Pathogens and blood feeding patterns of questing ticks in Maasai Mara wildlife ecosystem, Kenya

BY

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DECLARATION

Student

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or for any other award.

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DEDICATION

This thesis is dedicated to my beloved parents Kerina Oundo and Geofrey Oundo and to my brother Julius for their moral support and continued encouragement during the study.

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ABSTRACT

Questing ticks are particularly important in the epidemiology of tick-borne diseases, as they are likely to pick up and transmit pathogens across various vertebrate hosts during blood-feeding. Nevertheless, their role in wildlife habitats undergoing rapid changes in land usage and intensified encroachment by humans and livestock remains poorly understood. Therefore, this study was conducted to investigate diversity of questing ticks, their blood-meal hosts, and tick-borne pathogens in Kenya's Maasai Mara National Reserve (MMNR), an area with intensified human-wildlife-livestock interactions. Questing ticks were collected from 25 sites using flagging and direct handpicks from vegetation, and identified by both morphological and molecular methods. They were then pooled (1-11 adults, 1-25 nymphs or 1-20 larvae) by sex, species and sampling site and screened for blood meal sources and tick-borne pathogens of *Anaplasma*, *Babesia*, *Coxiella*, *Ehrlichia*, *Rickettsia* and *Theileria* species using PCR with high-resolution melting (HRM) analysis followed by sequencing of samples with unique melting profiles. A total of 1,465 host-seeking ticks were collected, including *Rhipicephalus appendiculatus* (n=1,125), *Rhipicephalus pulchellus* (n=6), *Rhipicephalus evertsi* (n=5), *Amblyomma hebraeum*-like *Amblyomma* sp., (n=178), *Amblyomma gemma* (n=145), *Amblyomma variegatum* (n=4), *Amblyomma* sp (n=1) and *Haemaphysalis leachi* (n=1). Bloodmeals of humans, blue wildebeest and African buffalo were identified in *Rhipicephalus appendiculatus*, humans in *Am. hebraeum*-like *Amblyomma* sp., goat in *Rh. evertsi*, sheep in *Am. gemma*, and cattle in *Am. variegatum*. *Rickettsia africae* was detected in the *Am. gemma* (1/25) and *Am. variegatum* (1/3) pools that had fed on sheep and cattle, respectively. *Rickettsia* spp. were found in *Am. gemma* (4/25 pools) and *Rh. evertsi* (1/4 pool), *Anaplasma ovis* in *Rh. appendiculatus* (1/172) and *Rh. evertsi* (1/4), and *Anaplasma bovis* (1/172) and *Theileria parva* (27/172) in *Rh. appendiculatus*. Further, species-specific *Coxiella* endosymbionts were amplified from 75.3% of tick pools. This baseline survey in the MMNR suggests that ticks feed on humans and diverse species of wildlife and livestock and that ticks may transmit diverse pathogens to humans and livestock in the region.

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LIST OF ABBREVIATIONS

AFPMB	Armed Forces Pest Management Board
ATBF	African tick bite fever
BLAST	Basic Local Alignment Search Tool
CDC	Centers for Disease Control and Prevention
CFT	Complement Fixation Test
COI	Cytochrome oxidase I
ECF	East Coast fever
ELISA	Enzyme-Linked Immuno-Sorbent Assay
HRM	High Resolution Melting
ICIPE	International Centre of Insect Physiology and Ecology
IFA	Immuno-Fluorescence Assay
ITS2	Internal transcribed spacer 2
MMNR	Maasai Mara National Reserve
MSF	Mediterranean spotted fever
ompB	Outer Membrane Protein B
PCR	Polymerase Chain Reaction
RMSF	Rocky Mountain spotted fever
SFG	Spotted fever group
TBPs	Tick-borne pathogens
TBDs	Tick-borne diseases

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Wildlife ecosystems are known to be hotspots for a range of emerging diseases that threaten human and livestock health (Jones *et al.,* 2008; Siembieda *et al.,* 2011; Wiethoelter *et al.,* 2015). Majority of the emerging pathogens are maintained asymptomatically by wildlife and are transmitted to humans and livestock by vectors such as ticks. Ticks transmit a wide variety of pathogenic microorganisms including viruses, bacteria, and protozoa (Jongejan and Uilenberg, 2004). An upsurge of emerging tickborne zoonoses such as Lyme borreliosis has been witnessed in several developed countries in the recent past (Schwartz *et al.,* 2017), while the burden of some of the "old" tick-transmitted diseases such as East Coast Fever (ECF) have persisted (Lawrence *et al.,* 2004; Gachohi *et al.,* 2012). There is thus a need for regular surveillance and detection of tick-borne diseases (TBDs) to assess the risk for disease outbreaks especially at the human-wildlife interface.

Generally, the ecology of tick-borne pathogens (TBPs) is complex and often involves both wildlife, domestic animals and humans. These hosts provide blood-meals that maintain tick populations and also serve as reservoirs and/or amplifiers of different TBPs (Mather and Howard, 1994; Parola and Raoult, 2001; Dantas-Torres *et al.,* 2012). Once ticks have obtained pathogens from an infected host, they are capable of maintaining infective status throughout their lifecycle (transtadially) and through multiple generations (transovarially) thereby optimizing pathogen transmission to susceptible hosts in their next blood-meals (Parola and Raoult, 2001). Control of TBPs therefore demands for a One-Health approach requiring knowledge of the tick species, their host feeding preferences, habitat and range (Dantas-Torres *et al.,* 2012).

Emergence and expansion of TBDs is increasingly linked to changes in the physical environment (Vanwambeke *et al.,* 2010; Dantas-Torres, 2015). Ecological changes driven by human activities on land use are likely to induce increased connectivity and probability of contact between vectors, animal and human hosts (Lambin *et al.,* 2010). This provides opportunities for pathogens from the wild to spill over to domestic animals and human beings (Jones *et al.,* 2008). The Maasai Mara ecosystem in south-western Kenya, represents one such fast-changing environment. This ecosystem has undergone drastic changes in land use in the last decades (Kimanzi and Wishitemi, 2001; Serneels and Lambin, 2001; Lamprey and Reid, 2004; Mundia and Murayana, 2009; Ogutu *et al.,* 2011; Maasai Mara Science and Development Initiative policy paper [MMSDI], 2015). The major drivers of the drastic changes in land-use in this ecosystem have been cited as intensification of crop and livestock farming, tourism activities and human settlement, which may have inadvertently increased the contact between wildlife, ticks (and other vectors) with humans and livestock. Therefore, the aim of this study was to investigate the diversity of questing ticks in this ecosystem as well as their blood meal sources and infection with *Rickettsia, Anaplasma, Ehrlichia, Coxiella, Babesia* and *Theileria* species. This study was based on questing ticks because this is the active stage of most ixodid ticks seeking vertebrate hosts for blood-meals and are likely to pick new pathogens which can be transmitted to humans and livestock.

1.2 Statement of the problem

In Kenya, TBPs have continued to exert significant levels of disease burden in humans (Potasman *et al.,* 2000; Rutherford *et al.,* 2004; Yoshikawa *et al.,* 2005; Kenya Zoonosis Disease Unit, 2014) and economic losses in the cattle industry (Grootenhuis, 1986; Mulei and Rege, 1989; Wesonga *et al.,* 2010; Gachohi *et al.,* 2012; Kiara *et al.,* 2014). Among these, pathogens within the spotted fever group (SFG) *Rickettsia*, *Anaplasma* and *Ehrlichia* species, account for an increasing

proportion of the emerging tickborne diseases (Parola and Raoult, 2001; Kernif *et al.,* 2016). In addition, diseases caused by the parasitic piroplasms such as *Theileria* and *Babesia* have persisted and are predicted to emerge in new foci despite concerted efforts towards their control (Gachohi *et al*, 2012; Kabi *et al*, 2014).

Tick borne pathogens are normally maintained in natural cycles involving ticks as vectors and wild and/or domestic animal as hosts (Parola and Raoult, 2001). Questing ticks are the most important in transmission of TBPs to humans and livestock. However, their role in disease transmission in African wildlife habitats has been widely neglected by both the public and veterinary health authorities. Further, the role of vertebrate hosts involved in maintaining the life cycle of ticks in these wildlife habitats are inadequately described. There is thus need to understand the diversity of questing ticks, their blood-meal hosts and the pathogens they harbor in order to predict disease outbreaks and develop effective control strategies.

1.3 Objectives

1.3.1 Broad objective

This study aims at providing an in-depth description of pathogens and blood feeding patterns of questing ticks in the fast-changing wildlife ecosystem of Maasai Mara,Kenya

1.3.2 Specific objectives

1. To assess the species distribution, abundance and diversity of questing ticks in MMNR.
2. To determine the diversity of tick-borne pathogens (*Anaplasma, Babesia, Coxiella, Ehrlichia, Rickettsia* and *Theileria* species) in questing ticks from MMNR.
3. To identify the vertebrate blood-meal hosts for ticks in MMNR.

1.4 Research hypothesis

1. Questing ticks are present and diverse in MMNR.
2. Tick-borne pathogens of veterinary and public health importance are present and diverse in MMNR
3. Vertebrate blood-meal hosts for ticks are present and diverse in MMNR

1.5 Research question

What are the species composition of questing ticks, their associated pathogens and vertebrate blood-meal hosts in MMNR?

1.6 Justification of the Study

Group ranches, communal lands and human settlements directly border the MMNR and this brings wildlife, livestock and humans in close proximity to one another (Lamprey and Reid, 2004; MMSDI policy paper, 2015). These close interactions among humans, wildlife and domestic animals creates an ideal opportunity for transmission of TBPs. Understanding the transmission dynamics of TBPs in terms of their presence, possible vectors and vertebrate blood meal hosts involved in supporting tick lifecycle is a critical step towards assessing the risk of TBDs and thus forestalling possible disease outbreaks in Maasai Mara ecosystem.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ticks

2.1.1 Taxonomy

Ticks are classified in the phylum *Arthropoda,* class *Arachnida,* and order *Acarina*. *Acarina* has three families *Argasidae, Ixodidae* and *Nuttalliellidae*. *Ixodidae* (hard ticks) and the *Argasidae* (soft ticks) are the major families of ticks, while the *Nuttalliellidae* family is the least among the tick families (Guglielmone *et al.,* 2010).

The family *Argasidae* has three genera including *Argas, Otobius* and *Ornithodoros,* but only the genus *Ornithodoros* is of medical importance. The family *Ixodidae* consists of *Amblyomma, Dermacentor, Hyalomma, Haemaphysalis, Ixodes, Margropus, Rhipicephalus, and Aponoma.* Hard ticks of medical importance belong to the genera *Ixodes, Dermacentor, Amblyomma, Haemaphysalis, Rhipicephalus* and *Hyalomma. Nuttalliedae* family is comprised of a single species which is of no medical importance and is confined to southern Africa (Guglielmone *et al.,* 2010; Service, 2012).

2.1.2 Morphology

Adult hard ticks are dorsoventrally flattened with an oval outline. The body size measure about 2–23mm long but varies depending on species and whether they are fed or unfed (Walker *et al.,* 2003). Adults and nymphs have four pairs of walking legs, while larvae have three. They lack the Coxal organs. The capitulum is visible from dorsal side, a feature that distinguishes adult ixodid ticks from argasid ticks (Fig. 1). The capitulum bears the mouthparts, including sensory organs, cutting organs, and the hypostome with numerous recurved teeth that anchor the tick to the host's skin. The palps are rigid, swollen and club-shaped unlike the leg-shaped palps in soft ticks.

Both the hypostome and chelicerae penetrate the host during feeding. The ixodids are characterized by the presence of dorsal plate known as scutum, which covers the dorsal surface of males, and in female ticks it covers only the anterior dorsal part (Fig. 1), thus allowing the remainder of the body to expand during feeding. Scutum of *Dermacentor, Amblyomma* and some *Rhipicephalus* species has enamelled coloured areas, and are thus known as ornate ticks (Walker *et al.,* 2003; Service, 2012). Festoons are present in the posterior margin of the body in *Dermacentor, Rhipicephalus* and *Haemaphysalis* species, although it may be invisible in fully engorged females (Walker *et al.,* 2003; Service, 2012).

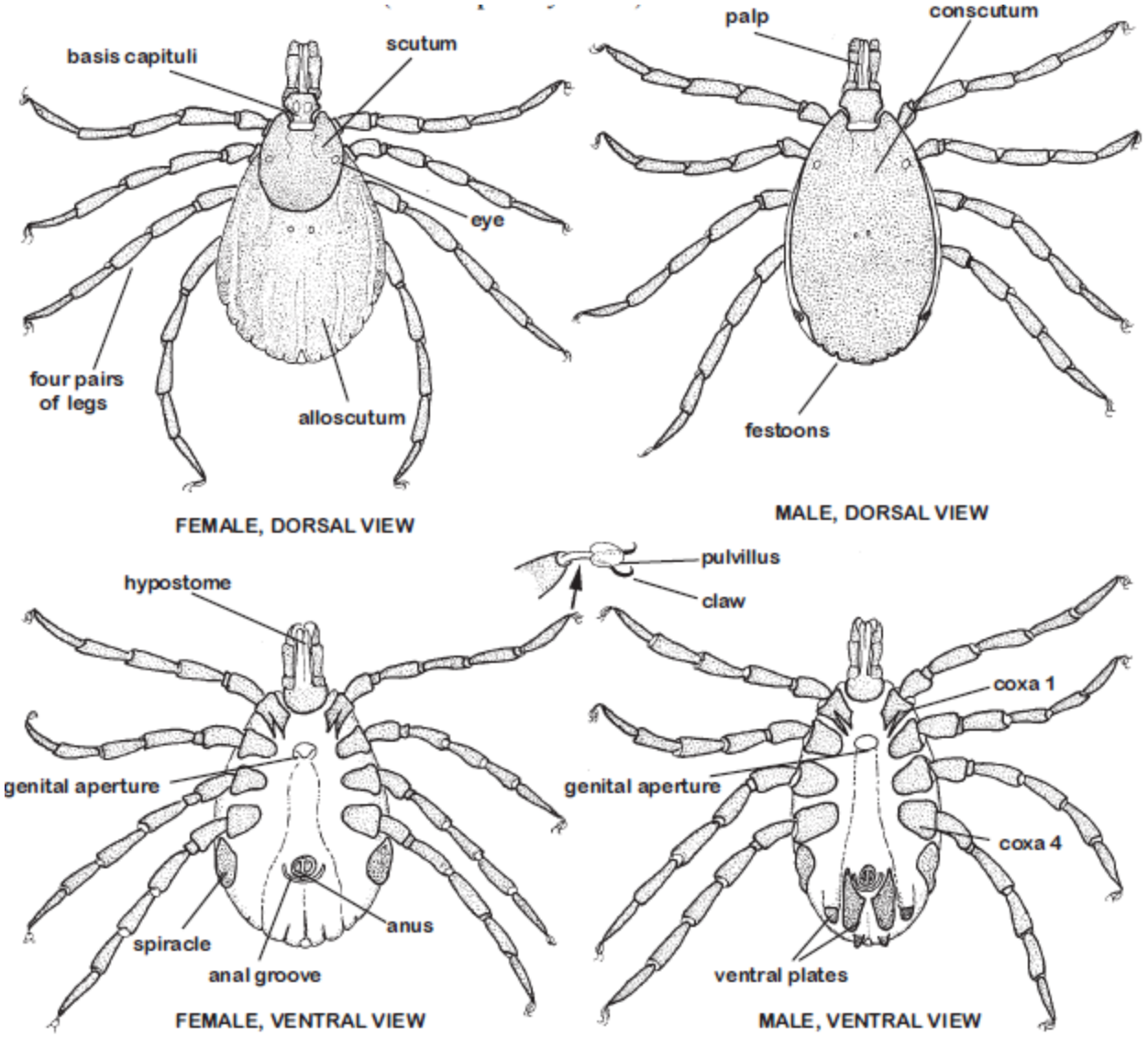

Figure 1. External structure of adult ixodid ticks (Adopted from Walker *et al.*, 2003).

