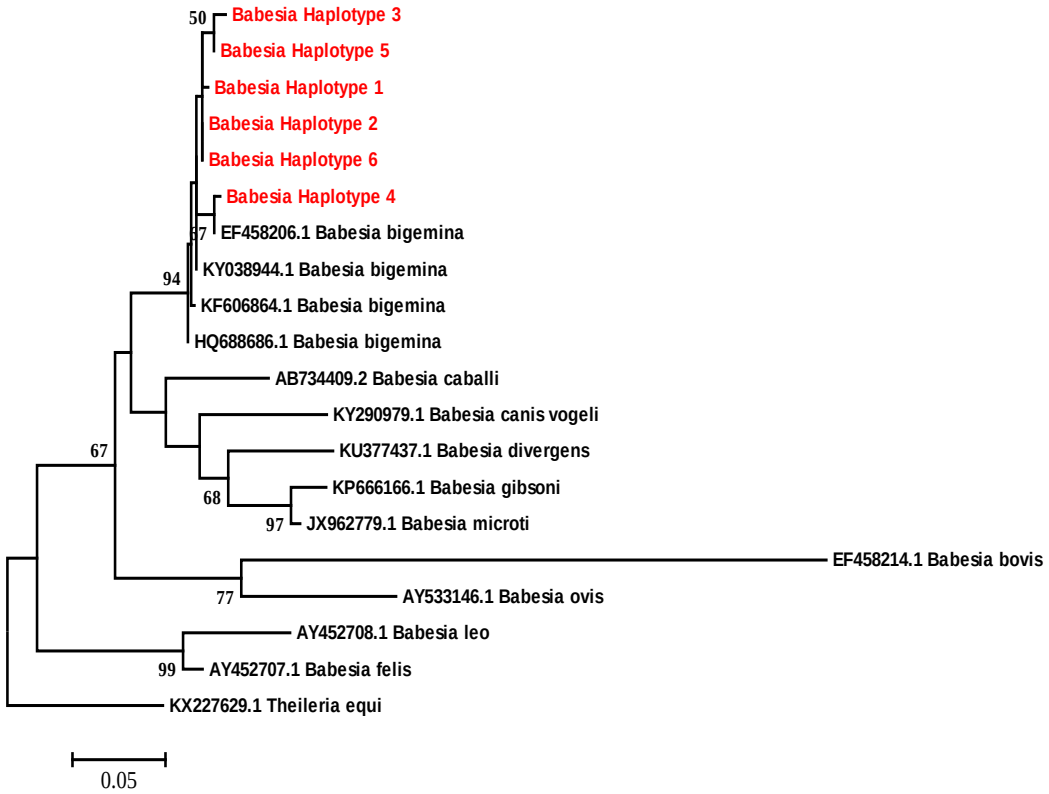


Figure 4.5: Molecular phylogenetic analysis of *Babesia* by Maximum likelihood method.

The evolutionary history of *Theileria* was inferred using the maximum likelihood method based on Kimura-2 Parameter model (Kimura, 1980), with a discrete Gamma distribution indicating rate differences among sites (5 categories (+*G*, parameter = 0.3256). The tree was drawn to scale with branch lengths measured in the number of substitutions per site. The analysis used a total of 417 nucleotide positions from 57 sequences. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.,* 2016). The percentage of trees in which the associated taxa clustered together was shown for each branch where bootstrap support is greater than 50% (figure 4.6). From the analysis, there were 23 haplotypes, of which haplotype 23 clustered with *T. mutans* and had 100% bootstrap support. Haplotype 22 clustered with *T. taurotragi* with 95% bootstrap support. Haplotype 15 and 21 clustered with *T. cf sinensis* with a bootstrap support of above 70%. The rest of the haplotypes had 60% bootstrap support and clustered with *T. parva* and *T. sp. ex. Syncerus caffer* clade. The analysis did not discriminate between *T. parva* and *T. sp. ex. Syncerus caffer* haplotypes.

Figure 4.6: Molecular phylogenetic analysis of *Theileria* by maximum likelihood method.

CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic and economic characteristics as factors associated with awareness and prevention practices on prevention of human tick-borne diseases

Based on the socio-economic and demographic features, the respondents in this study were a rural low income community that was highly dependent on livestock keeping and crop farming. These factors were associated with the risk of exposure to ticks and transmission of tick-borne diseases (Vanderburg *et al.,* 2014). Particularly, close contact with livestock, which are often reservoirs of pathogens and ticks (DePuy *et al.,* 2014), is strongly associated with increased human sero-positivity to tick borne pathogens such as *Coxiella burnetii*, the causal agent for Q – fever (Vanderburg *et al.,* 2014). Architectural design of most of the houses in the study coupled with their proximity to livestock enclosures further enhanced the risk of exposure to ticks on the entire household as was seen in a study in Tanzania where houses made of mud walls and earthen floors were found to be a suitable harbourage for tick infestation (Kisinza *et al.,* 2008).

5.1.2 Awareness on susceptibility to infection by *Theileria, Babesia* and other human tick-borne pathogens

The high proportions of affirmative responses to questions on ticks and transmission factors of tick-borne diseases suggested a high awareness level. Although a large proportion of the respondents had formal schooling, and many could distinguish ticks from other arthropods. A majority of respondents associated ticks with animal diseases and less as vectors of human diseases. This could be attributed to the fact that livestock is the backbone of rural economy in Kenya and that loss of their productivity, morbidity and mortality due to tick-borne diseases have been widely publicized. In the survey, a number of the respondents recognized East Coast Fever as a human tick-

borne disease yet it is a disease affecting cattle. Generally, in Africa, human tick-borne diseases are neglected and least documented. Lyme disease, a tick-borne bacterial disease caused by *Borrelia burgdorferi* is richly described in terms of its public health impact, epidemiology and diagnosis in the United States, Europe and parts of Asia. Conversely, in spite of the multiple species of *Borellia* causing relapsing fever in Africa little is known about them, especially information on epidemiology and public health burden (Fotso Fotso & Drancourt, 2015). According to Trape *et al.,* (2013), about 44 million people living in rural Africa are at risk of tick-borne relapsing fever and the justification for public health awareness campaigns on human tick-borne diseases (72.3%) as shown by this study is warranted.

Tick-borne diseases in humans are often associated with diverse symptoms, though fever is quite common. In this survey, the respondents rated highly skin rash, which is an immediate immune response on the site of a tick bite. Tick-borne symptoms are often masked by other fever-causing illnesses therefore leading to misdiagnosis and wrong medication, especially in malaria endemic regions (Crump *et al.,* 2013).

Eco-climatic factors are associated with tick abundance and distribution (Cumming, 2002). In this study, respondents indicated that intensity of tick bites coincides with dry season. This is consistent, specifically in terms of abundance, to observation from pastoralist communities in Northern Tanzania (Kioko *et al.,* 2015). Increased tick bite intensity is likely to enhance risks of tick-borne infections among pastoralists, given that dry season is associated with sparse pasture and they have to trek further and stay more in tick infested habitats.

5.1.3 Practices on prevention of tick bites and human tick-borne diseases

While majority of the respondents were aware that the area they occupied was infested with ticks and that they were at risk of infection with tick-borne diseases, they were less keen to take preventive measures against tick bites. It has been demonstrated that personal protective behaviours (PPBs) against tick bites such as wearing protective clothing, applying tick repellent on skin and clothing, checking for and removing ticks and avoiding tick habitats as described by Piesman & Eisen (2008) are less used,

even among knowledgeable people or people occupying areas with endemic diseases due to the inconvenience and discomfort especially during summer or in the hot tropics (Bartosik *et al.*, 2008).

According to Schreck *et al.,* (1986), repellents containing DEET applied on the skin and those containing permethrin applied to clothing or tents are effective in preventing tick bites. Although many respondents affirmed that use of repellents is effective in preventing tick bite, this was the least used protection method by the community. This was probably due to cost implications and their limited availability to rural communities in Africa.

The use of tick repellent as the least practised method of tick bite prevention has also been documented in various parts of the world including the United States (Eisen & Stafford, 2020) and Europe (Jepsen *et al.,* 2019) where the responents preferred other methods of tick-bite prevention. An exception was in Poland (Bartosik *et al.,* 2008) where most respondents reported using tick repellents more than the other prevention methods. Generally, there is need to raise awareness on the advantages of using tick repellents as a prevention measure against tick bites.