2.1.3 Life cycle

Ixodid ticks have hemimetabolous life cycles (i.e. incomplete metamorphosis) involving a larval and nymphal stage. The adult female lays only one batch of eggs (1000 - 10000 eggs in a batch) in a gelatinous mass which is formed in front and on top of the tick's scutum. As in argasid ticks, the eggs are coated with a waxy secretion produced by Gene's organ, which in ixodid ticks also helps transfer eggs from the genital opening to the scutum. The female hard tick engorges only

once and die shortly after the completion of oviposition, unlike soft tick females that engorge a number of times and oviposit after each feeding (Parola and Raoult, 2001; Walker *et al.,* 2003).

The oviposited eggs hatches into six-legged larvae (0.5–1.5mm long), which then climbs onto low vegetation where it quests for a host. Once on a host, the larvae will feed and engorge before dropping off to the ground and seek shelter under vegetation, stones or bury itself in the surface soil from where they will moult to an eight-legged nymph (Parola and Raoult, 2001; Service, 2012). The nymphs will then climb up vegetation, quest for a host, attach, blood-feed and the fully engorged nymphs drop to the ground and seek shelter under stones and vegetation.

The nymphs moult to produce male or female adult ticks. All hard ticks have only one nymphal stage, unlike argasid ticks which have several nymphal stages, ranging from 2-7 nymphal instars (Parola and Raoult, 2001; Service, 2012).

Newly formed adults will then climb vegetation and start questing for passing hosts, attach, mate on the host (except for *Ixodes* species), engorge and the females detach and drop off to the ground to lay eggs. The males may remain on a host and mate with several other females (Parola and Raoult, 2001). Hard ticks are termed non-nidicolous ticks i.e. they move away from burrows and homes of their animal hosts, unlike the argasid ticks which are nidicolous. The life cycle of ixodid ticks is takes approximately 2–3 years, but it may last to 6 years depending on environmental conditions such as temperature, relative humidity and sunlight (Parola and Raoult, 2001).

Hard ticks can be classified by the number of host species they parasitize during their life cycle. They include: one-host, two-host and three-host ticks. In one-host ticks, all the three stages of the life cycle i.e. larvae, nymph, and adults stay, feed and moult on only one animal host before the female drops off to the ground to lay her eggs. An example of a medically important one-host tick

is the *Rhipicephalus annulatus*, the vector for babesiosis (Kettle, 2000; Walker *et al.,* 2003; Service, 2012).

In two-host ticks, the larvae remain on the host after blood-feeding and moult to produce nymphs which feed on the same host. The engorged nymphs then drop off the host and moult to produce adults, which in turn feed on a different host. An example of a medically important two-host tick is the *Hyalomma marginatum*, the vector for Crimean-Congo viral hemorrhagic fever (Kettle, 2000; Walker *et al.,* 2003; Service, 2012).

In three-host ticks, each stage of the life cycle will feed on a different individual host, which may be of the same or different species, and moulting occurs on the ground. Examples of medically important three host feeders include *Ixodes*, *Dermacentor*, *Rhipicephalus*, *Haemaphysalis* and *Amblyomma* species (Kettle, 2000; Walker *et al.*, 2003; Service, 2012).

2.1.4 Questing and feeding behavior of ixodid ticks

Ixodid ticks display two typical patterns of host-seeking behavior, i.e. the ambush strategy and the hunter strategy. In the ambush strategy, ticks climb up vegetation, extend their first pair of front legs and wait for passing hosts (e.g. the brown dog tick, *R. sanguineus*). In the hunter strategy, ticks attack hosts. They emerge from their habitat and run toward their hosts and attack the host when they appear nearby (e.g., *Amblyomma hebraeum* and *Amblyomma variegatum*) (Parola and Raoult, 2001). Factors that alert and stimulate the ticks for the presence of a host include carbon dioxide, ammonia, phenols, odors, vibrations, air currents, body warmth/ heat, and interrupted light (Parola and Raoult, 2001).

Once on a host, a tick will crawl over the skin to find a suitable place to attach and feed (Service, 2012). The tick will then cut through the dermis with their mouthparts and break the capillary

blood vessels very close to the skin surface and thus creating a feeding wound. During attachment, the ixodid tick will secrete saliva containing various substances including a cement to hold the mouthparts firmly to the skin of the host; anesthetic to make the tick bite painless; enzymes; vasodilators; anticoagulants; and anti-inflammatory, antihemostatic, and immunosuppressive substances (Binnington and Kemp, 1980; Kemp *et al.,* 1982; Soneshine, 1991; Walker *et al.,* 2003). These salivary secretions are injected into the wound to facilitate successful blood feeding. Saliva of some species contain toxins that that may cause paralysis of the host (Soneshine, 1991). The salivary secretions also play the important role in the transmission of disease by serving as a medium of transport for the disease agents from the infected tick to the host (Binnington and Kemp, 1980). As the ticks continue to feed, they regurgitate excess water that has been extracted from the blood meal into the wound, thus enabling the tick to concentrate the blood in their body (Kaufman and Sauer, 1982). This process also increases the possibility for the transmission of pathogens from an infected tick to the vertebrate host. Undigested residues from the midgut and wastes from the excretory body are eliminated through the anus. Adult females require 5 to 20 days to fully engorge, nymphs 4 to 8 days and larvae 3 to 5 days. They will then detach from the host, drop to the ground and find a resting place where they can digest the blood meal and molt to the next feeding stage, or enter diapause (Walker *et al.,* 2003).

2.2 Tick-borne rickettsioses

2.2.1 Aetiology

Tick-borne rickettsioses are zoonotic diseases caused by obligate intracellular, gram-negative bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia*. Tick-transmitted rickettsioses include African tick-bite fever (caused by *Rickettsia africae*), Mediterranean spotted fever (caused by *R. conorii*) and Rocky Mountain spotted fever or Brazilian spotted fever (Caused

by *R. rickettsia*) (Parola and Raoult, 2001). Cases of African tick bite fever (ATBF) continue to be regularly reported in foreign travellers visiting Africa (Raoult *et al.,* 2001; Jensenius *et al.,* 2003b; Roch *et al.,* 2008), and its agent, *Rickettsia africae*, continues to be detected in ticks in many sub-Saharan Africa countries including Kenya (Macaluso *et al.,* 2003; Maina *et al.,* 2014), Ethiopia (Kumsa *et al.,* 2015), Uganda (Nakao *et al.,* 2013), Sudan (Nakao *et al.,* 2015), Senegal (Sambou *et al.,* 2014), Union of the Comoros (Yssouf *et al.,* 2014), Nigeria (Lorusso *et al.,* 2013). The principal reservoir hosts and vectors of *R. africae* are ticks of the genus *Amblyomma,* namely *A. hebraeum* and *A. variegatum* (Jensenius *et al.,* 2003a).

Rocky Mountain spotted fever (RMSF) is the predominant SFG rickettsiosis in the Americas and Mexico (Service, 2012; CDC, 2016). RMSF is spread by *Dermacentor variabilis* (the American dog tick) and *D. andersoni* (Rocky Mountain wood tick) in USA; *Rhipicephalus sanguineus* (the brown dog tick) in southwestern USA and Mexico; and *Amblyomma cajennense* (the cayenne tick) in Central and South America (Armed Forces Pest Management Board [AFPMB] (2012), 2012; Service, 2012; CDC, 2016). Both transstadial and transovarial transmission occur in ticks (Service, 2012).

Mediterranean spotted fever (MSF) is distributed in countries that surround the Mediterranean Sea and transmitted by *R. sanguineus.* Both MSF and ATBF have been described as the second most important causes of febrile illnesses in the travelers returning from Africa (Freedman *et al.,* 2006).

Many other tick-borne SFG rickettsioses have been described from other parts of the world including Queensland tick typhus caused by *Rickettsia australis*, Flinders Island spotted fever caused by *Rickettsia honei*, Japanese spotted fever caused by *Rickettsia japonica*, North Asian tick typhus (Siberian tick typhus) caused by *Rickettsia sibirica*, Boutonneuse fever caused by *Rickettsia mongolotimonae* and *Rickettsia slovaca* and lymphangitis-associated rickettsiosis

caused by *Rickettsia sibirica mongolotimonae* (Blair *et al.,* 2004; Jensenius *et al.,* 2004; Fournier *et al.,* 2005). Other pathogenic rickettsiae include *Rickettsia rhipicephali, Rickettsia aeschlimannii, Rickettsia heilongjiangensis, Rickettsia helvetica, Rickettsia montana, Rickettsia massiliae, Rickettsia amblyomii, Rickettsia cooleyi* and *Rickettsia marmionii* subspecies (Azad and Beard, 1998; Fournier *et al.,* 2003; Blair *et al.,* 2004; Jensenius *et al.,* 2004; Parola *et al.,* 2005; Moncayo *et al.,* 2010).

2.2.2 Tick-borne rickettsioses in Kenya

The prevalence of antibodies against SFG *Rickettsia* among febrile patients in Kenya has been found to range between 10-23% (Thiga *et al.,* 2015; Maina *et al.,* 2016; Omballa *et al.,* 2016). Human infection with *R. conorii* (main agent of MSF) has been reported in a missionary from the United States on the basis of the severity of the disease (Rutherford *et al.,* 2004), and in a Japanese traveler based on a laboratory diagnosis (Yoshikawa *et al.,* 2005).

Rickettsia africae has been detected in 15.8% of *Amblyomma variegatum* and 1% of *Rhipicephalus* species of ticks collected from livestock and wild animals from private and public land surrounding the Maasai Mara game reserve (Macaluso *et al.,* 2003). *Rickettsia africae*–genotype DNA was also found in 92.6% of adult *Am. variegatum* ticks collected from domestic ruminants in rural Western Kenya (Maina *et al.,* 2014). Maina *et al.* (2014) also detected high prevalence of *R. africae* variants in 95.2% of *Am. variegatum* ticks in western Kenya. Recently, Mwamuye *et al.* (2017) also documented *R. africae* infection in *Am. eburneum* ticks from the Shimba Hills National Reserve. Among domestic ruminants in Kenya, the prevalence of *R. africae* antibodies was reported as 43% in goats, 23% in sheep, and 1% in cattle in rural Western Kenya (Maina *et al.,* 2014).

Rickettsia aeschlimannii, another member of SFG rickettsiae, has been found in *Am. variegatum* (Maina *et al.,* 2014), *Rh. pulchellus* (Koka *et al.,* 2017; Omondi *et al.,* 2017), *Rh. annulatus* (Mutai *et al.,* 2013) and *Hyalomma* ticks (Mutai *et al.,* 2013; Koka *et al.,* 2017; Omondi *et al.,* 2017).

Other studies on the presence of SFG rickettsiae DNA in Kenya have been done by Mutai *et al.* (2013) who reported a prevalence of 16.3% in cattle, 15.1% in sheep and 7.1% in goats. Furthermore, 23.3% of ticks had rickettsiae with the highest detection in *Amblyomma* (62.3%), then *Rhipicephalus* (45.5%), *Hyalomma* (35.9%), and *Boophilus* (34.9%). They also characterized the SFG rickettsiae and found *Rickettsia africae* was present in 93% of the ticks, *Rickettsia aeschlimannii* in 1.9%, *Rickettsia mongolotimonae*, *Rickettsia conorii* subsp. *israelensis* and *Candidatus Rickettsia kulagini* in 0.96% of ticks, and *Rickettsia* spp. in 1.9% of the ticks.

2.2.3 Clinical characteristics

The clinical signs of SFG rickettsioses vary depending on the rickettsial species that are involved. The signs generally begin 6–10 days after a tick bite and include fever, headache, muscle pain, rash, local lymphadenopathy, and one or several inoculation eschars (Raoult and Roux, 1997). Thrombocytopenia and leukocyte count abnormalities are common, and hepatic enzyme levels are often elevated.

2.2.4 Diagnosis and treatment

Various serological tests are used in the diagnosis of rickettsioses including Immuno-Fluorescence Assay (IFA), Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Complement Fixation Test (CFT). Other methods used for diagnoses of rickettsioses include use of PCR to amplify the DNA of the organism in blood samples and tissues (La scola and Raoult, 1997; Parola and Raoult, 2001).

The treatment of choice of SFG rickettsioses is doxycycline, 200 mg/day, given for 1–7 days, depending on the severity of the disease (Parola and Raoult, 2001).

2.3 Q fever

2.3.1 Aetiology

Q fever is a zoonotic tick-borne disease caused by obligate intracellular gram-negative bacterium *Coxiella burnetii*. Human infection primarily occurs through ingestion or inhalation of virulent organisms from infected mammals and their products (urine, milk and birth products), most frequently goats, sheep, cattle, goats and cats (Marrie and Raoult, 1997; Maurin and Raoult, 1999). The role of ticks in human infections is minimal. Transovarial and transstadial transmission occurs in ticks (Service, 2012).

2.3.2 Q fever in Kenya

Largest outbreaks of Q fever in Kenya was reported in 4 of 50 international travelers who had visited a Maasai Manyata in Maasai Mara (Potasman *et al.,* 2000) and in 31 individuals in Baringo County (Kenya Zoonosis Diseases Unit, 2014). For the travelers, it was postulated that the infection was acquired through inhalation of fumes in a shack that was housing two goats (Potasman *et al.,* 2000). Previous serologic surveillance of Q fever in domestic ruminants in Laikipia indicated that *C. burnetii* was prevalent in 3-4% cattle, 13-20% in sheep, 31-40% in goats and 5-46% in camels (DePuy *et al.,* 2014). Serological surveys for *Coxiella burnetii* in western Kenya reported a prevalence of 30.9% in archived sera from patients and in 3% of patients with acute lower respiratory illness (Knobel *et al.,* 2013). Antibodies against *C. burnetii* were also detected in 28.3% of cattle, 32.0% of goats and 18.2% of sheep surveyed (Knobel *et al.,* 2013). Further, *C. burnetii* DNA was detected in 20% *Rhipicephalus Sanguineus,* 11.1% *Rh. appendiculatus,* 20% un-speciated *Rhipicephalus,* 20% of *Am. variegatum,* 50% of *Hyalomma*

leachi and 20% of *Rh*. (*Boophilus*) *decoloratus* ticks (Knobel *et al.,* 2013). Maina *et al.* (2016) also reported a sero-prevalence of 8.9% in febrile children in western Kenya.

2.3.3 Clinical characteristics

In humans, the disease occurs in either acute or chronic forms. Typical symptoms of acute Q-fever in humans include fever, chills, fatigue, headache, muscle aches, nausea, vomiting, diarrhea, chest pain, stomach pain, weight loss and non-productive cough (Parker *et al.,* 2006). Severe cases of Q fever are characterized by pneumonia and hepatitis (Mediannikov *et al.,* 2010). Infection during pregnancy may cause miscarriage, stillbirth, pre-term delivery, or low infant birth weight. Chronic Q fever on the other hand is characterized with inflammation of heart valves (endocarditis) (Parker *et al.,* 2006).

In domestic ruminants, Q fever is frequently asymptomatic. The clinical signs in animals include abortion, stillbirth, premature delivery, delivery of weak offspring, infertility, metritis, and mastitis (Njeru *et al.,* 2016).

2.3.4 Diagnosis and treatment

Q fever is commonly diagnosed by serology using either IFA, ELISA or CFT (Porter *et al.,* 2011). Another method for diagnosis is molecular methods by employing PCR amplification in EDTA blood, serum, tissue biopsies or bacterial cultures (Porter *et al.,* 2011).

Q fever can be prevented through consumption of pasteurized dairy products, proper handling and disposal of placenta, foetal membranes and aborted fetuses, and tick control in animals. Q fever is treated using tetracyclines (Bossi *et al.,* 2004).

2.4 Anaplasmosis

2.4.1 Aetiology

Human anaplasmosis (Human granulocytic anaplasmosis) is a zoonosis caused by the gram-negative bacteria *Anaplasma phagocytophilum.* On the other hand, anaplasmosis affecting domestic animals include *Anaplasma marginale, A. ovis* and *A. bovis*.

Anaplasma marginale is transmitted biologically by approximately twenty tick species, and mechanically by biting flies and blood-contaminated fomite such as such as needles, dehorning, castration or tattooing equipment (Kocan *et al.*, 2010; Aubry and Geale, 2011). Although clinical disease is most notable in cattle, other ruminants including water buffalo, bison, African antelopes, and some species of deer can be infected (Aubry and Geale, 2011). The severity of the disease in cattle increases with age (Aubry and Geale, 2011).

Anaplasma centrale, a less pathogenic organism than *A. marginale,* also infects the erythrocytes of cattle and occasionally causes a milder form of anaplasmosis (Kocan *et al.*, 2010). *A. centrale* is presently used as a live vaccine against *A. marginale* in South Africa, Israel, South America, and Australia (de la Fuente *et al.*, 2005; Kocan *et al.*, 2010; Aubry and Geale, 2011).

Anaplasma ovis, the agent of ovine anaplasmosis, may cause mild to severe disease in sheep, deer and goats but is not infectious for cattle (Aubry and Geale, 2011). *Anaplasma bovis* which is the agent of bovine anaplasmosis, may cause acute or chronic disease in cattle (Sreekumar *et al.*, 1996; Kawahara *et al.*, 2006). *Anaplasma platys*, the agent of infectious canine cyclic thrombocytopenia, may cause subclinical to mild disease in dogs (Hibler and Greene, 1986; Harrus *et al.*, 1997;). Both *Anaplasma ovis* and *Anaplasma platys* have zoonotic potential, being reported in several human clinical cases (Chochlakis *et al.*, 2010; Maggi *et al.*, 2013; Arraga-Alvarado *et al.*, 2014; Breitschwerdt *et al.*, 2014; Hosseini-Vasoukolaei *et al.*, 2014).

2.4.2 Anaplasmosis in Kenya

Reports of human infection with *A. phagocytophilum* is currently unavailable in Kenya. However, this pathogen has been identified in *Rh. maculatus* ticks sampled from Kenya's Shimba Hills National Reserve (Mwamuye *et al.,* 2017).

A longitudinal study of cohorts of calves in Busia County using reverse line blot hybridization assay found a high prevalence of *Anaplasma* spp. Omatjenne (42.7%) and *Anaplasma bovis* (39.9%) (Njiiri *et al.,* 2015). Study conducted by Omondi *et al.* (2017) detected DNA of *A. ovis*, *A. platys,* and *A. bovis* in ticks parasitizing livestock in Homa Bay and Baringo Counties. *Anaplasma platys* infection has also been detected in blood samples of free-roaming dogs and in *Rh. camicasi* ticks collected from negative hosts (Matei *et al.,* 2016). *A. marginale* DNA has been detected in blood sampled from cattle in Kajiado and Machakos counties (Adjou Moumouni *et al.,* 2015). *A. marginale* antibodies have also been reported in cattle from Machakos (Wesonga *et al.,* 2010), Mbeere (Gachohi *et al.,* 2010) and western Kenya (Okuthe *et al.,* 2006; Kiara *et al.,* 2014). Serological survey of *Anaplasma* antibodies in wildlife (eland, blue wildebeest, kongoni, impala, Thomson's gazelle, Grant's gazelle, giraffe and plains zebra) and domestic animal (cattle, sheep and goat) populations in wildlife/livestock interface areas of Kenya indicated a high seroprevalence of 72.7-100% in all species (Ngeranwa *et al.,* 2008). Serologic survey in captive and free-ranging ungulates from Central Kenya was able to detect *Anaplasma* spp. antibodies in one of the buffaloes from a ranch in Laikipia (Kimber *et al.,* 2002).

2.4.3 Clinical characteristics

Typical signs and symptoms of human anaplasmosis include fever, headache, muscle pain, malaise, chills, nausea, abdominal pain, cough, confusion, rash, mild anemia, thrombocytopenia

and leukopenia (CDC, 2017). Severe anaplasmosis is characterized with difficult breathing, hemorrhage, renal failure or neurological problems.

Clinical signs of bovine anaplasmosis include fever, weight loss, abortion, tachycardia, diarrhoea, lethargy, anaemia and icterus without haemoglobinemia and haemoglobinuria and sometimes death (Kocan *et al.*, 2003; Potgieter and Stoltsz, 2004).

2.4.4 Diagnosis and treatment

Human infection can be diagnosed through detection of DNA by PCR of whole blood and by demonstration of a four-fold change in IgG-specific antibody titer by IFA assay in paired serum samples (CDC, 2017). Bovine anaplasmosis can be diagnosed by finding *A. marginale* in Giemsa-stained blood smears from clinically infected animals, during the acute phase of the disease (Aubry and Geale, 2011). Additionally, the infection can be diagnosed by serology using CFT, capillary agglutination assay, card agglutination test (CAT), IFA, and ELISA. PCR can also be used to detect the presence of low-level infection in carrier cattle (Aubry and Geale, 2011).

Anaplasmosisis is treated with doxycycline (CDC, 2017).

2.5 Ehrlichiosis

2.5.1 Aetiology

Human ehrlichiosis is a zoonosis caused by three obligate intracellular, gram-negative bacteria belonging to the *Ehrlichiae* species (*Ehrlichia chaffeensis*, *E. ewingii* and *E. muris*-like (EML) agent) (CDC, 2016). *E. chaffeensis* and *E. ewingii* are transmitted by the lone star tick (*Amblyomma americanum*) while the EML agent is transmitted by the blacklegged tick (*Ixodes scapularis*) (CDC, 2016). Transstadial transmission of *E. chaffeensis* occurs within the tick with no transovarial transmission (AFPMB, 2012; Service, 2012).

The genus also includes pathogens of veterinary interest, namely *E. canis* and *E. ruminantium* (formerly *Cowdria ruminantium*) (Dumler *et al.,* 2001). *E. ruminantium* is the causative agent of heartwater or cowdriosis in some wild, and all domestic ruminants (Allsopp, 2010). It is transmitted by *Amblyomm*a ticks in Africa, the most important being *Am. variegatum* and *Am. hebraeum* (Allsopp, 2010). *E. canis* mainly affect canids, producing a monocytic ehrlichiosis or subclinical infections (Dumler *et al.,* 2001). Growing evidence indicates that *E. canis* and *E. ruminantium* may also be pathogenic in humans (Perez *et al.,* 1996; Allsopp *et al.,* 2005; Perez *et al.,* 2006; Allsopp, 2015).

2.5.2 Ehrlichiosis in Kenya

Although reports of human ehrlichiosis are currently unavailable in Kenya, *E. chaffeensis* have been identified in *Am. eburneum* ticks sampled from Kenya's Shimba Hills National Reserve (Mwamuye *et al.,* 2017).

Ehrlichia ruminatium, the aetiological agent of the tick-borne heart water disease of domestic and wild ruminants, has been detected in *Am. eburneum, Am. variegatum, Am. lepidum* and *Am. gemma* ticks in Kenya (Ngumi *et al.,* 1997; Mwamuye *et al.,* 2017; Omondi *et al.,* 2017). *Ehrlichia ruminantium* was also detected in *Am. falsomarmoreum* and *Am. nuttalli* ticks sampled from tortoises, and in *Am. sparsum* ticks sampled from both tortoises and cattle in Baringo County (Omondi *et al.,* 2017).

Ehrlichia canis, the aetiological agent of canine ehrlichiosis has been detected in *Rh. pravus, Rh. evertsi evertsi* and *Rh. pulchellus* tick pools sampled from livestock and domestic dogs in Baringo and Mbita, and *Am.* (*aponomma*) *latum* ticks parasitizing monitor lizards in Rusinga Island of Homa Bay County (Omondi *et al.,* 2017).

2.5.3 Clinical characteristics

Typical signs and symptoms of human ehrlichiosis include fever, headache, malaise, myalgia, nausea, vomiting, diarrhea, abdominal pain, confusion and conjunctival infection (Dumler and Bakken, 1995, 1998; Parola and Raoult, 2001). Severe ehrlichiosis may occur in immunocompromised individuals, which may be due to HIV/ AIDS, malignancy and corticosteroid therapy. Complications of *E. chaffeensis* ehrlichiosis may include leukopenia, thrombocytopenia and elevated liver enzymes (Parola and Raoult, 2001; CDC, 2016).

Heartwater disesase in livestock is characterized by fever, pulmonary oedema, marked respiratory distress, and nervous signs including ataxia, chewing movements, circling and aggression (Allsopp *et al.*, 2010, 2015).

2.5.4 Diagnosis and treatment

Ehrlichiosis is diagnosed by observing morulae in leukocytes after Wright's or Giemsa staining of blood smears, by molecular methods, serological testing (IFA), and by isolation of organisms in cell culture systems (Parola and Raoult, 2001). Ehrlichioses is treated using tetracyclines, doxycycline or rifampin (Parola and Raoult, 2001; CDC, 2017).

Ehrlichiosis is treated using doxycycline (CDC, 2017). *Ehrlichia chaffeensis* is resistant to erythromycin, cotrimoxazole, penicillin, and quinolones (Brouqui and Raoult, 1992)

2.6 Theileriosis

2.6.1 Aetiology

Theileriosis is caused by obligate intracellular protozoan parasites of the genus *Theileria*. It infects the lymphocytes of a wide range of wild and domestic ruminants and thus resulting to diseases with varying degrees of severity (Bishop *et al.*, 2004). Theileriosis in livestock results in enormous

economic losses through morbidity, mortality, productivity losses and the cost of control (de Castro, 1997; Jongejan and Uilenberg, 2004). The most severe forms of the disease in Africa are tropical theileriosis and East Coast fever (ECF). Tropical theileriosis, which originated from water buffaloes (*Bubalus bubalis*), is distributed in Northern Africa where it is caused by *T. annulata* and transmitted by *Hyalomma* ticks (Bishop *et al.,* 2004). On the other hand, ECF is distributed in Eastern, Central and Southern Africa, and it is caused by *Theileria parva* and transmitted mainly by *Rhipicephalus appendiculatus*. The African Cape buffalo (*Syncerus caffer*) is the natural host of *T. parva* and also serves as source of infection for cattle. Cattles that recover from acute or primary ECF infections following treatment or spontaneous recovery usually remain persistently infected and may act as reservoirs for tick vectors (Kariuki *et al.,* 1995; Kabi *et al.,* 2014). Benign forms of theileriosis are caused by *T. mutans*, *T. taurotragi*, *T. sergenti/ buffeli/orientalis* (referred to as *T. orientalis* complex) and *T. velifera* (Bishop *et al.,* 2004).

2.6.2 Theileriosis in Kenya

Theileriosis is ranked among the most common causes of economic losses in cattle industry in Kenya (Mulei and Rege, 1989; Kanyari and Kagira, 2000). East Cost fever has been associated with high mortality rates of 40–80% in unvaccinated calves in Maasai pastoralist herds (Homewood *et al.,* 2006; Di Giulio *et al.,* 2009). A longitudinal assessment of the serological response to *Theileria* species from birth to one year in a cohort of indigenous calves in western Kenya reported a high prevalence of *T. parva* (77%) and *T. mutans* (82%) (Kiara *et al.,* 2014). Similarly, a longitudinal study conducted by Njiiri *et al.* (2015) on cohorts of calves in Busia County found a high prevalence of *T. mutans* (71.6%), *T. velifera* (62.8%), *Theileria* sp. (sable) (32.7%), *T. parva* (12.9%), and *T. taurotragi* (8.5%). In other studies, the prevalence of *Theileria*

equi, the causative agent of equine piroplasmosis, was 100% in Grevy's zebras and 72% in donkeys in northern Kenya (Hawkins *et al.,* 2015).

2.6.3 Clinical characteristics

The clinical signs of theileriosis include fever, respiratory distress, coughing, nasal and lachrymal discharges, diarrhoea, weightloss, weakness ataxia, petechiation of the mucous and lymph node enlargement (Irvin and Mwamachi, 1983; Lawrence *et al.,* 2004). There may be blood and mucus in the faeces, and the pregnant cows may abort (Lawrence *et al.,* 2004). In the terminal stages of the disease, dyspnoea develops with an increased respiratory rate, a watery cough and a discharge of frothy fluid from the nostrils. The enlarged superficial lymph nodes begin to regress, the rectal temperature falls to subnormal levels, and the animal becomes recumbent and dies in a coma (Lawrence *et al.,* 2004).