5.1.4 Association between the socio-demographic and economic characteristics of the respondents and the level of awareness and practice on prevention of human tick-borne diseases

This study determined that awareness on ticks and human tick-borne diseases was strongly associated with gender and level of education. This suggests that awareness was driven by the complex interaction between the various socio-demographic factors within the community. The level of education was also associated with awareness where respondents with higher levels of education had higher scores in awareness. However, gender also had a bearing on the level of education with more males attaining higher levels of education compared to the females. The cultural gender-bias for formal education among pastoralist communities disadvantages the females also on issues of health (Caulfield *et al.,* 2016). Conversely, since livestock herding is dominantly a male responsibility, the occupation similarly disadvantaged this group

by putting them at risk of tick-borne diseases as seen in studies in Tanzania and other parts o Africa where cases of Q-fever have been reported among patients who interacted with animals (Crump *et al.,* 2013; Heinrich *et al.,* 2015; Vanderburg *et al.,* 2014). A public health awareness campaign thus should actively seek to engage women, groups engaged in livestock keeping and occupations that deal with animals.

5.1.5 Association between the socio-demographic and economic characteristics of the respondents and practices on prevention of human tick-borne diseases

Although the study population had some level of awareness on ticks and tick-borne diseases, they were generally indifferent to taking measures that prevent tick bites. Except occupation, there was no statistically significant association between gender, level of education and livestock ownership on practices towards prevention of tick bites and human tick-borne diseases. The analysis demonstrated that people who interacted with animals were more likely to take personal preventive measures against tick bites compared to those who did not. This discrepancy that favoured positive preventive behaviour on individuals who interacted with animals was probably influenced by the type of interaction.

For instance, a herder is likely to have direct contact with cattle through touch and milking thereby exposing himself to tick bites as opposed to individuals who did not interact with any animals.

Occupation also had a statistically significant influence on the use of protective clothing. For instance, wardens, mixed farmers, office workers and herders were more likely than pastoralist and business people to use protective clothing to minimize tick bites and tick-borne diseases. It is likely that inconvenience and discomfort is the main cause for the discrepancy in the use of protective clothing among pastoralists, but for business people this could be as a result of their occupation not having any direct interaction with animals. It is common for employees, such as wardens and herders, to adhere to formal work uniforms that likely come with protective advantages.

Personal Preventive Behaviours (PPBs) were associated with gender and occupation. Females were more likely to use tick repellent and check their bodies for ticks and avoid tick infested areas compared to males. This has also been document in studies from Europe (Jepsen *et al.,* 2019) and in United States (Phillips *et al.,* 2001). Conversely, occupation took precedence over gender with regard to tick habitat avoidance. For instance those who worked with animals (wildlife wardens and herders) are least likely to avoid tick habitats compared to individuals in other occupations. It is probable that wildlife wardens would not care to avoid tick habitats because ticks are likely to be ubiquitous in the entire wildlife habitat. Further, wildlife wardens usually wear uniforms that include long pants that could be tucked in boots, thus protect them against ticks. Generally, males in this study were likely to use protective clothing than females, a practice that has been observed in other communities (Phillips *et al.,* 2001) and suggests that gender influences the choice of personal protective measures.

Further, in terms of occupation, pastoralists are naturally a high tick exposure group and since they are mostly males, they least cared for any preventive practice against ticks compared to other occupations. This could be due to the inconvenience of wearing protective clothing given that herders and pastoralists have to trek long distances with cattle under scorching heat, in rugged terrain and bushy habitats. This suggests that male herders are more at risk to tick-borne pathogens compared to females as have been shown by high sero-positivity to rickettsial exposure in a pastoral Tanzanian community (Heinrich *et al.,* 2015).

The results also showed that occupation was not associated with the practice of seeking medical attention. The highest risk group members of the community, the herders and pastoralists, were least likely to seek medical care if they had fever which could be attributed to tick-borne illnesses. The practice of seeking medical attention was associated with the level of education and interaction with animals.

5.1.7 Proportion of *Theileria* and *Babesia* infections among sympatric humans, cattle and African buffalos at Ol Pejeta Conservancy and the surrounding community

The findings of this study show that sympatric humans did not habour piroplasms, but cattle and African buffalos haboured *Theileria* and *Babesia* piroplasms. The negative results in humans could be attributed to low parasitaemia. Samples from patients with low parasitaemia contain low levels of the pathogen's DNA, this low levels are further degraded or lost during the extraction and purification stages before the PCR is done (Gonçalves-de-Albuquerque *et al.,* 2014). The other reason for the negative results could be the fact that piroplasms are better adapted to cattle and buffaloes than humans as the primary hosts.

A negative PCR does not eliminate the potential of an on-going infection. Studies have shown that microbial DNA is rapidly cleared from the blood in the absence of microbial replication so that the detectable presence of DNA is an indication of an active infection (Krause *et al.,* 1998). Most persons with *Babesia* infections resolve the infections spontaneously without any need for anti-microbial therapy. The use of nested PCR for analysis gave more conclusive results with the human blood samples because it increased the sensitivity and specificity of detecting the target amplicon which may not be detectable after 30 to 40 cycles of regular PCR (Paxson, 2008). The reason for the false positive results with bands that are of the exact size as the desired target gene shown at the beginning of the molecular analysis could have been as a result of cross- contamination or usage of less specific primer pairs (RLB primer).

Laikipia County is seen as a migratory route for many animals both domestic and wild from the northern part of Kenya towards the central part of the country. Ol Pejeta Conservancy lies at the centre of Laikipia County next to a major route (Rumuruti road) that separates the conservancy from the northern community which the pastoralists and wild animals use to access the Aberdare ranges for pasture and water during drought. Studies have also shown that ticks do not move far but depend on the movement of hosts including birds which can fly long distances to move them into new habitats or introduction of animals from other habitats into the new habitats with

ticks infected by a new variety of tick borne pathogens (Hasle, 2013; Mathers *et al.,* 2011). The study showed that *T. cf sinesis* was being reported in Kenya and in the African buffalos for the first time. Previously, this *Theileria* spp has been reported in China (Chen *et al.,* 2014). This suggests the possibility of the humans and animals living in such an ecosystem being exposed to pathogens that are spread by migratory hosts.

When the ratio of infected cattle was compared across the 3 communities it was clear that OPC cattle had a low infection rate despite the cattle sharing pasture with other wild herbivores including the African buffalos. This low prevalence at OPC may be attributed to the tick control measures and the animal husbandry practises at the Conservancy.

5.1.8 Genetic diversity of *Theileria* and *Babesia* species in Ol Pejeta Conservancy and the surrounding community

The results showed that there was no difference between the species of piroplasms found within the Conservancy and the cattle from the community surrounding the Conservancy. However, the presence of more *B. bigemina* infection in cattle at OPC pointed towards inefficient control of the one host tick. This is because this pathogen is spread by a one host tick (*Boophilus decoloratus*) which develops resistance to acaricides faster than other two and three host ticks. As a result, they require higher concentration or more frequent application of acaricide for elimination. However, the few ticks that remain in the herd also confer herd immunity against piroplasm by ensuring that the herd does not become totally naïve to piroplasm infection. This is important in conferring innate immunity to the herd (Ahmed *et al.,* 2008).

This study also demonstrated that in the African buffalo, *Theileria* was the predominant piroplasm. The molecular characterization tool revealed a high level of genetic diversity among the buffalo derived *T. parva* compared to the cattle derived *T. parva*. This is supported by previous studies which have shown that only a limited subset of the total *T. parva* gene pool within the buffalos ends up being established in cattle in the cattle-buffalo interface (Morrison *et al.,* 2020). This could be the reason

why they are suspected to be the source of *T. parva* infection for cattle (Bigalke, 1994). *Theileria parva* shows extensive genotype diversity and undergoes frequent genetic recombination while in the ticks. The *T. parva* maintained in African buffalos is more genotypically diverse than the cattle-maintained population as shown by the *Theileria* phylogenetic tree in this study. However, the *T. parva* transmitted from buffalo to cattle does not differentiate to the tick transmissible stages while in the cattle. As a result, the population of *T. parva* in cattle and buffalos have remained as separate populations (Morrison *et al.,* 2020) .