2.6.4 Diagnosis and treatment

Theileriosis is diagnosed by observing the piroplasm in Giemsa stained blood smears, by serological assays (ELISA), by PCR amplification of blood sample, and by observation of clinical symptoms. Treatment of theileriosis is limited to supportive care and symptomatic treatment. Buparvaquone, is used in the treatment of mild infection (Kakati, 2015). The spread of the disease can be prevented by minimizing stress and movement of affected cattle. Cattle can be protected against ECF by immunization (International Livestock Research Institute [ILRI], 1999; Babo Martins *et al.,* 2010).

2.7 Babesiosis

2.7.1 Aetiology

Babesiosis is caused by obligate intra-erythrocytic protozoan parasites of the genus *Babesia* (). The parasites infect a wide range of domestic and wild animals, and occasionally humans. Human

babesiosis is caused by several species of *Babesia* including *B. microti*, *B. divergens*, *B. duncani* (CDC, 2015a). Bovine babesiosis is caused by *B. bovis*, *B. bigemina*, *B. divergens*, *B. ovata* and *B. major* (Bock *et al.,* 2004; Suarez and Noh, 2011). *Babesia ovis* and *B. motasi* infect sheep and goats (Bock *et al.*, 2004; Uilenberg, 2006;).

In Africa, bovine babesiosis (redwater fever) is caused by *Babesia bovis* and *B. bigemina* and the disease is transmitted by *Rhipicephalus* ticks (Bock *et al.,* 2004). Althoughcollars *B. bigemina* is more widespread, *B. bovis* infection is the most virulent because of its neurological symptoms (Uilenberg, 2006). Animals that recover from babesiosis often become persistent carriers of the infection (Suarez and Noh, 2011).

2.7.2 Babesiosis in Kenya

The main species of babesia that infect cattle in Kenya is *Babesia bigemina* and is transmitted by *R. decoloratus* (Wesonga *et al.,* 2010). Studies of babesiosis in Kenya showed that the sero-conversion rates to *B*abasia were 25-53% on cattle in Machakos County (*Wesonga et al.,* 2010). No human sero-surveys on babesiosis have been reported so far. A molecular survey of *Babesia* in Ngong farm reported a high prevalence of *B. bigemina* (71%) and *B. bovis* (12.3 %) (Adjou Moumouni *et al.*, 2015).

2.7.3 Clinical characteristics

Human babesisos can range from subclinical to severe. Clinical symptoms include fever, chills, sweats, headache, body aches, loss of appetite, nausea, or fatigue. Some patients have splenomegaly, hepatomegaly, or jaundice. Severe symptoms may occur in elderly patients, asplenic and immunosuppressed individuals. Complications of babesiosis may include low and unstable blood pressure, hemolysis, thrombocytopenia, malfunction of vital organs such as the kidneys, lungs, and liver or even death (CDC, 2015a).

The signs of bovine babesiosis include fever, tachypnea, depression, weakness and a reluctance to move. Some cattles may have anaemia and jaundice. Muscle wasting, tremors, and recumbency develop in advanced cases followed by coma (De Vos *et al.,* 2004).

2.7.4 Diagnosis and treatment

Human babesiosis is usually diagnosed by demonstration of the characteristic parasites on Giemsa-stained thin blood films and by PCR amplification of blood sample (Persing *et al.,* 1992; CDC, 2015a). It is usually treated with a combination of two prescription medications, either atovaquone plus azithromycin or clindamycin plus quinine for at least 7-10 days (CDC, 2015a).

Bovine babesiosis is diagnosed by observing the characteristic parasites in Giemsa stained blood smears, by serological assays (IFA), and by PCR amplification of blood sample (Oliveira-Sequeira *et al.,* 2005).

2.8 Tick control and tick-borne disease prevention

2.8.1 Removal of ticks in humans and animals

A thorough inspection and removal of ticks from humans, domestic animals and pets immediately after visiting tick-infested habitats or after handling tick-infested animals may help to reduce the attachment period of the tick and thus greatly reduce probability of transmission of TBPs (AFPMB, 2012; CDC, 2015b).

2.8.2 Use of insecticides

Application of repellents containing *N*,*N*-diethyl-*m*-toluamide (DEET), IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester) and picaridin (1-piperidinecaboxylic acid, 2-[2-hydroxyethyl], 1-methlypropyl ester) on the outer clothing (e.g., shirts and pants) and the exposed

skin surfaces helps to repel ticks and decrease the chances of tick bite as well as killing ticks on contact with the clothes (Carroll *et al.,* 2010; Jordan *et al.,* 2012; Pages *et al.,* 2014;).

Acaricidal products such as impregnated collars, acaricidal shampoos and sprays, pour-on and dip solutions can be applied directly to wild or domestic hosts to kill attached ticks and disrupt tick feeding. Residual insecticide sprays and dust can also be applied in structures occupied by domestic animals (Piesman and Eisen, 2008; CDC, 2015b).

However, use of chemical acaricides is often accompanied by serious drawbacks including the selection of acaricide-resistant ticks and contamination of the environment and animal products with residues (Piesman and Eisen, 2008; CDC, 2015b).

2.8.3 Use of protective clothing

Wearing protective clothing such as long-sleeved shirts, pants, socks and closed shoes when visiting tick-infested habitats makes it easier to see and remove attached ticks. Tucking shirt into pants and pants into socks (Fig. 2) also provides an additional protection for preventing crawling of ticks inside the clothes and subsequent attachment of ticks on the skin (Piesman and Eisen, 2008; AFPMB, 2012).

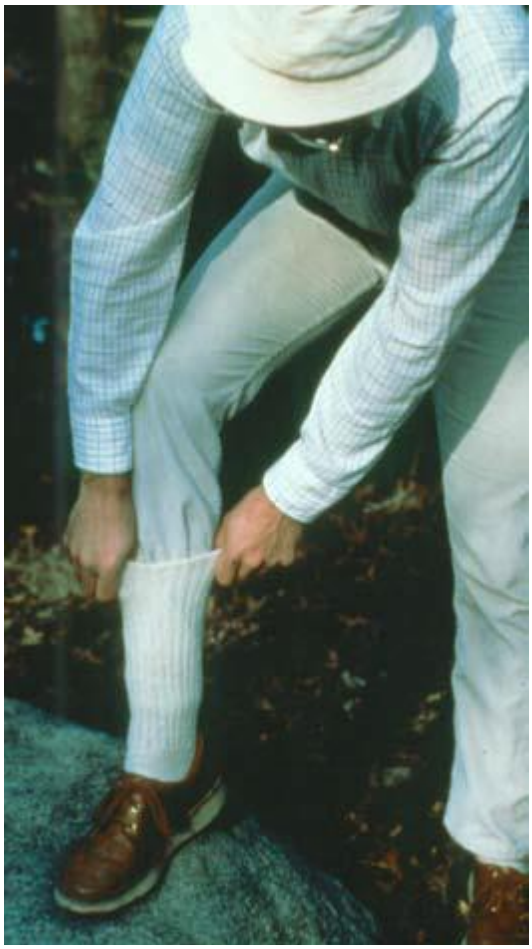

Figure 2. Protective clothing for preventing tick bites.

2.8.4 Habitat modifications

The presence and abundance of ticks in a given area is influenced by the availability of favorable habitats. Therefore, altering the landscape of these habitats e.g. by clearing vegetation, debris, and refuse may render the habitat less hospitable to ticks because of increased sunlight and low humidity (Stafford, 2007).

2.8.5 Use of biological methods

Although ticks have relatively few natural enemies, use of natural predators, parasites and bacterial pathogens of ticks; the mass release of sterile males; and the immunization of hosts against ticks

have been examined for biological control of ticks (Jongejan and Uilenberg, 1994; Samish Rehacek, 1999).

Parasitic nematodes such as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* have been found to induce mortality in engorged female *Rhipicephalus appendiculatus*, *Rh. evertsi*, *Amblyomma variegatum, Am. gemma* and *Boophilus decoloratus* under experimental conditions, but not un-fed females and immature stages (Kaaya *et al.*, 2000; Samish and Glazer, 2001).

Metarhizium anisopliae is a naturally occurring soil fungus that is considered nonpathogenic to mammals. The fungus infects host insects (and ticks) when conidia (spores) attach to the host cuticle, germinate, penetrate the cuticle and hyphae (filaments) grow. This penetration is helped by secretion of the chitinolytic enzyme CHIT 30 by *M. anisopliae* (Da silva *et al*., 2005). Other enzymes implicated in the degradation of the tick cuticle by *M. anisopliae* during the infection process include chitinases and proteases (de Moraes *et al*., 2003). *Metarhizium anisopliae* has been evaluated in laboratory and field conditions for control of adult *Boophilus microplus* and *Ixodes scapularis,* and various life cycle stages of *Boophilus annulatus*, *Hyalomma excavatum* and *Rhipicephalus sanguineus* with promising potential (Zhioua *et al.*, 1997; Kaaya and Hassan, 2000; Onofre *et al.*, 2001; Benjamin *et al.*, 2002; Gindin *et al.*, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

This study was conducted in Maasai Mara National Reserve (MMNR), one of Kenya's wildlife conservation sites located in Narok County in southwestern Kenya (Fig. 3). It is contiguous with Serengeti national park in Tanzania and stretches 1,510 km^2.

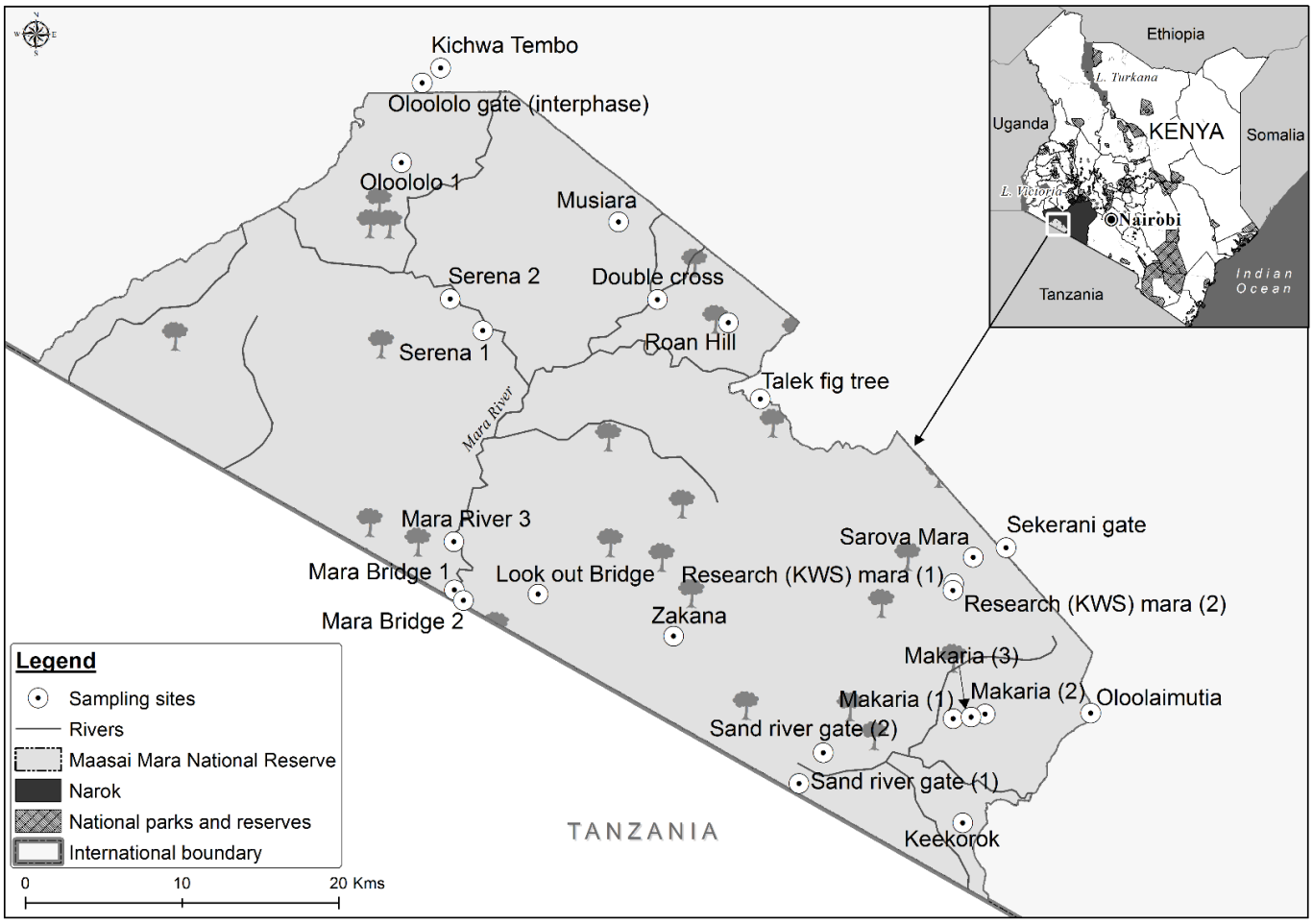

Figure 3. Map of Maasai Mara National Reserve indicating the sampling sites from where ticks were collected.

3.1.2 Flora and fauna

The MMNR is predominantly a savannah ecosystem, characterzed with short and tall grasslands interspersed by acacia woodlands, shrub lands, thicket and riverine forests (Lamprey & Reid, 2004). The expansive grasslands in the reserve forms the major attraction for nomadic pastoralists from the region, who routinely migrate into the reserve during dry seasons with their livestock to access pasture. The MMNR also supports an extremely high diversity of large mammals including all members of the 'Big Five' animal group (lion, leopard, African elephant, African buffalo, and black rhinoceros). The wildebeest are the dominant herbivores, and their numbers are estimated in millions. Around July, these animals migrate north from the Serengeti plains in search of fresh pasture, and return to the south around October. This great migration involves approximately 1.3 million wildebeest, 200,000 zebras and hundreds of thousands of Thomson's gazelles, topi, and elands (MMSDI policy paper, 2015). The migrants are followed by predators, most notably lions and hyenas. Other herbivores present include Thomson's and Grant's gazelles, impalas, topi, elands, duikers, Coke's hartebeests, zebras and Maasai giraffes.

3.1.3 Climate

The mean annual rainfall in the Maasai Mara ranges between 800-1200 mm. The Mara region also has a bimodal rainy season occurring from March to mid-June (long rainy season) and mid-October to December (short rainy season). The dry season falls in Jan – Feb (short dry season) and July-October (long dry season).