The phylogenetic tree also shows that there are is very little distinction between *T. parva* and *T sp Syncerus caffer*. This is shown by the low bootstrap support of less than 50% which indicates that there is limited genetic exchange through occasional sexual recombination between the two populations.

Suprisingly, when we look at the results in cattle, they had a wider genetic variety of piroplasms by having both *Theileria* and *Babesia* infections within the herds compared to buffalos that only had *Theileria* infection. This is supported by other studies done in East Africa that did not find any *Babesia* infection in the African buffaloes (Oura *et al.,* 2011) even though *Babesia* infections have been reported in African buffaloes in South Africa (Eygelaar *et al.,* 2015) This could mean that cattle are more susceptible to *Babesia* infections compared to the African buffaloes.

5.2. Conclusion

In reference to the objective of this study which was to determine the level of awareness and practices on prevention of human tick-borne diseases and genetic diversity of *Theileria* and *Babesia* infections in sympatric human, cattle and African buffalo in the fenced Ol Pejeta Conservancy (OPC) and the community adjacent to OPC in Laikipia County. The findings conclude that

The socio-economic and demographic factors of this community were associated with the level of awareness on ticks and human tick-borne diseases and the practices towards prevention of tick bites and human tick-borne diseases.

There was a complex association between socio-demographic and economic factors, awareness and practice. Gender, formal education and occupation were the main social factors that were associated with awareness and practices on prevention of tick bites and tick-borne diseases.
In addition, individuals who had higher education levels and also interacted with animals were more likely to apply measures that reduced exposure to tick bites and tick-borne diseases.

Although the respondents were aware that ticks are vectors of diseases, majority of them did not take any personal preventive measures against tick-bites thereby putting themselves at risk of getting tick-borne diseases.

The study also illustrated that there were piroplasm infections among the sympatric African buffalos and cattle but not in humans. The molecular analysis showed that 94.6% of the African buffalos were infected. In OPC, the proportion of cattle infected was 21.2%. In the Northern community 100% of the cattle were infected while in the south 94.1% were infected.

Cattle had both *Theileria* and *Babesia* infections, the predominant piroplasm in cattle was *Babesia bigemina* especially at OPC. There was a high degree of genetic diversity within the *Theileria* species with the predominant species being *T. sp. ex. Syncerus caffer* in the buffalos followed by *T. parva.* When the rate of infection in cattle was compared across the three communities, the results showed that the cattle that co-grazed with wildlife had a lower infection rate compared to the other sites that had almost 100% infection rates. This could be attributed to tick vector control practices and the animal husbandry practices at the Conservancy.

In summary, the study has shown a strong support for the health behavior model by illustrating that factors such as higher levels of education, gender, location and occupations shaped the communities awareness on human tick borne diseases and drove specific practices towards prevention of tick bites and tick borne diseases which

would include *Theileria* and *Babesia* infections. The respondents strongly advocated for public health awareness campaign on human tick-borne diseases.

5.3 Recommendations

This study recommends the following:

Although the study did not establish the presence of *Theileria* or *Bebesia* infection in humans, there is need to create public health awareness on human tick-borne diseases such as the tick borne relapsing fever and Q fever which have been reported in Laikipia County.

The public health campaign should be designed to enhance the perception of the risk of tick bites and to also improve the uptake of prevention practices aimed towards prevention of tick bites and tick-borne diseases.

Tick repellents was the least used method of tick bite prevention yet it has been documented as the most effective. Arising from this, there is need to create awareness on the importance of its use in tick endemic areas. Tick repellents also need to be made available at affordable costs.

There is need to sensitize personnel whose occupation involved handling animals or continuous interaction with animals to always seek medical attention to that human tick-borne diseases can be identified and be treated appropriately since this group was the least likely to seek medical attention when they developed fever.

Further research is required to identify and establish the burden of human tick-borne diseases in this community based on the symptoms that the respondents listed as being associated with tick bites in this study.

From the finding of high prevalence of piroplasm especially in the community owned cattle, there is need for adoption of evidence based acaricide tick control for livestock especially for the community cattle in order to reduce the high infection rate of the animals with piroplasms. The practices within OPC can also be applied in the community around the Conservancy to reduce the infection rate in cattle.

The results of this study on the piroplams circulating in the sympatric cattle and African buffalos and the molecular sequences generated from this study could be used to re-analyse and improve the vaccines produced for use in areas where cattle interact with African buffalos.

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APPENDICES

Appendix I: Questionnaire on the Awareness and Practices on Prevention of Human Tick-Borne Diseases.

Study title: Awareness and pratices on prevention of human tick-borne diseases and genetic diversity of *Theileria* and *Babesia* at the human, cattle and African buffalo *(Syncerus caffer*) interface in the Ol-Pejeta Conservancy, Laikipia County, Kenya.

Date: _____

Enumerator: _____ Number: _____

SECTION A: SOCIO-DEMOGRAPHIC AND ECONOMIC CHARACTERISTICS

1. Name of village: _____

2. Gender of interviewee: Female: ☐
   Male: ☐

3. How old are you? _____

4. What is the highest academic qualification? Tick only one box.
   - No formal schooling ☐
   - Incomplete primary school ☐
   - Complete primary school ☐
   - Incomplete secondary school ☐
   - Complete secondary school ☐
   - Post-secondary e.g. certificate, diploma, degree ☐
   - Degree and above ☐

5. What is your occupation? _____

6. Do you keep livestock? Yes ☐ No ☐

7. Where are your livestock housed?
   - In a livestock enclosure ☐
   - Inside the house ☐

8. What is the household size? _____

9. Type of house?

a) Permanent b) Semi-permanent c) Temporary

10. What material is your house wall made of?

(a) Stone (b) brick/block (c) mud (d) wood (e) Iron sheets

(f) Other (specify) _____

11. What material is the floor of your house made of?

(a) Cement (b) wood (c) earth (d) tiles

(e) Other (specify) _____

12. What material is the roof of your house made of?

(a) Grass (b) iron sheets (c) tiles (d) Other (specify) _____

13. Where does your household obtain water for domestic use?

(a) In house tap (b) piped to the compound (c) pipe in the local area (d) bore-hole

(e) River/ stream (f) vendors (g) other (specify) _____

14. What type of toilet facility is available for your household?

(a) Own flush toilet (b) shared flush toilet (c) Own pit latrine

(d) Shared pit latrine (e) No toilet (f) other (specify) _____

15. What is your source of energy for cooking?

(a) Electricity (b) kerosene (c) firewood

(d) Others (specify) _____

16. What is your source of lighting?

(a) Electricity (b) Kerosene lamp (c) Wax candle

(d) Other (specify) _____

SECTION B: AWARENESS

17. Which of the following insect is a tick? (Tick only one box)

1. ☐ 2. ☐ 3. ☐

18. Do ticks transmit diseases to livestock?

Yes ☐ No ☐ I do not know ☐

19. Do ticks transmit diseases to wild animals?

Yes ☐ No ☐ I do not know ☐

20. Do ticks transmit diseases to human beings?

Yes ☐ No ☐ I do not know ☐

21. Are there diseases that are shared between livestock and wild animals?

Yes ☐ No ☐ I do not know ☐

22. Which of the following diseases is spread by ticks to humans? Choose all the correct answers.

Malaria ☐
Typhoid ☐
Tick fever ☐
Babesiosis ☐
Anaplasmosis ☐
East coast fever ☐
Cholera ☐
None ☐

23. Which of the following signs or symptoms have you observed on yourself after a tick bite? Tick all boxes that apply)

Generalized weakness	☐
Reduced appetite	☐
Fever	☐
Headache	☐
Dark urine	☐
Nausea	☐
Vomiting	☐
Muscle pain	☐
Sensitivity to light	☐
Difficulty in breathing	☐
Skin rash	☐
Yellowing of the skin	☐
No symptoms after tick bite	☐

24. Which of the following animals do you stay with or interact with daily? (Choose only one answer).

Cattle ☐ Cattle and wildlife ☐ Wildlife ☐ None ☐

25. Which season do you experience a lot of tick bites? (Tick the most appropriate)

Wet season ☐ Dry season ☐ All year round ☐ I don't know ☐

Please indicate your agreement with the following statements by ticking the answer. Please select only one answer for the following questions.

Key: Strongly disagree (1), Disagree (2) Neither agree nor disagree (3), Agree (4), strongly agree (5)

		I strongly disagree	**I disagree**	**I neither agree nor disagree**	**I agree**	**I strongly agree**
26	I believe tick repellents are effective	☐	☐	☐	☐	☐
27	I am at risk of catching a disease spread by ticks	☐	☐	☐	☐	☐
28	By use of proper prevention strategies, I can prevent diseases spread by ticks	☐	☐	☐	☐	☐
29	Diseases spread by ticks occur in my area	☐	☐	☐	☐	☐
30	A tick can spread more than one disease	☐	☐	☐	☐	☐
31	There should be more education about diseases spread by ticks	☐	☐	☐	☐	☐

SECTION C: PRACTICES

Please indicate your agreement with the following statements using the following scale. Select only one answer for the following questions.

Key: Never (1), Sometimes (2), always (3)

		Never	Sometimes	Always
32	When in areas that may increase my chances of exposure to ticks, I use tick repellents	☐	☐	☐
33	I wear long sleeved shirts and trousers whenever I go to areas where ticks occur	☐	☐	☐
34	After exposure to tick habitats I thoroughly check my body for ticks	☐	☐	☐
35	I avoid areas where ticks occur whenever possible	☐	☐	☐
36	When in a tick infested area I tuck my trouser into socks or boots	☐	☐	☐
37	After possible tick exposure I remove my clothes and wash them	☐	☐	☐
38	When bitten by a tick I apply a disinfectant to the skin where the bite occurred after removal of the tick.	☐	☐	☐
39	When walking in a tick infested area I check my body for ticks	☐	☐	☐
40	I stick to clear pathways when walking in a tick infested area.	☐	☐	☐
41	If I develop a fever, I seek medical care from medical centres	☐	☐	☐

Appendix II: Questionnaire on the Awareness and Practices on Prevention of Human Tick-Borne Diseases (Swahili Version).

Kichwa cha utafiti: Mambo yanayohusiana na maambukizi ya binadamu na maumbile tofauti ya *Theileria* na *Babesia* katika eneo lenye binadamu, ng'ombe na nyati katika Ol Pejeta Conservancy kaunti ya Laikipia, Kenya.

Tarehe:_____

Muorodheshaji: _____ Nambari: _____

SEHEMU A: HULKA ZA KIJAMII NA UCHUMI

8. Jina la kijiji: _____
9. Jinsia la mhojiwa: kike: ☐
   kiume: ☐
10. Una umri gani? _____

11. Umehitimu kiwango kipi cha juu zaidi? (chagua jibu moja).
    sina elimu rasmi ☐
    sikukamilisha shule ya msingi ☐
    nilikamilisha shule ya msingi ☐
    sikukamilisha shule ya upili ☐
    nilikamilisha shule ya upili ☐
    Baada ya shule ya upili kama vile cheti, stashahada, ☐
    Shahada ya kwanza na zaidi ☐

12. Kazi yako ni ipi? _____

13. Je una mifugo? Ndio ☐ La ☐

14. Je, mifugo wako wanalala wapi?
    kwenye zizi ☐
    Ndani ya nyumba ☐

8. Jamii yako ina watu wangapi? _____

9. Nyumba yako ni ya aina gani?

a) Ya kudumu b) Angalau ya kudumu c) Nyumba ya muda

10. Ukuta wa nyumba yako umetengenezwa kwa kifaa kipi?

(a) Jiwe (b) Tofari la udongo/tofali la saruji (c) Tope (d) Mbao (e) Mabati (f) Vifaa vinginevyo (elezea) _____

11. Sakafu ya nyumba yako imetengenezwa kwa kifaa kipi?

(a) Saruji (b) Mbao (c) Udongo (d) Vigae

(e) Vifaa vinginevyo (elezea) _____

12. Paa la nyumba yako limetengenezwa kwa kifaa kipi?

(a) Nyasi (b) Mabati (c) Vigae (d) Vifaa vinginevyo (eleza) _____

13. Jamii yako hutoa wapi maji ya matumizi nyumbani?

(a) Mfereji ulio kwenye nyumba (b) Mfereji ulio nje ya nyumba ndani ya ua

(c) Mfereji kwenye eneo la mtaa (d) kisima (e) mto (f) wachuuzi

(g) sehemu nyinginezo (elezea) _____

14. Jamii yako inatumia choo cha aina gani?

(a) Choo chenye maji cha ubinafsi (b) Choo chenye maji cha ujumla

(c) Choo cha shimo cha ubinafsi (d) Choo cha shimo cha ujumla (e) Hatuna choo

(f) Zingine (eleza) _____

15. Unatumia nini kupika?

(a) Umeme (b) Mafuta taa (c) Kuni

(d) Zingine (eleza) _____

16. Unatumia nini kupata mwangaza?

(a) Umeme (b) Mafuta taa (c) nta wa mshumaa

(d) Zingine (eleza) _____

SECTION B: UFAHAMU

17. Ipi kati ya wadudu wafuatao ni kupe? Chagua jibu moja tu.

1☐ 2.☐ 3. ☐

18. Je kupe husambaza ugonjwa kwa mifugo?

Ndio ☐ La ☐ Sijui ☐

19. Je, Kupe husambaza ugonjwa kwa wanyama pori?

Ndio ☐ La ☐ Sijui ☐

20. Je, Kupe husambaza ugonjwa kwa binadamu?

Ndio ☐ La ☐ Sijui ☐

21. Je, kuna magonjwa ambayo husambazwa kati ya mifugo na wanyama pori?

Ndio ☐ La ☐ Sijui ☐

22. Kati ya magonjwa yafuatayo ni yapi yanayoenezwa na kupe kwa binadamu?

Chagua jibu zote zilizo sawa.

Malaria ☐
Homa ya matumbo ☐
Homa ya kupe ☐
Ugonjwa wa kukojoa damu ☐
Ndigana baridi ☐
Ndigana ☐
Kipindupindu ☐
Hakuna ☐

23. Ni ipi kati ya dalili zifuatazo umewahi kuwa nayo baada ya kuumwa na kupe? Chagua jibu zote zinazofaa.

Udhaifu wa jumla	☐
Kupunguka kwa hamu ya chakula	☐
Homa	☐
Kuumwa kwa kichwa	☐
Mkojo mwekundu	☐
Kichefuchefu	☐
Kutapika	☐
Uchungu wa misuli	☐
Usikivu wa mwanga	☐
Shida ya kupumua	☐
Upele kwenye ngozi	☐
Ngozi kubadili rangi kuwa manjano	☐
Hakuna dalili zozote baada ya kuumwa	☐

24. Ni wanyama wapi kati ya wanyama wafuatao unaokaa nao ama kuingiliana nao kila siku?

(Chagua jibu moja tu)

Ng'ombe ☐ Ng'ombe na wanyama pori ☐ wanyama pori ☐
Hakuna ☐

25. Ni msimu upi ambao wewe huumwa sana na kupe? (Chagua jibu moja)

Msimu wa mvua ☐ Msimu wa kiangazi ☐ Mwaka mzima ☐

Sijui ☐

Tafadhali onyesha kuridhishwa kwako na kauli zifuatazo kwa kuweka sahihi kwa jibu mwafaka. Chagua jibu moja.