3.1.4 Human activities in Maasai Mara ecosystem

The Mara region is dominated mainly by the Maasai tribe who are mainly pastoralists whose livelihoods are dependent on livestock keeping, mainly goats, camels, cattle and sheep. Human activities within and around the MMNR include tourism, land cultivation and livestock grazing

(Lamprey and Reid, 2004; MMSDI policy paper, 2015). The MMNR is also surrounded by community-owned group ranches (4870 km^2) that act as wildlife dispersal areas, thus allowing a remarkable interaction between wildlife and livestock as they compete for the same resource (Lamprey and Reid, 2004; MMSDI policy paper, 2015). These form an important wildlife/livestock/human interface, and expose humans to risks of contracting tick-borne zoonotic infections. This human-animal interface is enhanced further by the conflicts between wildlife and humans due to the proximity of the reserve to human settlement (Lamprey and Reid, 2004; MMSDI policy paper, 2015).

3.2 Study design

This was a field-based cross-sectional survey. This survey was done within established 100 m × 100 m survey plots which were purposively selected to include vertebrate resting areas, burrows, host routes and watering holes.

3.3 Tick collection and identification

Questing ticks were collected from June – July of 2016 in 25 localities around MMNR (Fig. 3). This period coincided with the beginning of dry season and the great wildebeest migration from the Serengeti National Park. Questing ticks were sampled within these plots between 10am and 5pm using a combination of flagging and hand picks from vegetation (Ginsberg and Ewing, 1989). Flagging was carried out by slowly dragging a 1 m^2 white cotton cloth over the vegetation along 100m transects. All tick stages (adult, nymph and larva) attached to the cloth were collected using forceps after each 10 m drag. These was to ensure that ticks collected early in each transect did not fall off before collection. Questing ticks on leaf blades were also collected using a pair of forceps. Following collection, ticks were put in sterile falcon tubes labelled with the collection site and date, frozen on dry ice while in the field. They were then transported to the Martin Lüscher

Emerging Infectious Diseases Laboratory at the International Centre of Insect Physiology and Ecology in Nairobi for deep freezing (-80°C) until further analysis.

Adult ticks were identified to genus and/or species level under a microscope (Stemi 2000-C, Zeiss, Oberkochen, Germany) based on taxonomic keys developed by Walker *et al.* (2003). They were also photographed using a microscope-mounted Axio-cam ERc 5s digital camera (Zeiss). The ticks were then pooled by species, sampling site and sex into groups of 1-11 adults, 1-25 nymphs or 1-20 larvae. They were then stored at -80°C prior to DNA extraction.

3.4 DNA extraction from tick pools

Tick pools were homogenized for 90 s in 1.5 ml screw-cap tubes filled with a lysis matrix consisting of 750 mg of 2.0 mm, 150 mg of 0.1 mm zirconia/yttria stabilized zirconium oxide beads (Glen Mills, Clifton, NJ, USA), and 650 µl PBS (pH =7.4) using a Mini-Beadbeater-16 (BioSpec, Bartlesville, OK, USA) ((Crowder et al., 2010). The genomic DNA was extracted from these homogenates using the in-house protein precipitation method. Briefly, 300ul of sterile cell lysis buffer (10 mM Tris-HCL [pH 8.0], 5 mM EDTA, 0.5% sodium dodecylsulfate) was added and the homogenate was incubated at 65°C for 60 minutes. Following this incubation, 100 µl of protein precipitation solution (8 M ammonium acetate, 1mM EDTA) was added, and the tube vortexed for 30 seconds and centrifuged for 10 min at 14,000 x g. The supernatant was then precipitated by adding 300 µl isopropanol, vortexing for 30 seconds and centrifuging for 30 minutes at 14,000 x g. The pellet was rinsed with ice-cold 70% ethanol and air-dried for 12 hours before suspension in 50 µl of single-distilled sterile water. DNA extracts were quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher scientific) and stored at -20°C until further use.

3.5 PCR amplification of the internal transcribed spacer 2 (ITS-2) gene of ticks

PCR amplification of the internal transcribed spacer 2 (ITS2) gene was performed for purposes of molecular confirmation of the tick species as well as genetic diversity. For this analysis representative pools of mature and immature tick species were targeted. The PCRs were performed in 10 µl reaction volumes that included 5 µl PCR water, 2 µl of DNA template (50 ng), 2 µl of 5 x HOT FIREPol® Blend Master Mix (Solis Biodyne, Estonia) and 0.5 µl of 10 µM forward and reverse primers (Table 1). A The mixture was amplified in ProFlex PCR System Thermal Cycler (Applied Biosystems) under the following conditions: initial denaturation at 95◦C for 15 minutes followed by 35 cycles of 95◦C for 20 seconds, 65◦C for 30 seconds and 72◦C for 1 minute. Final extension was at 72◦C for 1 minute. A no-template control with double distilled water in place of DNA was included in each run. PCR products were electrophoresed on a 1% (w/v) agarose gel stained with ethidium bromide, and the size of the amplicons estimated against a 1 kb DNA ladder (Solis Biodyne). PCR products were purified using ExoSAP-IT Enzymatic PCR Product Clean-Up kit (USB Corporation, Cleveland, OH, USA) according to the manufacturer's instructions and sent to Macrogen (Netherlands) for capillary sequencing.

3.6 Detection of tick-borne pathogens

DNA samples were screened for *Anaplasma, Babesia, Coxiella, Ehrlichia, Rickettsia* and *Theileria* species using PCR followed by high-resolution melting (HRM) analyses in a HRM capable RotorGene Q thermocycler (Qiagen, Hannover, Germany). All assays were carried out in 10 µl reaction volume, containing 2 µl of 5x HOT FIREPol EvaGreen HRM mix (no ROX) (Solis BioDyne, Estonia), 0.5 µl of 10 pmol of each primer (Table 1), 5 µl PCR water and 2 µl of template DNA (50 ng). DNA samples of *Anaplasma phagocytophilum*, *Ehrlichia ruminantium*, and *R. africae* confirmed by sequencing in an earlier study (Mwamuye *et al.*, 2017) were used as positive

controls and no-template controls were included. The thermal cycling profile used included an initial denaturation step at 95°C for 15 minutes, followed by ten cycles at of 94°C for 20 seconds, 63.5 to 53.5°C (the temperature was decreased by 1°C between consecutive steps) for 25 seconds, and 72°C for 30 seconds. These cycles were followed by 25 cycles of 94°C for 25 seconds, 50.5°C for 20 seconds, 72°C for 30 seconds, and then a final extension step of 7 minutes at 72°C. For *C. burnetii* -specific primers, cycling conditions described by Fard and Khalili (2011) was used, with an initial denaturation of 95°C for 15 minutes. The PCR cycling was immediately followed by HRM analysis that involved gradual increase of temperature from 75°C to 90°C at 0.1°C increments after every 2 seconds. Melting profiles were assessed with Rotor-Gene Q Software 2.1.0. Melting profiles were visualized with pre- and post-melt fluorescence signals normalized to values of 100 and 0. Amplicons with unique HRM profiles were purified for sequencing.

3.7 Blood meal analysis using PCR-HRM

Blood meals were identified following established protocols (Omondi *et al.*, 2015; Ogola *et al.*, 2017). Genomic DNA from individual tick pools were analyzed by PCR amplification of vertebrate cytochrome b (*cyt b*) and 16S ribosomal (r) RNA genes. The reaction was made at a final volume of 10 µl containing 5 µl of PCR water, 0.5 µM of each primer, 2 µl of 5× Hot Firepol Evagreen HRM Mix (Solis BioDyne, Tartu, Estonia), and 2 µl of DNA template (50ng). Thermal cycling conditions included initiation at 95°C for 15 min; 35 cycles of 95 ◦C for 30 s, 56°C for 20 seconds, 72°C for 30 seconds followed by a final extension at 72°C for 7 minutes. This was directly followed by HRM analysis carried out by gradually increasing the temperature by 0.1 °C after every 2 seconds from 75 to 90 °C. DNA extracted from voucher wildlife specimens (obtained from the Kenya Wildlife Service) and livestock species previously confirmed by sequencing were included as positive controls. These included: Blue wildebeest (*Connochaetes taurinus*), giraffe

(*Giraffa camelopardalis*), impala (*Aepyceros melampus*), buffalo (*Syncerus caffer*), warthog (*Phacochoerus africanus*), Grant's gazelle (*Nanger granti*), hartebeest (*Alcelaphus buselaphus*), waterbuck (*Kobus ellipsiprymnus*), plain's zebra (*Equus quagga*), Kirk's dik-dik (*Madoqua kirkii*), Sable antelope (*Hippotagus niger*), lion (*Panthera leo*), cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*). Amplicons with unique *cyt b* and *16S* rRNA HRM melt curves were purified for sequencing as previously stated.

Table 1. PCR primer pairs used in this study.

	Target gene	**Primer name**	**Primer pair sequence (5'-3')**	**Amplicon size (bp)**	**Reference**
Tick species	ITS 2	ITS2F1 ITS2R1	CGAGACTTGGTGTGAATTGCA TCCCATACACCACATTTCCCG	920 - 1850	(Chitimia *et al.,* 2009)
***Rickettsia* spp.**	16S rRNA	Rick-F Rick-R	GAACGCTATCGGTATGCTTAACACA CATCACTCACTCGGTATTGCTGGA	364	(Nijhof *et al.,* 2007)
	Rickettsia gltA	CS-1069 CS-239	CAGGGTCTTCGTGCATTTCTT GCTCTTCTCATCCTATGGCTATTAT	834	(Labruna *et al.,* 2004)
	Rickettsia ompB	ompB 120–2788 ompB 120–3599	AAACAATAATCAAGGTACTGT TACTTCCGGTTACAGCAAAGT	856	(Roux and Raoult, 2000)
***Ehrlichia* spp.**	*Ehrlichia* 16S rRNA	*Ehrlichia* 16S F *Ehrlichia* 16S R	CGTAAAGGGCACGTAGGTGGACTA CACCTCAGTGTCAGTATCGAACCA	200	(Tokarz *et al.,* 2009)
	Ehrlichia 16S rRNA	*Ehrlichia*JV F *Ehrlichia*JV R	GCAACCCTCATCCTTAGTTACCA TGTTACGACTTCACCCTAGTCAC	300	(Mwamuye *et al.,* 2017)
***Coxiella* spp.**	IS1111	IS1111F IS1111R	GCTCCTCCACACGCTTCCAT GGTTCAACTGTGTGGAATTGATGAGT		(Tokarz *et al.,* 2009)
Coxiella burnetii	IS1111	Trans 1 Trans 2	TATGTATCCACCGTAGCCAGTC CCCAACAACACCTCCTTATTC	687	(Hoover *et al.,* 1992)
***Anaplasma* spp.**	16S rRNA	*Anaplasma* 16S F *Anaplasma* 16S R	GGGCATGTAGGCGGTTCGGT TCAGCGTCAGTACCGGACCA	112 – 200	(Tokarz *et al.,* 2009)
	16S rRNA	*Anaplasma*JV F *Anaplasma*JV R	CGGTGGAGCATGTGGTTTAATTC CGRCGTTGCAACCTATTGTAGTC	300	(Mwamuye *et al.,* 2017)
***Theileria* and *Babesia* spp.**	18S rRNA	RLB F RLB R	GAGGTAGTGACAAGAAATAACAATA TCTTCGATCCCCTAACTTTC	450	(Georges *et al.,* 2001)

Blood meal analysis	Vertebrate cyt b	Cytb For	CCCCTCAGAATGATATTTGTCCTCA	383	(Boakye *et al.,* 1999)
		Cytb Rev	CATCCAACATCTCAGCATGATGAAA		
	Vertebrate 16S rRNA	Vert 16S For	GAGAAGACCCTRTGGARCTT	200	(Omondi *et al.,* 2015)
		Vert 16S Rev	CGCTGTTATCCCTAGGGTA		

3.8 Genetic and phylogenetic analyses

The returned nucleotide sequences were edited and aligned using the MAFFT plugin in Geneious software version 11.1.4 (created by Biomatters) (Kearse *et al.*, 2012). Sequence identities were revealed by querying the GenBank nr database using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST/). The aligned DNA sequences were used to construct maximum likelihood phylogenetic trees using PHYML v. 3.0 (Guindon *et al.*, 2010). The phylogenies employed the Akaike information criterion for automatic model selection and tree topologies were estimated using nearest neighbor interchange (NNI) improvements over 1,000 bootstrap replicates. Phylogenetic trees were depicted using FigTree v1.4.2 (Drummond and Rambaut, 2007).

CHAPTER FOUR

RESULTS

4.1 Species distribution, abundance and diversity of ticks in MMNR

A total of 1,465 questing ticks comprising of 1,137 adults, 42 nymphs and 286 larvae were collected. Adult ticks were morphologically identified as *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi, Rhipicephalus pulchellus, Amblyomma gemma, Amblyomma variegatum,* and *Haemaphysalis leachi* (Plate 1). For some *Amblyomma* specimens, morphological identification at species level was not possible and were thus supported by ITS2 gene sequencing. The ITS2 sequence (submitted GenBank accession MH996473) of a commonly sampled *Amblyomma* sp. 'F' (*Am. hebraeum*-like), which shared most key features with *Am. hebraeum* according to Walker *et al.* (2003), but had distinct patterns (plate 1c) from reference photographs in the BOLD database, shared 98-100% nucleotide identity with *Amblyomma eburneum* (GenBank accession KP862667), *Amblyomma hebraeum* (GenBank accession KY457490), and *Amblyomma variegatum* (GenBank accession HQ856771, submitted GenBank accession MH996475). The ITS2 sequences of *Amblyomma* larvae and nymphs (submitted GenBank accessions MH996466-MH996472, MH996474) fell within the same cluster as the adult *Amblyomma* sp. 'F' (*Am. hebraeum*-like) sequence (Fig. 4). The ITS2 sequence of another morphologically distinct male *Amblyomma* sp. 'HippoUK' adult (Plate 1d, submitted GenBank accession MH996465) shared 99% nucleotide identity with a nostril tick (*Amblyomma* sp.) removed from a traveler returning from Lope National Park in Gabon (GenBank accession KJ619637). The ITS2 sequences of *Rh. appendiculatus* nymphs (submitted GenBank accession MH996476) shared 100% nucleotide identity reference *Rh. appendiculatus* sequences (GenBank accession KY457500). In general, *Rh. appendiculatus* was the most frequently collected species (n=1125, 76.84%), followed by

Amblyomma sp. 'F' (*Am. hebraeum*-like) (n=178, 12.16%), *Am. gemma* (n=145, 9.90%), *Rh. pulchellus* (n=6, 0.41%) (submitted ITS2 GenBank accession MH996477), *Rh. evertsi* (n=5, 0.34%), *Am. variegatum* (n=4, 0.27%), *H. leachi* (n=1, 0.07%), and *Amblyomma* sp. 'HippoUK' (n=1, 0.07%).

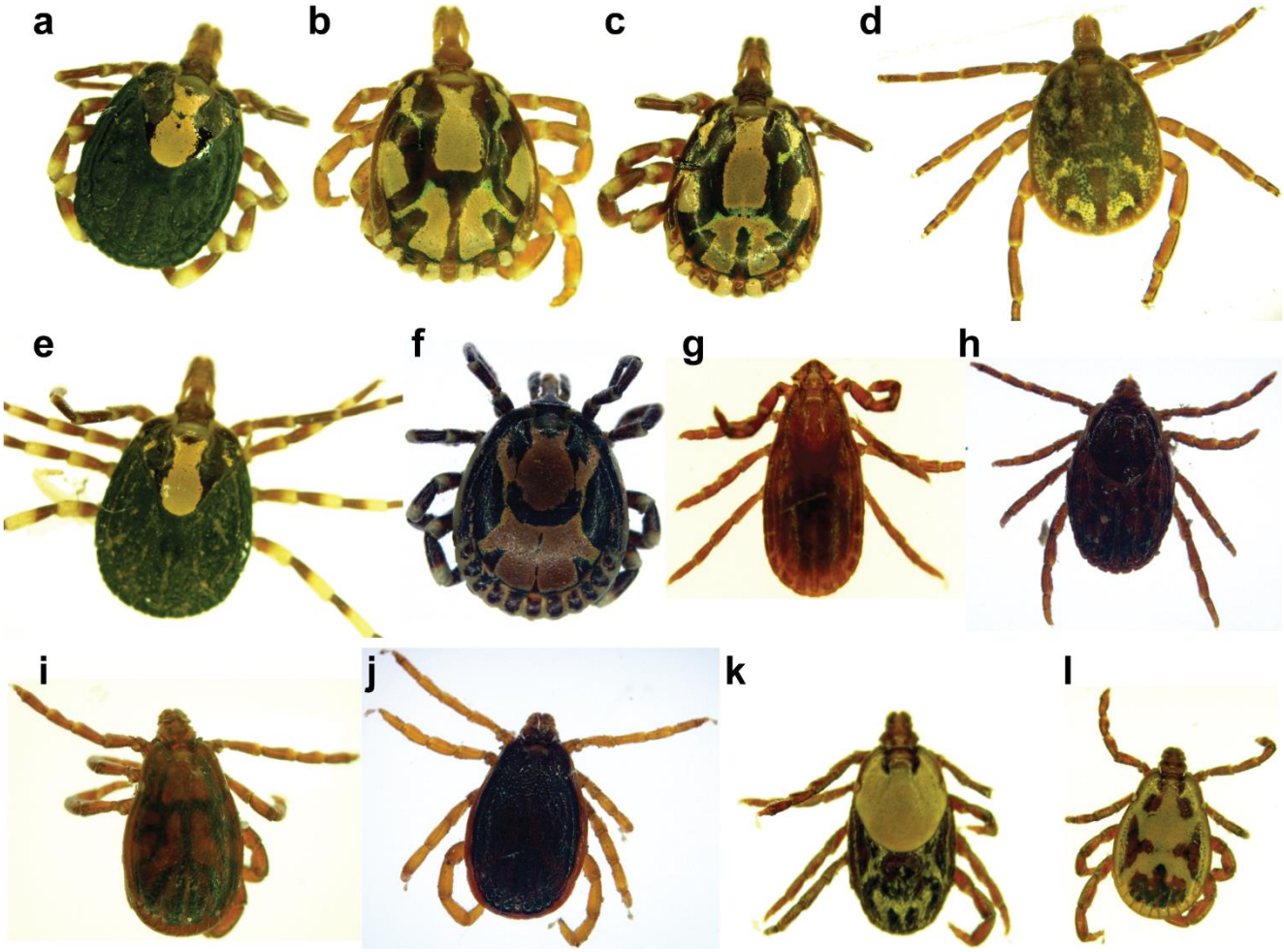

Plate 1. Images of selected ticks collected from the Maasai Mara National Reserve. The ticks are illustrated as follows: (a) *Am. gemma*, adult female; (b) *Am. gemma*, adult male; (c) *Amblyomma* sp. 'F' (*Am. hebraeum*-like), adult male; (d) *Amblyomma* sp. (nose tick-like), adult male; (e) *Am. variegatum*, adult female; (f) *Am. variegatum*, adult male; (g) *H. leachi*, adult male; (h) *Rh. appendiculatus*, adult female; (i) *Rh. appendiculatus*, adult male; (j) *Rh. evertsi*, adult male; (k) *Rh. pulchellus*, adult female; (l) *Rh. pulchellus*, adult male.

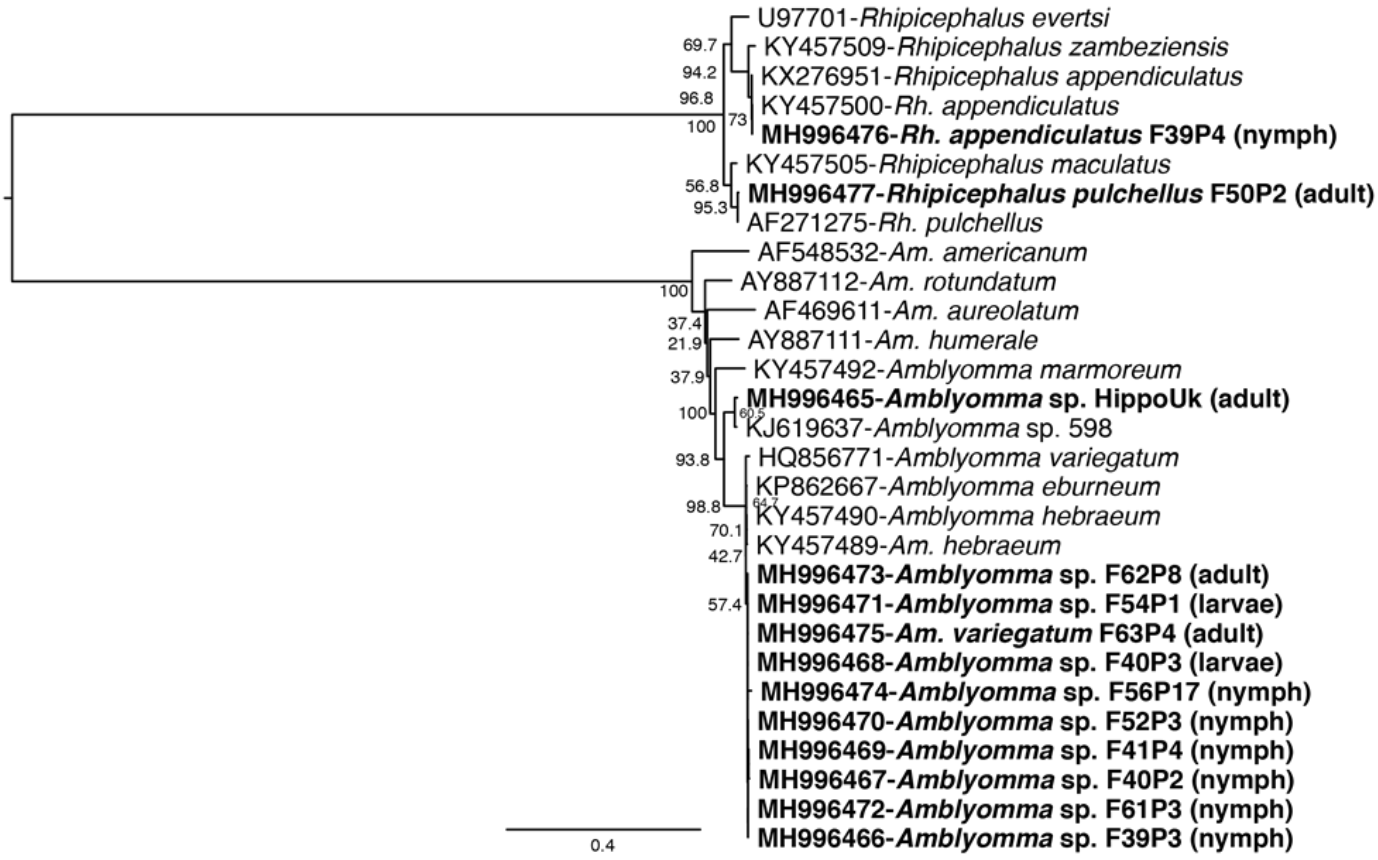

Figure 4. Maximum likelihood phylogenetic tree of tick ITS2 gene sequences (739-1139 nt). GenBank accession numbers, species identifications, and isolates, and tick life stages (in brackets) are indicated for each sequence. Sequences from this study are bolded. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site.

The total number of ticks collected in the different sites varied as shown in (Table 2). Highest abundance of tick population was collected from Makaria 1 (N=275) followed by Sarova Mara (N=263). No tick species was collected at Roan hill and Oloolaimutia. The number of *Rhipicephalus appendiculatus* was high at the Makaria 1 (N=275), an area frequented by elephants and hyenas. A majority of the *Rhipicephalus evertsi* ticks were collected at Kichwa tembo, an area frequented by elephants. *Rhipicephalus pulchellus* were mainly collected at Mara River 3, an area highly dominated with hippopotamus, elephant and impala. *Amblyomma gemma* and *Amblyomma* sp. 'F' (*Am. hebraeum*-like) were mainly obtained from the Sarova Mara, an area frequented by

buffalo and hippopotamus. The numbers of *Amblyomma variegatum* were high at Mara Bridge 1, an area frequented by hippopotamus, impala, giraffe and vervet monkey. *Haemaphysalis leachi* was collected from Zakaria, an area frequented by Thomson gazelle and topi. The adult *Amblyomma* sp was found at Serena 2, an area frequented by hippopotamus, elephant and grants gazelle.

Table 2: Distribution and abundance of questing ticks across the ecological sites in MMNR.

Location	No. of tick species collected								Total number of ticks
	Rh. appendiculatus	***Rh. evertsi***	***Rh. pulchellus***	***Am. gemma***	***Amblyomma* sp. F**	***Am. variegatum***	***Amblyomma* sp. 'HippoUK'**	***H. leachi***	
Sekenani gate	7	0	0	0	1	0	0	0	8
Sarova Mara	46	0	0	112	105	0	0	0	263
KWS Mara research station	21	0	0	0	4	0	0	0	25
KWS Mara research station 2	132	0	1	5	3	0	0	0	141
Sand river gate	14	0	0	0	0	0	0	0	14
Sand river gate 2	10	0	0	0	0	0	0	0	10
Makaria 1	275	0	0	0	0	0	0	0	275
Makaria 2	8	0	0	0	0	0	0	0	8
Makaria 3	71	0	0	0	0	0	0	0	71
Oloolaimutia	0	0	0	0	0	0	0	0	0
Keekorok	86	0	0	0	1	0	0	0	87
Talek/fig tree	50	0	1	0	0	0	0	0	51
Double cross	50	1	0	0	0	0	0	0	51
Musiara	2	1	0	0	1	0	0	0	4
Zakaria	29	0	0	0	0	0	0	1	30
Mara Bridge 1	50	0	1	1	55	3	0	0	110
Mara Bridge 2	22	0	0	0	0	0	0	0	22
Look out	174	0	1	9	1	0	0	0	185
Roan Hill	0	0	0	0	0	0	0	0	0
Mara river 3	14	0	2	0	0	0	0	0	16
Serena 1	2	0	0	0	0	0	0	0	2
Serena 2	26	0	0	0	1	0	1	0	28
Oloololo 1	13	0	0	1	2	0	0	0	16
Oloololo 2	13	0	0	17	4	0	0	0	34
Kichwa Tembo	10	3	0	0	0	1	0	0	14
Total	1225	5	6	145	178	4	1	1	**1465**

4.2 Tick-borne pathogens identified

A total of 231 pools (172 *Rh. appendiculatus*, 4 *Rh. evertsi*, 5 *Rh. pulchellus*, 25 *Am. gemma*, 3 *Am. variegatum*, 20 *Amblyomma* sp. F, one *H. leachi* and one *Amblyomma* sp. 'HippoUK') were screened for *Anaplasma, Babesia, Coxiella, Ehrlichia, Rickettsia* and *Theileria* pathogen diversities. Positive pools were identified for *Anaplasma, Rickettsia* and *Theileria* species, while none of the pools were positive for *Babesia*., *Ehrlichia*. and *C. burnetii* infection. Further, this study detected near universal infection rates on *Coxiella* sp. endosymbionts.

Anaplasma sp. were detected in adult *Rh. evertsi* (1/4 pools, 25%) and *Rh. appendiculatus* (2/172 pools, 1.2%). HRM analysis of the 16S rRNA amplicon revealed unique profiles of the different *Anaplasma* species (Fig. 5a). BLAST searches of the 16S rRNA sequences amplified in the *Rh. evertsi* tick pool (F63P5) (submitted GenBank accession MK026419) and one *Rh. appendiculatus* pool (F56P24) (submitted GenBank accession MK026421) showed 100% nucleotide identity to *Anaplasma ovis* described in sheep from China (GenBank accession KX579073). The other *Rh. appendiculatus* pool (F51P3) had sequences (submitted GenBank accession MK026420) with 100% nucleotide identity to *Anaplasma bovis* (GenBank accessions U03775).