Ufunguo: Sikubaliani kabisa (1), sikubaliani (2), sikubali wala kukataa (3), ninakubali (4), ninakubali kabisa (5)

		Sikubali kabisa	Sikubali	Sikubali wala kukataa	Ninakubali	Ninakubali kabisa
26	Ninaamini madawa yanayopakwa kwenye ngozi kuzuia kupe zina ufanisi	☐	☐	☐	☐	☐
27	Niko katika hatari ya kuambukizwa ugonjwa unaoenezwa na kupe	☐	☐	☐	☐	☐
28	kwa matumizi ya mikakati sahihi nitazuia magonjwa yanayoenezwa na kupe	☐	☐	☐	☐	☐
29	Magonjwa yanayoenezwa na kupe hutokea katika eneo langu	☐	☐	☐	☐	☐
30	kupe anaweza kueneza ugonjwa zaidi ya moja	☐	☐	☐	☐	☐
31	Tunahitaji elimu zaidi juu ya magonjwa yanayoenezwa na kupe	☐	☐	☐	☐	☐

SEHEMU C: DESTURI

Tafadhali onyesha makubaliano yako na kauli zifuatazo kwa kuchagua jibu moja tu kwa kila suali lifuatalo

Funguo: kamwe (1), wakati mwingine (2), kila mara (3)

		kamwe	**wakati mwingine**	**Kila mara**
32	Nikiwa kwenye maeneo yenye kupe mimi hutumia madawa ya kuzuia kupe kwenye ngozi au mavazi	☐	☐	☐
33	Mimi huvaa shati ya mikono mirefu na suruali wakati ninapoenda kwenye eneo lenye kupe	☐	☐	☐
34	Nikitoka kwenye eneo lenye kupe mimi huangalia mwili wangu kwa makini ikiwa nina kupe mwilini	☐	☐	☐
35	Mimi huepuka maeneo yenye kupe wakati wowote iwezekanavyo.	☐	☐	☐
36	Ninapokuwa kwenye maeneo yenye kupe mimi huingiza suruali yangu katika soksi au buti.	☐	☐	☐
37	Ninapovamiwa na kupe mimi hutoka nguo zangu na kuziosha	☐	☐	☐
38	Ninapoumwa na kupe mimi husafisha ngozi na dawa ya kuua viini vya maradhi baada ya kumuondoa kupe yule.	☐	☐	☐
39	Ninapotembea katika eneo lenye kupe mimi hujiangalia ikiwa mwili wangu una kupe	☐	☐	☐
40	Mimi hutembea kwenye barabara zisizo na nyasi wakati ninapopita kwenye maeneo yenye kupe	☐	☐	☐
41	Ninapojipata na homa mimi hutafuta huduma za afya	☐	☐	☐

Appendix III: Questionnaire for Patients

Study title: Awareness and pratices on prevention of human tick-borne diseases and genetic diversity of *Theileria* and *Babesia* at the human, cattle and African buffalo *(Syncerus caffer*) interface in the Ol-Pejeta Conservancy, Laikipia County, Kenya.

Date: _____ P/No: _____

Sample No: _____

1. Age _____
2. Sex Male ☐ Female ☐
3. Residence _____
4. Occupation _____

Clinical Presentation

5. Duration of symptoms: _____
6. Symptoms

Generalized weakness	☐
Reduced appetite	☐
Fever	☐
Headache	☐
Dark urine	☐
Nausea	☐
Vomiting	☐
Muscle pain	☐
Sensitivity to light	☐
Difficulty in breathing	☐
Skin rash	☐
Yellowing of the skin	☐

7. Have you experienced these symptoms before?

   Yes ☐ No ☐ Not sure ☐

8. Temperature: _____ ° C

Appendix IV: Questionnaire for Patients (Swahili Version)

Kichwa cha utafiti: Mambo yanayohusiana na maambukizi ya binadamu na maumbile tofauti ya *Theileria* na *Babesia* katika eneo lenye binadamu, ng'ombe na nyati katika Ol Pejeta Conservancy kaunti ya Laikipia.

Tarehe: _____ P/No: _____

Namba ya sampuli: _____

1. Umri _____
2. Jinsia Kiume ☐ Kike ☐
3. Makao _____
4. Kazi _____

Dalili zinazoonekana kwa mgonjwa

5. Muda wa dalili: _____
6. Dalili

Udhaifu wa jumla	☐
Kupunguka kwa hamu ya chakula	☐
Homa	☐
Kuumwa kwa kichwa	☐
Mkojo mwekundu	☐
Kichefuchefu	☐
Kutapika	☐
Uchungu wa misuli	☐
Usikivu wa mwanga	☐
Shida ya kupumua	☐
Upele kwenye ngozi	☐
Ngozi kubadili rangi kuwa manjano	☐

7. Je, umekuwa na dalili hizi wakati mwingine?

   Ndio ☐ La ☐ Sina uhakika ☐

8. Joto ya mwili: _____ º C

Appendix V: Informed Consent for the Awareness and Practice Study on Prevention of Human Tick-Borne Diseases

INFORMED CONSENT FORM

Title of the study

Awareness and Practices on prevention of human tick-borne diseases and the genetic diversity of *Theileria* and *Babesia* at the human, cattle and African buffalo interface in the Ol Pejeta Conservancy, Laikipia County, Kenya.

PART A

Introduction

Ticks are known to bite livestock and wildlife as well as people. Tick bites transmit parasites which cause diseases such as East Coast fever (Theileriosis) and Babesiosis in cattle and wildlife. Tick bites can also transmit these parasites to people and cause disease. In Laikipia County and many other parts of Kenya the burden of tick transmitted parasites to people is not known. Information on tick transmitted diseases will help to improve community health and prevention of tick bites.

Purpose of the study

This study aims to determine the knowledge and practice of the people living within and around Ol Pejeta conservancy regarding diseases that are spread by ticks to human beings.

Who is carrying out this study?

This study is being carried out by Marion Amulyoto, a student at the Institute of Tropical Medicine and infectious diseases (ITROMID) who is the principal investigator. It is not a funded project.

What will it involve for me if I agree?

If you agree to take part in the study I will ask you simple questions on your knowledge on tick borne diseases and what you do routinely to prevent them. There is no cost to you if you participate in this study.

Are there any risks or disadvantages to me taking part?

There are no risks in this study. All the mentioned procedure will be explained to you.

Are there any benefits to me taking part in the study?

There are no direct benefits for you. However, the results of the study will be used to improve the understanding of diseases that are spread by ticks from animals to humans.

This will in turn inform policy in order to improve human health through increased public health education on prevention of tick bites to prevent such diseases.

What happens if I refuse to participate?

Your participation in this research is voluntary. It is your decision to participate or not. If at any time you wish to withdraw from participating in this study, you can do so freely without any consequences against you.

Who will have access to information about me in this research?

All the research records will be stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to access this information.

Who has allowed this research to take place?

The Kenyatta University ethics review committee has looked carefully at this work and agreed that the research is important, relevant to Kenya and follows nationally and internationally agreed research guidelines. This includes ensuring that all participants' safety and rights are respected.

Contacts and questions

The Principal investigator in this research is **Marion Amulyoto.** You may ask any questions you have now, or if you have any questions later, you are encouraged to contact her through the mobile telephone number: **0722 826645** or email **marionamulyoto@gmail.com**

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the following:

The Director, Institute of Tropical Medicine and Infectious Diseases (ITROMID), Jomo Kenyatta university of Agriculture and technology (JKUAT) P.O. Box 62000 – 00200 Nairobi. Telephone: 020 2722541, Email: director@itromid.jkuat.ac.ke.

Or

The secretary, Kenyatta University Ethics Review Committee. P.O. Box 43884-00100 Nairobi Kenya.

PART B

CONSENT FORM

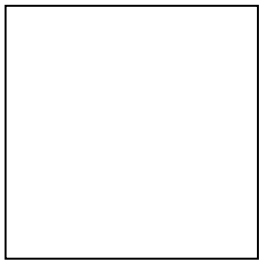
I, Mr/Miss/Mrs_____ have had the research explained to me. I have understood all that has been read/ explained and had my questions answered satisfactorily.

☐ I agree to take part in this research

I understand that I can change my mind at any stage and it will not affect me in any way

Respondent's name: _____

Signature or left thumb print



Signature: _____

Date: _____

Where subject cannot read, ensure a witness observes consent process and signs below. I attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the subject

Witness' signature: _____

Date**:** _____

Witness Name**:** _____

Name of the person taking consent: _____

Signature: _____ Date: _____

Name of the investigator: _____

Signature: _____ Date: _____

Appendix VI: Informed Consent for the Awareness and Practice Study on Prevention of Human Tick-Borne Diseases (Swahili Version).

Anwani ya utafiti

Mambo yanayohusiana na maambukizi ya magonjwa yanayoletwa na kupe kwa binadamu na maumbile tofauti ya *Theileria* na *Babesia* kwa binadamu, ng'ombe na nyati kwenye Ol Pejeta Conservancy kaunti ya Laikipia.