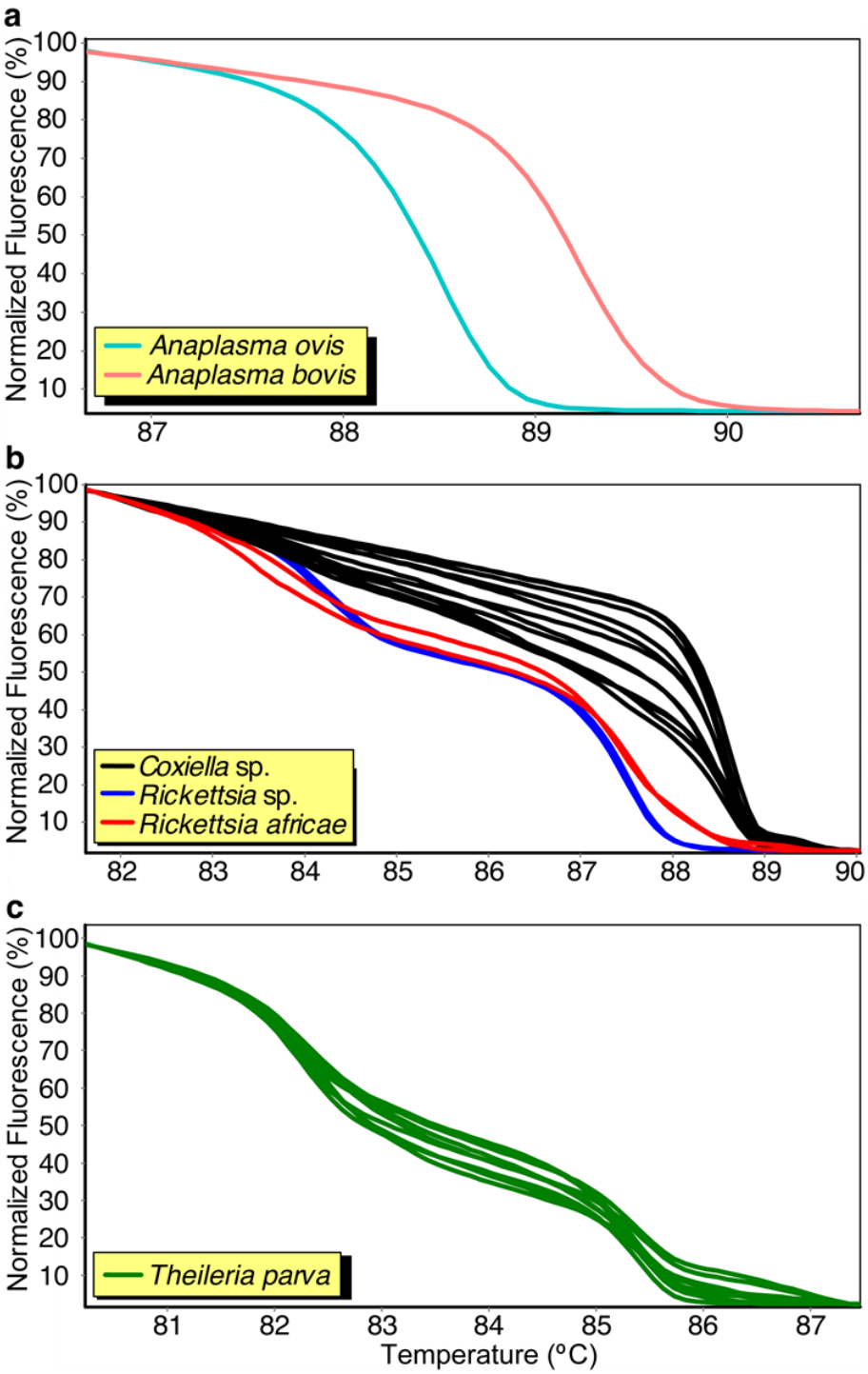

Figure 5. Normalized HRM profiles of representative (a) *Anaplasma* 16S rRNA, (b) *Coxiella* and *Rickettsia* 16S rRNA amplicons, and (c) *Theileria* 18S rRNA PCR amplicons.

Rickettsia spp. were detected in adult *Am. gemma* pools (4/25 pools, 16%), *Am. variegatum* (1/3 pools, 33.3%), and *Rh. evertsi* (1/4 pools, 25%). HRM analysis of the 16S rRNA amplicon revealed unique profiles of the different *Rickettsia* species (Fig. 5b). BLAST searches of the sequences from one *Am. gemma* adult pool (F63P6) (submitted GenBank accession MK026418) and one *Am. variegatum* adult pool (F63P4) (submitted GenBank accession MK026417) showed 100% identity with *Rickettsia africae* described in Ugandan ticks (GenBank accession AB934393). Furthermore, 16S rRNA sequences from two *Am. gemma* ticks (F42P3 and F54P12) (submitted GenBank accessions MK026415, MK026416) and one *Rh. evertsi* tick (F52P2) (submitted GenBank accession MK026414) was 99-100% identical to *Rickettsia* sp. Suedafrika1547 described in a mouse from South Africa (GenBank accession KX944390). Rickettsial *ompB*, genes for *Am. gemma* tick (F42P1 and F42P3) were further re-amplified but did not resolve the identity of this rickettsia species. The Rickettsial *ompB* gene sequence (submitted GenBank accessions MH997912- MH997913) in these two ticks had 97% identity to *Rickettsia* sp. (GenBank accession KT835128), *R. aeschlimannii* (GenBank accession MF002557), *R. rhipicephali* (GenBank accession CP013133), *R. massiliae* (GenBank accession KT835123) and *R. raoultii* (GenBank accession FN651773). The maximum likelihood phylogenetic analysis of *Rickettsia* based on the partial sequence of the 16S rRNA gene indicated that *Rickettsia* sp. (F42P3, F54P12 and F52P2) are in the same clade with other SFG rickettsiae, including *R. massiliae* (GQ144453), *R. rhipicephali* (NR_074484), *R amblyommii* (U11012) and *R. raoultii* (GenBank accession KJ410261) (Fig.5).

Surprisingly, the 16S rRNA *Rickettsia* primers amplified diverse *Coxiella* sp. endosymbionts in all three tick genera collected in this study with the melting profiles being clearly differentiated from all *Rickettsia* sp. 16S rRNA melt profiles (Fig. 5b). No *Coxiella* sp. endosymbionts yielded

positive PCR result when the samples were screened with *C. burnetii*-specific primers. *Coxiella* sp. endosymbionts were detected in *Rh. appendiculatus* (138/172 pools, 80%), *Rh. pulchellus* (5/5 pools, 100%), *Rh. evertsi* (1/4 pools, 25%), *Am. gemma* (18/25 pools, 72%), *Amblyomma* sp. F (*Am. hebraeum*-like) (9/20 pools, 45%), *Am. variegatum* (2/3 pools, 67%), and the single *H. leachi* pool. The endosymbionts were only absent in the single nostril tick-like *Amblyomma* sp. (HippoUK). The nucleotide similarity of *Coxiella* sp. endosymbionts detected in this study were submitted to the GenBank and assigned the accession numbers MK026402-MK026413. Two *Coxiella* sp. endosymbiont-positive ticks were co-infected with *A. bovis* and *A. centrale.* There were no instances in which both *Coxiella* sp endosymbionts and *Rickettsia* sp. were detected within the same tick pools. The sequences of *Coxiella* sp. endosymbionts are in distinct clades from pathogenic *C. burnetii* sequences in the phylogenetic tree (Fig. 6).

Figure 6. Maximum likelihood phylogenetic tree of *Rickettsia* and *Coxiella* 16S rRNA gene sequences (288-370 nt). GenBank accession numbers, species identifications, and isolates, with tick or vertebrate host species and country of origin in brackets, are indicated for each sequence. Sequences from this study are bolded. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site. The gaps indicated in the branches to the *Coxiella* and *Rickettsia* clades represent 0.16 substitutions per site.

Out of 172 pools of adult *Rh. appendiculatus* tested, 27 (15.7%) were positive for *Theileria* sp. HRM analysis of the 18S rRNA amplicon revealed unique profile of the *Theileria* species (Fig. 5c). BLAST searches of the 18S rRNA sequences (submitted GenBank accessions MH929321-MH929324) returned a 100% nucleotide identity to *Theileria parva* detected in Zambia (GenBank accession MG952926), Kenya (GenBank accession MG952923), Uganda (GenBank accession MG952922) and Tanzania (GenBank accession MG952924) (Fig. 7).

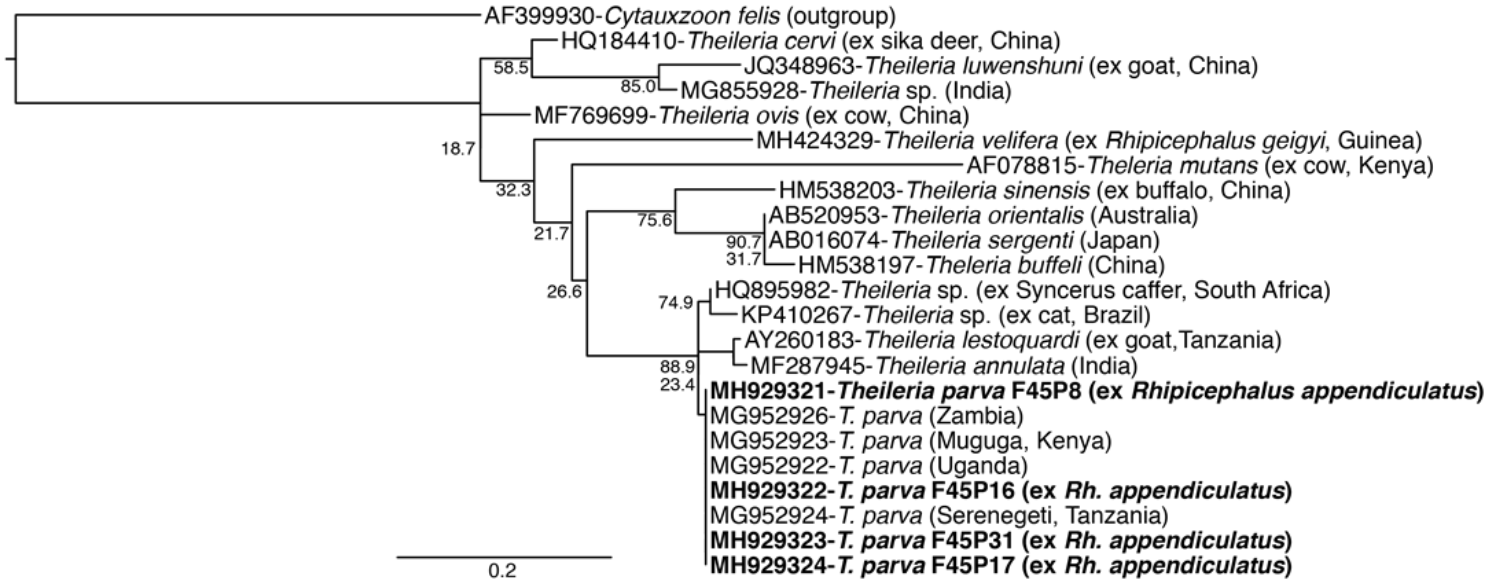

Figure 7. Maximum likelihood phylogenetic tree of *Theileria* 18S rRNA gene sequences (403-503 nt). GenBank accession numbers, species identifications, and isolates, with tock or vertebrate host species and country of origin in brackets, are indicated for each sequence. Sequences from this study are bolded. *Cytauxzoon felis* was used as outgroup. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site.

4.3 Blood-meal sources of the collected questing ticks

Of the 231 tick pools screened in this study, nine were single-tick pools with identifiable blood-meals from humans, blue wildebeest, African buffalo, goat, sheep and cattle (Table 3, Fig. 8). An *Am. gemma* blood-meal was from sheep (*Ovis sp.*) (2/25 pools) whereas *Am. variegatum* had fed on cattle (*B. taurus*) (1/3 pools) (Fig. 8). *Rickettsia africae* infections were observed in one of the *Am. gemma* ticks (F63P6) with a blood-meal from sheep and the *Am. variegatum* (F63P4) with a blood-meal from cattle.

Tick species	**Identified blood meal host**	**Submitted GenBank accession**
Rhipicephalus evertsi	Goat (*Capra hircus*)	MH997915
Rhipicephalus appendiculatus	Human (*Homo sapiens*) Blue wildebeest (*Connochaetes taurinus*) African buffalo (*Syncerus caffer*)	MH997917 MH997918-MH997919 MH997914
Amblyomma hebraeum-like *Amblyomma* sp.	Human (*Homo sapiens*)	MH997916

Table 3. Vertebrate blood-meal host of questing ticks in Maasai Mara National Reserve.

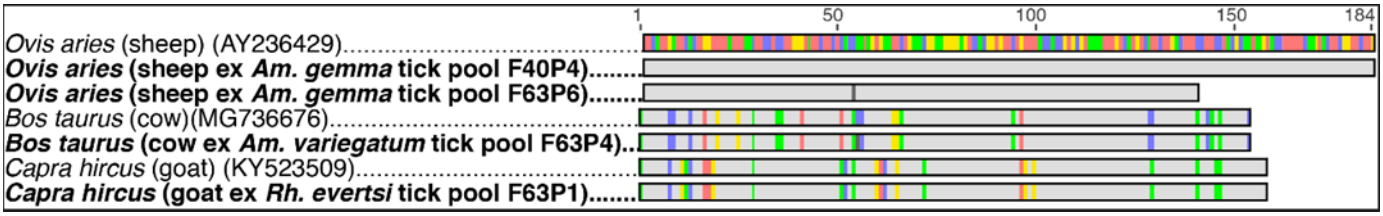

Figure 8. Alignment of short blood-meal vertebrate 16S rRNA sequences amplified in the study (bold) with closest sequences available in GenBank. Grey = consensus with top reference sequence; Red = Adenine; Blue = Cytosine; Green = Thymine; Yellow = Guanine.

CHAPTER FIVE

DISCUSSION

This study provides molecular evidence showing that questing ticks in MMNR are infected with diverse zoonotic pathogens such as *R. africae* and uncharacterized *Rickettsia* sp. and veterinary pathogens such as *A. bovis, A. ovis,* and *T. parva.* However, none of the ticks were positive for *Babesia, Ehrlichia,* and *C. burnetii* infection. Further, *Coxiella* sp. endosymbionts were detected in the majority of tick pools across all the tick genera collected in this study. Molecular detection of remnant blood-meal in ticks identified humans, blue wildebeest, African buffalo, goat, sheep and cattle as blood-meal sources of the ticks, thus potential reservoirs of TBPs identified in this study. The findings presented here are important to public and veterinary health in mitigating possible disease outbreaks in these foci.

5.1 Tick abundance

Analysis of the diversity of questing ticks in MMNR revealed *Rh. appendiculatus* as the most abundant species. Other tick species were *Am. gemma, Rh. pulchellus, Am. variegatum, Rh. evertsi,* and *H. leachi* identified morphologically to species level and confirmation by DNA sequencing. These findings on diversity of ticks in Maasai Mara agree with those of previous studies (Macaluso *et al.,* 2003; Ndeereh *et al.,* 2017) who found similar diversities of ticks within this region. According to Walker *et al.* (2003), all these tick species are found in Savanna environments such as the MMNR where they infest domestic animals such as cattle, sheep and goats, and wildlife, albeit with some differences in their preferential hosts.

An interesting finding of this study was the identification of two novel *Amblyomma* sp. comprising of 179 individual ticks which could not be morphologically classified to species level based on the taxonomic keys. These individuals were identified by sequencing of mitochondrial ITS-2 gene, as

Am. hebraeum-like sp. F (178 individual ticks) and *Amblyomma* sp. HippoUK (one individual). The *Am. hebraeum-like* sp. F ticks were morphologically distinct, but closely related to *Am. hebraeum*, *Am. variegatum*, and *Am. eburneum* based on ITS2 gene sequence analysis, whereas the single *Amblyomma* sp. HippoUK specimen was most closely related to a tick harboring an SFG group *Rickettsia* sp. that was removed from a traveler from Lope National Park in Gabon (Lopez-Velez *et al.,* 2015). These findings demonstrate the possibility of existence of species complex or hybrids making up novel sub-species diversity in the Maasai Mara, Kenya. Indeed, interspecific mating and hybridization in ixodid ticks have been previously demonstrated through genetic studies, with more evidence of natural hybrid populations being shown for *Ixodes* sp. (Kovalev *et al.,* 2015, Kovalev *et al.,* 2016; Patterson *et al.,* 2017). Although earlier studies in eastern Africa also showed that *Am. hebraeum* and *Am. variegatum* females respond to interspecific pheromones and mate readily, lower hatch rates were seen in eggs produced by interspecific mating, and natural hybrids were rare in nature (Rechav *et al.,* 1982; Yunker *et al.,* 1990). Given the high frequency of *Am. hebraeum*-like sp. F ticks in this study, further surveys of tick diversity in the Maasai Mara ecosystem (and other wildlife habitats in SSA) should be conducted to characterize existence of hybrids and complexes of ticks species more conclusively, and their possible roles in nature e.g. in transmission of pathogens.