SEHEMU A

Utangulizi

Kupe anajulikana kwa kuuma mifugo na wanyama pori. Kupe husambaza vimelea wanaosababisha magonjwa kama vile ndigana na ugonjwa wa kukojoa damu kwa ng'ombe na wanyama pori. Kupe anaweza pia kusambaza vimelea hivi kwa binadamu na kusababisha magonjwa. Katika kaunti ya Laikipia na sehemu nyingine nyingi za Kenya, mzigo wa vimelea vinavyosambazwa na kupe kwa binadamu haujabainika. Taarifa juu ya magonjwa yanayosambazwa na kupe itasaidia kuboresha afya ya jamii na kuzuia kuumwa na kupe.

Lengo la utafiti

Utafiti huu unalenga kubaini ufahamu na desturi ya watu wanaoishi ndani ya Ol Pejeta Conservancy na vijiji vilivyo pembezoni kuhusu magonjwa yanayo yanayosambazwa na kupe kwa binadamu.

Ni nani anayetekeleza utafiti huu?

Utafiti huu unatekelezwa na Marion Amulyoto, mwanafunzi kwenye taasisi ya utafiti wa madawa na magonjwa ya kuambukiza (ITROMID) ambaye ni mtafiti mkuu. Utafiti huu hauja gharamiwa na taasisi yoyote.

Nitahusishwa vipi katika utafiti huu ikiwa nitakubali?

Ikiwa utakubali kushiriki kwenye utafiti huu, nitakuuliza maswali rahisi juu ya elimu yako ya magonjwa yanayosambazwa na kupe na matendo unayofanya mara kwa mara ili kuzuia magonjwa hayo. Haitakugharimu chochote kushiriki kwenye utafiti huu.

Je, kuna hatari yoyote au hasara kwangu ikiwa nitashiriki?

Hakuna madhara yoyote inayotarajiwa kwa mshiriki wa utafiti huu. Utaelezwa utaratibu utakaofuatwa.

Je, kuna faida yoyote kwangu ikiwa nitashiriki?

Hakuna faida ya moja kwa moja kwako. Hata hivyo matokeo ya utafiti huu itaboresha kuelewa kwetu kwa magonjwa yanayosambazwa na kupe kutoka kwa wanyama hadi kwa binadamu. Itachangia pia kuboresha afya ya binadamu kwa kuelimisha umma juu ya umuhimu wa binadamu kujikinga na kupe ili kuzuia magonjwa kama haya.

Je, ni nini kitatokea kama nitakataa kushiriki?

Kushiriki katika utafiti huu ni kwa hiari. Ni uchaguzi wako kushiriki au la. Unaweza kuacha kushiriki wakati wowote bila madhara yoyote kwako.

Je, ni nani atakayeweza kusoma habari kunihusu katika utafiti huu?

Rekodi zote za utafiti huu zitahifadhiwa kwa usalama katika makabati zinazofungika na kwenye tarakilishi zilizo na nywila. Watu wachache walio kwenye utafiti huu ndio watakaoweza kusoma habari hii.

Ni nani ameruhusu utafiti huu kutendeka?

Kamati ya maadili ya utafiti ya chuo kikuu cha Kenyatta imechunguza kazi hii kwa makini na walikubaliana kwamba utafiti huuni muhimu kwa Kenya na kwamba imefuata miongozo ya kitaifa na Kimataifa inayohusiana na utafiti. Hii pamoja na kuhakikisha kuwa usalama na haki zote za washiriki zinaheshimiwa.

Mawasiliano na maswali

Mtafiti anayetekeleza utafiti huu ni **Marion Amulyoto**. Unaweza kuuliza maswali yoyote uliyonayo sasa ama ikiwa utakuwa nayo baadaye, unahimizwa kuwasiliana naye kupitia nambari ya simu ya mkono: **0722 826645** au barua pepe **marionamulyoto@gmail.com**.

Ikiwa una maswali yoyote kuhusu utafiti huu na ungependa kuongea na mwengine asipokuwa mtafiti, unahimizwa uwasiliane na wafuatao:

Mkurugenzi, Taasisi ya utafiti ya madawa na magonjwa ya kuambukiza, Chuo kikuu cha kilimo na Teknologia cha Jomo Kenyatta, S.L.P. 62000- 00200 Nairobi. Nambari ya simu: (020) 2722541 Barua pepe: director@itromid.jkuat.ac.ke

Au

Katibu, Kenyatta University Ethics Review Committee. S.L.P. 43884-00100 Nairobi Kenya.

SEHEMU B

IDHINI YA MSHIRIKI

Mimi Bw/Bi _____ nimeelezwa kuhusu utafiti huu na nimeelewa yote ambayo nimeelezwa na maswali yangu yote yamejibiwa kwa kiwango cha kuridhisha

☐ Nimekubali kushiriki katika utafiti huu

Jina la mhojiwa: _____

Sahihi au alama ya kidole gumba

Sahihi:_____

Tarehe: _____

Ikiwa mshiriki hawezi kusoma, hakikisha kuwa kuna shahidi ambaye ataona mchakato wa ridhaa na atatia sahihi chini.

Nimeshuhudia kwamba taarifa kuhusu utafiti huu umeelezwa kwa usahihi kwa mshiriki na mshiriki ameelewa na kutoa ridhaa yake kwa uhuru.

Sahihi la shahidi: _____

Tarehe**:** _____

Jina la shahidi**:** _____

Jina la anayepewa ruhusa: _____

Sahihi: _____ Tarehe: _____

Jina la mtafiti: ____

Sahihi: ___________________________ Tarehe: _________________________

Appendix VII: Informed Consent for Patients

Title of the study

Awareness and pratices on prevention of human tick-borne diseases and genetic diversity of *Theileria* and *Babesia* at the human, cattle and African buffalo *(Syncerus caffer*) interface in the Ol-Pejeta Conservancy, Laikipia County, Kenya.

PART A

Introduction

Ticks are known to bite animals and wildlife as well as people. Tick bites transmit parasites which cause diseases such as East Coast Fever (Theileriosis) and Babesiosis in cattle and wildlife. Tick bites can also transmit these parasites to people and cause disease. In Laikipia County and many other parts of Kenya, the burden of tick transmitted parasites to people is not known. Information on tick transmitted diseases will help improve community health and prevention of tick bites.

Who is carrying out this study?

This study is being carried out by Marion Amulyoto, a student at the Institute of Tropical Medicine and infectious diseases (ITROMID) who is the principal investigator. It is not a funded project.

What will it involve for me if I agree?

Blood will be drawn from your arm with a needle by an experienced phlebotomist. The blood will be slightly less than a teaspoon (3ml). This blood will be analysed in a laboratory for the presence or absence of Tick transmitted parasites (*Babesia* and *Theileria*). You will also fill a questionnaire on your knowledge and practice in regards to tick borne diseases. There is no cost to you to participate in the study

Are there any risks or disadvantages to me taking part?

The risk that you will be injured during collection of blood is minimal, but it is possible that there may be slight discomfort or pain during blood collection at the site of the injection. Afterwards, there may be some bruising or swelling. The process will take very short time and will adhere to the usual health care standards in the facility.

Are there any benefits to me taking part?

There are no direct benefits for you. However, the results of the study will provide information that Government can use to improve health care and tick management.

What happens if I refuse to participate?

Your participation in this research is voluntary. It is your choice whether to participate or not. If you chose not to participate you will receive all the services at the clinic and nothing will change.

What happens to the samples after analysis?

The research tests will be done in Nairobi at the Kenya Wildlife Service (KWS) Laboratory and later at the International Livestock Research Institute (ILRI). After the research, a small portion of the blood will be stored at the Kenya Medical Research Institute (KEMRI) for duration of two (2) years. This will allow sufficient time to use the samples for any further investigation that may be necessary as a result of the outcome of this initial study. The future research must first be approved by a national independent expert committee to ensure participants safety and rights are respected. The samples will be destroyed by incineration after the completion of the study.

Who will have access to information about me in this research?

All the information related to this project will be confidential. Your name will not be put on the samples; instead codes will be used to ensure that samples can be linked to the participants by the principle investigator only. All the records from this research will be kept safely in lockable cabinets and in computers that are secured with passwords.

Who has allowed this research to take place?

The Kenyatta University ethics review committee has looked carefully at this work and agreed that the research is important, relevant to Kenya and follows nationally and internationally agreed research guidelines. This includes ensuring that all participants' safety and rights are respected.