5.2 Tick-borne pathogens and endosymbionts identified

The genus *Anaplasma* contains TBPs that are pathogenic to both humans and animals. The detection of *A. bovis* in *Rh. appendiculatus* ticks from MMNR is of veterinary importance, as they have been linked to cases of bovine anaplasmosis in Africa (Walker *et al.,* 2003). In Kenya, a high prevalence of *A. bovis* (39.9%) has also been reported in a cohort of calves in Busia County (Njiiri *et al.,* 2015). This bacterium has also been detected in other *Rhipicephalus* species, namely *Rh.*

evertsi, *Rh. pulchellus,* and *Rh. praetextatus* ticks sampled from Baringo County (Omondi *et al.,* 2017). The detection of *A. bovis* DNA in *Rh. appendiculatus* ticks in this study suggests that *Rh. appendiculatus* may serve as a vector of bovine anaplasmosis in the Maasai Mara region.

Anaplasma ovis is the agent of ovine anaplasmosis and may cause mild to severe disease in sheep and goats (Aubry and Geale, 2011). The present study detected *A. ovis* infection in one *Rh. appendiculatus* and one *Rh. evertsi* tick pool. The presence of *A. ovis* in other *Rhipicephalus* species has also been reported in Kenya, including *Rh. pulchellus* and *Rh. appendiculatus* tick (Omondi *et al.,* 2017). These findings highlight the probable role of rhipicephaline ticks in the transmission of *A. ovis* in Maasai Mara ecosystem. Recent reports have indicated that *A. ovis* may also be pathogenic in humans (Chochlakis *et al.,* 2010; Hosseini-Vasoukolaei *et al.,* 2014). Thus, herders, veterinarians and physicians in Maasai Mara region should be aware of the risk of *A. ovis* infection in humans.

Spotted fever group (SFG) rickettsia is an emerging infectious disease that causes acute febrile illness in humans. The present study detected *R. africae,* the causative agent of African tick bite fever (ATBF), in *Am. gemma* and *Am. variegatum* ticks. These findings corroborate those of Macaluso *et al.* (2003), who previously identified SFG *Rickettsia* in this ecosystem. The presence of *R. africae* in *Am. gemma* and *Am. variegatum* ticks also agree with other reports elsewhere in eastern Africa, including Sudan (Morita *et al.,* 2004; Nakao *et al.,* 2015), Uganda (Lorusso *et al.,* 2013; Nakao *et al.,* 2013), Ethiopia (Kumsa *et al.,* 2015), and Kenya (Mutai *et al.,* 2013; Maina *et al.,* 2014; Omondi *et al.,* 2017).

Rickettsia africae infection is maintained through transovarial and transstadial transmission in *Am. variegatum* (Socolovschi *et al.,* 2009), and as a result all life stages of the ticks may transmit infection to humans. Because *Amblyomma* species readily feed on people, the increased contact

with humans due to the adverse changes in land use in MMNR poses a high risk for the emergence of ATBF in people living in the vicinity and those visiting the reserve. ATBF presents a high risk to tourists and has increasingly been reported in travelers returning from SSA including MMNR (Raoult *et al.,* 2001; Jensenius *et al.,* 2003a,b; Roch *et al.,* 2008, Althaus *et al.,* 2010). High levels of seroprevalence to SFG rickettsiae has been reported among the indigenious African populations, with antibodies against SFG *Rickettsia* having been detected among 10 - 23% of febrile patients in Kenya (Thiga *et al.,* 2015; Maina *et al.,* 2016; Omballa *et al.,* 2016). Therefore, there is need for the medical practitioners in Narok County to consider diagnosis of SFG rickettsiosis as a differential diagnosis to febrile cases.

Further, *Rickettsia* spp. of unknown pathogenicity were detected in *Rh. evertsi* and *Am. gemma* ticks. Although they cannot be associated with human illness, the potential pathogenicity of these rickettsiae cannot be overlooked as many tick-isolated *Rickettsia* initially characterized as nonpathogenic are now recognized as pathogens (Raoult and Roux, 1997). Cases in point are *Rickettsia slovaca, R. parkeri, R. massiliae* and *R. africae* which were first isolated from ticks and considered nonpathogenic rickettsiae until they were found to be pathogenic in humans (Raoult and Roux, 1997; Parola *et al.,* 2005). Additionally, ompB is a cell surface antigen present in rickettsial pathogens and has been demonstrated to be important in invasion and adherence to host cells (Chan *et al.,* 2009; Riley *et al.,*2012). Thus, the amplification of ompB gene in two of the unidentified *Rickettsia* sp. in *Am. gemma* ticks implicates it as a potential human pathogen.

This study found *Theileria parva* only in *Rh. appendiculatus* in MMNR. *T. parva* is the causative agent for East Coast Fever (ECF), the most economically important parasitic disease in cattle in eastern and southern Africa. Maasai pastoralists in Kenya (Bedelian *et al.,* 2007) have ranked it as the top disease associated with mortality rates of 40–80% in unvaccinated calves (Homewood *et*

al., 2006; Di Giulio *et al.,* 2009; Gachohi *et al.,* 2012). The distribution of *T. parva* has been associated with *Rh. appendiculatus* ticks and the African buffalo (*Syncerus caffer*), which has been cited as the natural reservoir host (Norval *et al.,* 1992; Walker *et al.,* 2003). In Kenya, *T. parva* has a wide distribution and has been detected in cohorts of calves in Busia County (Njiiri *et al.,* 2015) and in apparently healthy cattle in Ngong and Machakos (Adjou Moumouni *et al.,* 2015). The detection of *T. parva* in this study highlights the persistent risk of ECF to livestock and the need to intensify vaccination against *T. parva* in this area, in light of the presence of the natural reservoirs (buffalo) and infected ticks.

Coxiella burnetii is a zoonotic pathogen causing Q fever in both humans and domestic ruminants (Njeru *et al.,* 2016). Their apparent absence in questing ticks from MMNR suggests that the risk of acquiring Q fever from questing ticks could be negligible. These finding contrast those of Ndeereh *et al.* (2017), who reported *C. burnetii* in a range of rhipicephaline ticks in this area. Duron (2015) cited that C. *burnetii* is often misidentified in ticks and underscored the need to distinguish *C. burnetii* from diverse *Coxiella*-like bacteria which are commonly found in ticks. Nevertheless, outbreaks of Q fever in Kenya have been reported in international travelers (Potasman *et al.,* 2000) and local communities in Baringo County (Kenya Zoonosis Diseases Unit, 2014). Seroprevalence surveillance reports in Kenya have also indicated that *C. burnetii* was prevalent in humans and in domestic ruminants (Knobel *et al.,* 2013; DePuy *et al.,* 2014; Wardrop *et al.,* 2016; Maina *et al.,* 2016). To date, no patient cases of Q fever from Kenya have been unquestionably linked to tick bites.

Inadvertently, diverse *Coxiella* sp. endosymbionts were detected in 75% of adult ticks across the three genera sampled in the MMNR, forming distinct clades in the phylogenetic tree. In addition, one larvae and one nymph of *Rh. appendiculatus* also had *Coxiella* sp. endosymbionts, supporting

the notion that endosymbionts can be found in all life stages of ticks (Cowdry, 1925; Noda *et al.*, 1997). No *Coxiella* sp. endosymbionts yielded positive PCR result when the samples were screened with *C. burnetii*-specific primers. Therefore, *Coxiella* infection in ticks must be interpreted with caution.

The role of *Coxiella* sp. endosymbionts is not clear, but it has been suggested that they contribute to the fitness of the tick, possibly by providing nutrients and defense against pathogens (Duron, 2015). Treatment of *Amblyomma americanum* ticks with antibiotics that affect *Coxiella* bacteria reduced reproductive fitness based on observations of delayed oviposition, decreased numbers of hatched ticks, decreased numbers of larvae per tick, and the decreased size of ticks ticks treated with antibiotics (Zhong *et al.*, 2007). *Coxiella* symbionts are also potential vitamin sources for *Am. americanum* ticks (Smith *et al.*, 2015). The presence of a *Coxiella*-like bacterium in *Am. americanum* tick has also been suggested to impact the colonization and transmission of other pathogens in the same infected ticks (Lively *et al.*, 2005). Interestingly, two positive pools of *Rh. appendiculatus* were co-infected with *A. ovis* and *A. bovis.* However, none of the *Rickettsia* spp. infected tick pools had *Coxiella* endosymbionts. Therefore, co-infection of *Coxiella* sp. endosymbionts and their potential role in limiting specific TBPs in ticks warrants further investigation.

5.3 Blood‑meal sources of questing ticks collected

Ticks are obligate hematophagous ectoparasites that feed once in each active stage and ingest massive amounts of blood, which provides the energy necessary for molting or oviposition. The identification of blood-meal hosts in questing ticks from across the MMNR has given insight into the feeding ecology of ticks, and TBDs risk in this area. Blood meals were found only in nine of the questing ticks identified as *Rh. evertsi, Rh. appendiculatus, Am. gemma, Am. variegatum,* and

Am. hebraeum-like sp. F. Goat, sheep, blue wildebeest, African buffalo, cattle and humans were the common source of blood-meal for these tick species. This suggests that the host-seeking pattern of ticks in the MMNR is dynamic.

The findings of this study indicate that *Rh. appendiculatus* ticks had fed on humans, blue wildebeest and African buffalo. These findings are consistent with previous reports that *Rh. appendiculatus* infests a wide range of Bovidae, but found most commonly on cattle and buffalo (De Vos, 1981; Norval *et al.,* 1992; Walker *et al.,* 2003). The identification of human blood meal in *Rh. appendiculatus*, suggests more diverse host-seeking pattern in MMNR. Nonetheless, the African buffalo (*Syncerus caffer*) is an important reservoir of *Theileria parva, Anaplasma ovis* and *Theileria parva* (Bengis *et al.,* 2002; Olwoch *et al.,* 2008; Fyumagwa *et al.*, 2013), and may thus serve as important conduits of livestock diseases in Maasai Mara region. *Rh. appendiculatus* does not prefer to feed on humans. Therefore, detection of human blood-meal in these ticks suggest diverse host-seeking pattern of *Rh. appendiculatus* ticks in MMNR. Although the preferred domestic hosts of adult *Rh. evertsi* are horses, donkeys, cattle and sheep (Walker *et al.*, 2003), this study was able to detect blood-meal from goat.

The present investigation also found that *Am. variegatum* from MMNR had fed on cattle. This finding is consistent with a previous report that all stages of this tick infest cattle, sheep and goats (Walker *et al.,* 2003). *Amblyomma* sp. 'F' (*Am. hebraeum*-like) from MMNR had fed on humans. Although no pathogens were identified in this novel tick species, it nevertheless highlights the species as a human parasite, and is consistent with previous reports which show that *Amblyomma* species commonly attack humans, usually on the legs with attachment often behind the knee, in the groin, or in the axilla (Estrada-Peña and Jongejan, 1999; Jensenius *et al.,* 2003a).

Domestic livestock particularly cattle serve as important hosts for both *Am. hebraeum* and *Am. variegatum,* and there is some evidence that they may play a role in the maintenance of human pathogenic rickettsiae (Kelly *et al.,* 1991; Walker *et al.,* 2003). In this study, *Rickettsia africae* infection was detected in one *Am. gemma* with blood-meal from sheep and in one *Am. variegatum* that had fed on cattle. This suggests that sheep and cattle may be important in the epidemiology of *R. africae* in MMNR. *R. africae* antibodies have previously been detected in goats, sheep and cattle in rural Western Kenya (Maina *et al.,* 2014), suggesting that these domestic ruminants seem to be frequently exposed to these bacteria. Therefore, rickettsial infections should be taken into consideration when diagnosing acute febrile illnesses in pastoralists of Maasai Mara area, who keep large herds of cattle, sheep and goats.

The study was unable to detect the blood meal sources in the remaining questing ticks. These may be because questing ticks may have had their last blood-meal in their previous life stage, up to 1 year before collection (Randolph *et al.,* 2002). Consequently, the host DNA may have been degraded by digestive and hemolytic processes in the tick midgut (Kirstein and Gray, 1996; Sojka *et al.*,2013). Nevertheless, published studies on laboratory fed and captive-reared ticks have shown that host DNA can be detected up to 10 months after the blood-meal (Kirstein and Gray, 1996; Pichon *et al.,* 2003).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

i. The findings of this study suggest that *Am. hebraeum* may constitute a species complex.

ii. This study demonstrates the presence and possible circulation of TBPs that are etiological agents of anaplasmosis and theileriosis that may be a serious constraint to livestock production in MMNR.

iii. The findings also raise the awareness profile of the existence of *R. africae* in the region that could be useful to clinicians in diagnosing febrile cases in humans.

iv. Furthermore, this study identified *A. ovis* that have been suspected to cause human diseases. Thus, dog and livestock owners, veterinarians and physicians in MMNR region should be aware of the risk of infection with these pathogens.

v. This study reports that ticks from MMNR harbor diverse *Coxiella* sp. endosymbionts.

vi. This study shows that goat, sheep, blue wildebeest, African buffalo, cattle and humans are the important blood meal sources to the tick vectors in MMNR.

vii. The findings also suggest that sheep and cattle are most likely responsible for amplifying *R. africae* in MMNR.

6.2 Recommendations

i. This study recommends a roll-out of the ECF vaccine in the Maasai pastoral ecosystem so as to reduce the risk of bovine theileriosis.

ii. Spotted fever group rickettsiosis should be included in the differential diagnosis of febrile illnesses in Maasai Mara ecosystem and that clinicians should also be educated on some of the clinical features that distinguish malaria and other febrile illnesses from rickettioses.

iii. The epidemiological significance of the detected *Rickettsia* spp. and their tick hosts is still unclear and should be further assessed.

iv. Despite the absence of *Coxiella* and *Ehrlichia* spp. in screened samples, further studies should be conducted regarding their occurrence and prevalence in Maasai Mara ecosystem.

v. Further studies should be conducted to understand the role of *Coxiella* sp. endosymbionts in tick physiology and vector competence.

vi. Replicate this study in other human-wildlife-livestock interfaces in Kenya which can facilitate transmission of infectious pathogens across different species.

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