Contacts and questions

The Principal investigator in this research is **Marion Amulyoto.** You may ask any questions you have now, or if you have any questions later, you are encouraged to contact her through the mobile telephone number: **0722 826645** or email **marionamulyoto@gmail.com**

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the following:

The Director, Institute of Tropical Medicine and Infectious Diseases (ITROMID), Jomo Kenyatta university of Agriculture and technology (JKUAT) P.O. Box 62000 – 00200 Nairobi. Telephone: (020) 2722541 Email: director@itromid.jkuat.ac.ke

Or

The secretary, Kenyatta University Ethics Review Committee. P.O. Box 43884-00100 Nairobi Kenya.

PART B

CONSENT FORM

I, Mr/Miss/Mrs_____ have had the research explained to me. I have understood all that has been read/ explained and had my questions answered satisfactorily.

☐ I agree to take part in this research

☐ I agree to samples being stored and used for future research

I understand that I can change my mind at any stage and it will not affect me in any way.

Respondent's name: _____

Signature or left thumb print

Signature: _____

Date: _____

Where subject cannot read, ensure a witness observes consent process and signs below

I attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the subject

Witness' signature: _____

Date: _____

Witness's name: _____

Name of the person taking consent: _____

Signature: _____ Date: _____

Name of the investigator: _____

Signature: _____ Date: _____

Appendix VIII: Informed Consent for Patients (Swahili Version)

Anwani ya utafiti

Mambo yanayohusiana na maambukizi ya magonjwa yanayoletwa na kupe kwa binadamu na maumbile tofauti ya *Theileria* na *Babesia* kwa binadamu, ng'ombe na nyati kwenye Ol Pejeta Conservancy kaunti ya Laikipia, Kenya

SEHEMU A

Utangulizi

Kupe anajulikana kwa kuuma mifugo na wanyama pori. Kupe husambaza vimelea wanaosababisha magonjwa kama vile ndigana na ugonjwa wa kukojoa damu kwa ng'ombe na wanyama pori. Kupe anaweza pia kusambaza vimelea hivi kwa binadamu na kusababisha magonjwa. Katika kaunti ya Laikipia na sehemu nyingine nyingi za Kenya, mzigo wa vimelea vinavyosambazwa na kupe kwa binadamu haujabainika. Taarifa juu ya magonjwa yanayosambazwa na kupe itasaidia kuboresha afya ya jamii na kuzuia kuumwa na kupe.

Ni nani anayetekeleza utafiti huu?

Utafiti huu unatekelezwa na Marion Amulyoto, mwanafunzi kwenye taasisi ya utafiti wa madawa na magonjwa ya kuambukiza (ITROMID) ambaye ni mtafiti mkuu. Utafiti huu hauja gharamiwa na taasisi yoyote.

Nitahusishwa vipi katika utafiti huu ikiwa nitakubali?

Damu itatolewa kwenye mkono wako kwa sindano na muuguzi aliye na uzoefu. Damu hiyo itakuwa kiasi cha chini ya kijiko kidogo (mililita tatu). Damu hii itachambuliwa kwenye maabara kubaini kuwepo au kutokuwepo kwa vimelea *Theileria* na *Babesia* ambazo husambazwa na kupe. Pia utajaza dodoso juu ya ufahamu na desturi juu ya magonjwa yanayosambazwa na kupe. Haitakugharimu chochote kushiriki katika utafiti huu.

Je, kuna hatari yoyote au hasara kwangu ikiwa nitashiriki?

Hatari ya kujeruhiwa wakati wa ukusanyaji wa damu ni ndogo sana. Lakini inawezekana kwamba huenda kukawa na usumbufu kidogo au maumivu kidogo wakati wa ukusanyaji wa damu.

Baada ya hapo kuna uwezekano ya uvimbe kutokea. Zoezi hili litachukua muda mfupi sana na utaambatana na viwango vya kawaida vya huduma za afya katika hiki kituo.

Je, kuna faida yoyote kwangu ikiwa nitashiriki?

Hakuna faida ya moja kwa moja kwako. Hata hivyo matokeo ya utafiti itatoa taarifa kwa serikali amabayo itatumiwa kuboresha huduma za afya na kudhibiti kupe.

Je, ni nini kitatokea kama nitakataa kushiriki?

Kushiriki katika utafiti huu ni kwa hiari. Una uhuru wa kushiriki au la. Ukichagua kutoshiriki bado utapokea huduma zote katika kliniki hii kama kawaida.

Je, sampuli zangu zitafanyiwa nini?

Utafiti wa maabara utafanyika jijini Nairobi kwenye maabara ya huduma ya wanyama pori (KWS) na baadaye katika taasisi ya kimataifa ya utafiti wa mifugo (ILRI). Baada ya utafiti, sehemu ndogo ya damu itahifadhiwa katika maabara ya taasisi ya utafiti wa kimatibabu ya Kenya (KEMRI) kwa mda wa miaka miwili (2). Sampuli itakayowekwa itatumika kwa utafiti zaidi ikiwa kutakuwa na hitaji baada ya matokeo ya utafiti wa kwanza. Utafiti wowote utakaofanywa siku zijazo kwanza utapitishwa na kamati ya kitaifa la wataalam wa maadili kuhakikishia washiriki kuwa usalama na haki zao zinaheshimiwa. Sampuli zote zitaharibiwa kwa kuchomwa mwisho wa utafiti.

Je, ni nani atakayeweza kusoma habari kunihusu katika utafiti huu?

Taarifa zote kuhusiana na utafiti huu utahifadhiwa kwa siri. Jina lako halita wekwa kwenye sampuli. Badala yake, kificho kitatumiwa kuhakikisha kuwa sampuli inaweza kuhusishwa na mshiriki na mtafiti mkuu peke yake. Rekodi zote za utafiti huu zitahifadhiwa kwa usalama katika kabati zinazofungika na kwenye tarakilishi zilizo na nywila.

Ni nani ameruhusu utafiti huu kutendeka?

Kamati ya maadili ya utafiti ya chuo kikuu cha Kenyatta imechunguza kazi hii kwa makini na walikubaliana kwamba utafiti huuni muhimu kwa Kenya na kwamba imefuata miongozo ya kitaifa na Kimataifa inayohusiana na utafiti. Hii ni pamoja na kuhakikisha kuwa usalama na haki zote za washiriki zinaheshimiwa.

Mawasiliano na maswali

Mtafiti anayetekeleza utafiti huu ni **Marion Amulyoto**. Unaweza kuuliza maswali yoyote uliyonayo sasa ama ikiwa utakuwa nayo baadaye, unahimizwa kuwasiliana naye kupitia nambari ya simu ya mkono: **0722 826645** au barua pepe **marionamulyoto@gmail.com**.

Ikiwa una maswali yoyote kuhusu utafiti huu na ungependa kuongea na mwengine asipokuwa mtafiti, unahimizwa uwasiliane na wafuatao:

Mkurugenzi, Taasisi ya utafiti ya madawa na magonjwa ya kuambukiza, Chuo kikuu cha kilimo na Teknologia cha Jomo Kenyatta, S.L.P. 62000- 00200 Nairobi. Nambari ya simu (020) 2722541 Barua pepe: director@itromid.jkuat.ac.ke

Au

Katibu, Kenyatta University Ethics Review Committee. P.O. Box 43884-00100 Nairobi Kenya.

SEHEMU B

IDHINI YA MSHIRIKI

Mimi Bw/Bi _____ nimeelezwa kuhusu utafiti huu na nimeelewa yote ambayo nimeelezwa na maswali yangu yote yamejibiwa kwa kiwango cha kuridhisha.

- ☐ Nimekubali kushiriki katika utafiti huu
- ☐ Nimekubali sampuli yangu kuhifadhiwa na kutumika kwa ajili ya utafiti baadaye

Naelewa kwamba naweza kubadili mawazo yangu katika hatua yoyote na sita athirika kwa njia yoyote.

Jina la mhojiwa: _____

Sahihi au alama ya kidole gumba

Sahihi:_____

Tarehe: _____

Ikiwa mshiriki hawezi kusoma, hakikisha kuwa kuna shahidi ambaye ataona mchakato wa ridhaa na atatia sahihi chini.

Nimeshuhudia kwamba taarifa kuhusu utafiti huu umeelezwa kwa usahihi kwa mshiriki na mshiriki ameelewa na kutoa ridhaa yake kwa uhuru.

Sahihi la shahidi: _____

Tarehe: _____

Jina la shahidi: _____

Jina la anayepewa ruhusa: _____

Sahihi: _____ Tarehe: _____

Jina la mtafiti: _____

Sahihi: _____ Tarehe: _____

Appendix IX: Approval Letter from Kenyatta University Ethics Research Committee for Research at Ol Pejeta Conservancy

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: KU/R/COMM/51/816

Date: 2nd November, 2016

Marion Amulyoto
Jomo Kenyatta University of Agriculture & Technology
P.O. Box 62000-00100
NAIROBI

Dear Marion,

APPLICATION NUMBER **PKU/557/E52** – "FACTORS ASSOCIATED WITH HUMAN INFECTION AND GENETIC DIVERSITY OF THEILERIA AND BABESIA AT THE HUMAN, CATTLE AND AFRICAN BUFFALO (SYNCERUS CAFFER) INTERFACE IN THE OL-PEJETA CONSERVANCY, LAIKIPIA COUNTY" – VERSION 2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic **"Factors Associated with Human Infection and Genetic Diversity of Theileria and Babesia at the Human, Cattle and African Buffalo (Syncerus Caffer) Interface in the Ol-Pejeta Conservancy, Laikipia County"** Version 2 received on 26th October, 2016 and discussed on 1st November, 2016.

2. APPLICANT
Marion Amulyoto

3. SITE
Ol-Pejeta Conservancy, Laikipia County, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 2nd November, 2016.**

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN P.O. BOX 43844 ETHICS REVIEW COMMITTEE 03 NOV 2016

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I MARION AMULYOTO accept the advice given and will fulfill the conditions therein.

Signature.... Dated this day of 3rd NOVEMBER 2016.

cc. Vice-Chancellor
DVC-Research Innovation and Outreach

Appendix X: Authorization Letter from Laikipia County Department of Health

REPUBLIC OF KENYA
COUNTY GOVERNMENT OF LAIKIPIA
DEPARTMENT OF MEDICAL SERVICES AND PUBLIC HEALTH

OFFICE OF THE COUNTY DIRECTOR
P.O. Box 1271-10400,
Nanyuki, Kenya

REF: CGL/HEALTH/RESEARCH/VOL.1/11 **DATE**: 26th January, 2017

TO WHOM IT MAY CONCERN

RE: PERMISSION TO UNDERTAKE A RESEARCH STUDY

Amulyoto Marion (TM306-1164/2013) is a graduate student in Institute of tropical medicine and infectious diseases (ITROMID) a joint programme between Kenya Medical Research Institute (KEMRI) and Jomo Kenyatta University of Agriculure and Technology (JKUAT).

She has been granted permission to undertake a research on **"Factors associated with human infection and genetic diversity of theileria and babesia at the human, cattle and African buffallo (*Syncerus Caffer*) interface in the Ol-Pejeta Conservancy, Laikipia County".**

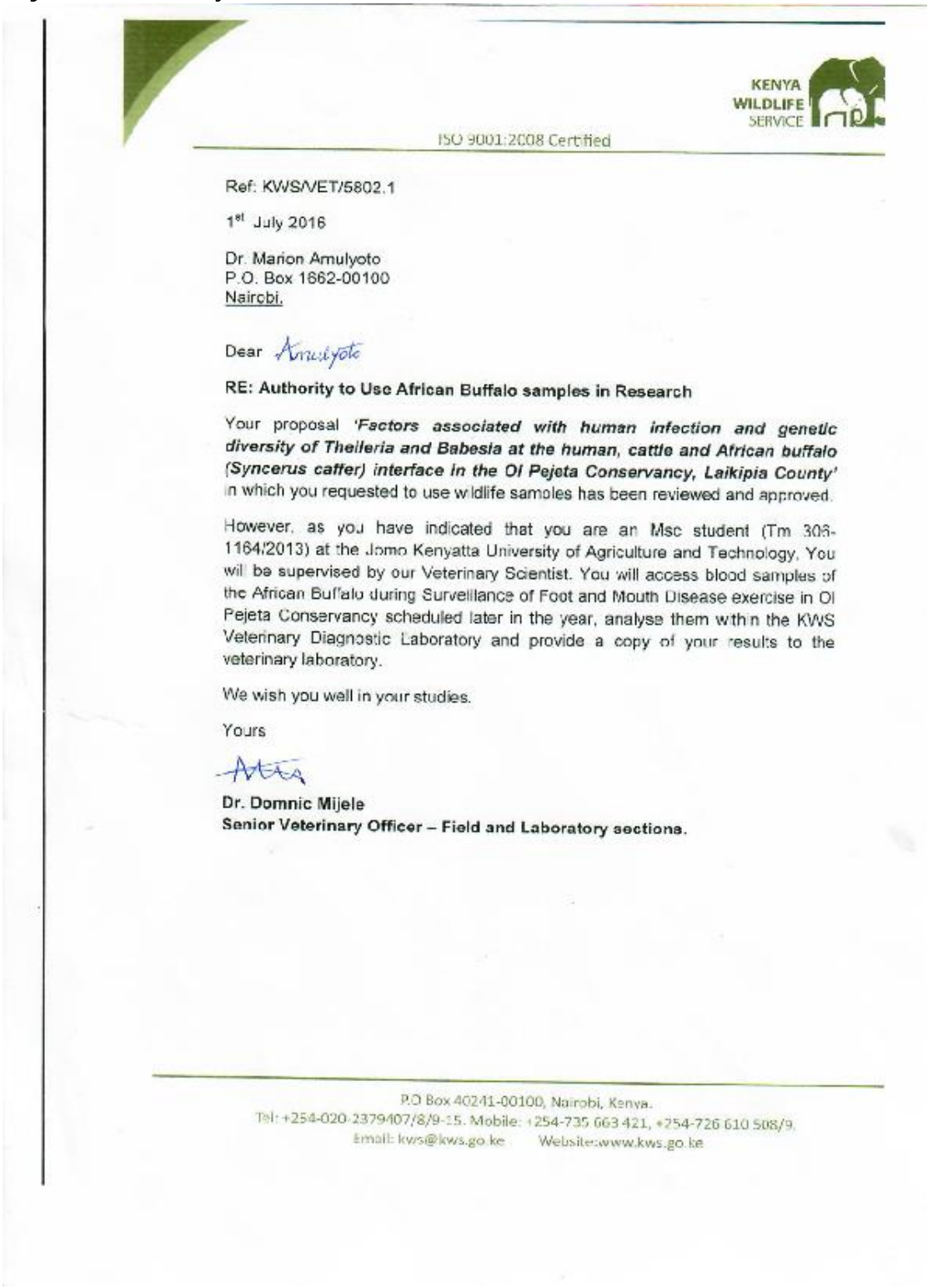
This is therefore to request for your support to enable her to accomplish the study.

Thank you.

26 JAN 2017

DR. WAIHENYA MWANGI
COUNTY DIRECTOR MEDICAL SERVICES
LAIKIPIA COUNTY

Appendix XI: Approval Letter from Kenya Wildlife Service for Research At Ol Pejeta Conservancy On African Buffalos



KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

Ref: KWS/VET/5802.1

1st July 2016

Dr. Marion Amulyoto
P.O. Box 1662-00100
Nairobi.

Dear Amulyoto

RE: Authority to Use African Buffalo samples in Research

Your proposal ***'Factors associated with human infection and genetic diversity of Theileria and Babesia at the human, cattle and African buffalo (Syncerus caffer) interface in the Ol Pejeta Conservancy, Laikipia County'*** in which you requested to use wildlife samples has been reviewed and approved.

However, as you have indicated that you are an Msc student (Tm 303-1164/2013) at the Jomo Kenyatta University of Agriculture and Technology, You will be supervised by our Veterinary Scientist. You will access blood samples of the African Buffalo during Surveillance of Foot and Mouth Disease exercise in Ol Pejeta Conservancy scheduled later in the year, analyse them within the KWS Veterinary Diagnostic Laboratory and provide a copy of your results to the veterinary laboratory.

We wish you well in your studies.

Yours

Dr. Domnic Mijele
Senior Veterinary Officer – Field and Laboratory sections.

P.O Box 40241-00100, Nairobi, Kenya.
Tel: +254-020-2379407/8/9-15. Mobile: +254-735 663 421, +254-726 610 508/9.
Email: kws@kws.go.ke Website:www.kws.go.ke