MOLECULAR EPIDEMIOLOGY OF TICKS AND TICK-BORNE PATHOGENS IN DROMEDARY CAMELS (*Camelus dromedarius*) AND CO-HERDED SHEEP IN MARSABIT COUNTY, NORTHERN KENYA

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Molecular epidemiology of ticks and tick-borne pathogens in dromedary camels (*Camelus dromedarius*) and co-herded sheep in Marsabit County, northern Menya

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Molecular Biology and Bioinformatics of the Jomo Kenyatta University of Agriculture and Technology

2022

DECLARATION

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

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This thesis is dedicated to my parents, brothers and sisters.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACDS	Acute camel death syndrome
DNA	Deoxyribonucleic acid
ECF	East Coast fever
EDTA	Ethylene diaminetetraacetic acid
ELISA	Enzyme-linked immuno-sorbent assay
FAO	Food and Agriculture Organization
gDNA	Genomic DNA
GDP	Gross domestic product
HRM	High-resolution melting
NCBI	National Centre for Biotechnology Information
PCR	Polymerase chain reaction
CDC	Centre for Disease Control
SDS	Sodium dodecyl sulphate
TBD	Tick-borne diseases
TBP	Tick-borne pathogens
SSA	Sub-Saharan African

ABSTRACT

The camel industry is a vital part of the Kenyan livestock economy and greatly contributes to the developing local dairy industry. However, camel health and production is constrained by ticks and tick-borne pathogens (TBPs). Currently, information on molecular epidemiology of ticks and TBPs of Kenyan camels is limited. This study aimed to investigate the diversity of ticks and TBPs in dromedary camels in Marsabit county, northern Kenya, a semi-arid and arid area, using morphological and molecular tools. Two hundred and ninety-six blood samples and 2610 ticks from camels and 77 blood samples and 88 ticks from co-herded sheep in 12 different sites in Marsabit County were screened for *Ehrlichia, Anaplasma, Theileria/Babesia, Coxiella*, and *Rickettsia* spp. using genus-specific polymerase chain reaction - high resolution melting (PCR-HRM) assays and confirmed through gene-sequencing. Morphological examination, confirmed through gene sequencing, revealed that the collected ticks (n = 2610) belonged to eight different species: *Hyalomma dromedarii* (n = 919; 35.2%), *Hyalomma rufipes* (n = 810; 31.0%), *Amblyomma lepidum* (n = 330; 12.6%), *Hyalomma impeltatum* (n = 221; 8.5%), *Amblyomma gemma* (n = 129; 4.9%), *Rhipicephalus pulchellus* (n = 104; 4.0%), *Rhipicephalus camicasi* (n = 72; 2.8%), and *Hyalomma truncatum* (n = 25; 1.0%). "*Candidatus* Anaplasma camelii" (233/296; 78.8%), "*Candidatus* Ehrlichia regneryi" (43/296; 14.5%), and *Coxiella burnetii* (10/296; 3.4%) were detected in camel blood samples using molecular tools. A wide range of pathogens, including *Ehrlichia ruminantium* (1.3%), *Ehrlichia chaffeensis* (0.1%), "*Candidatus* Ehrlichia regneryi" (2.8%), *Ehrlichia* sp. (0.9%), *Coxiella burnetii* (1.5%), *Rickettsia africae* (1.7%), *Rickettsia aeschlimannii* (3.9%), "*Candidatus* Anaplasma camelii" (3.9%) and *Coxiella* endosymbionts (0.7%) were detected in tick samples using molecular tools. *Erlichia ruminantium* (1.3%), *E. chaffeensis* (2.60%), *Anaplasma ovis* (88.3%), and *Theileria ovis* (88.3%) were detected in blood from co-herded sheep. *Erlichia rimuninatium* (3.5%), *R. africae* (7.1%), *A. ovis* (14.1%) and *T. ovis* (1.2%) were detected in ticks collected from sheep. These findings provide information on the broad range of ticks and TBPs affecting camel production in Marsabit county, northern Kenya. The results also demonstrate that camels and their associated ticks may be reservoirs of *C. burnetii*, a zoonotic pathogen causing Q fever. The finding of zoonotic pathogens, such as *E. ruminantium*, *C. burnetii*, *R. africae, R. aeschlimannii* in ticks collected from camels underscores the need for increased surveillance and monitoring of TBPs in Kenyan camels by the county and national government veterinary authorities.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tick-borne diseases (TBD) constitute major constraints in livestock production in Kenya and the Horn of Africa (Wesonga *et al*., 2010). The losses caused by ticks and tick-borne pathogens (TBPs) in livestock production in Kenya is very high, approximately KShs. 32 billion per year (Nyariki & Amwata, 2019). TBPs may have a serious economic impact since they lead to decreased animal productivity, increased cost of control, and lowered working efficiency (Bornstein & Younan, 2013; Kagunyu & Wanjohi, 2014). Tick-borne diseases such as heartwater, babesiosis, anaplasmosis, and theileriosis are economically important and widely distributed animal diseases (Abdel-Shafy *et al*., 2012; Eskezia, 2016).

Camel production is a major source of livelihood in northern Kenya and the Horn of Africa (Somalia, Somaliland, Djibouti) (Anderson *et al*., 2016; Dirie & Abdurahman, 2003). About 6% of the African camel population is found in the arid and semi-arid parts of Kenya (Dirie & Abdurahman, 2003; Watson *et al*., 2016), while approximately 35% of the camels worldwide are found in the Horn of Africa region (FAOSTAT, 2020). The latest census (2020) reported a total of 4.7 million camels in Kenya (FAOSTAT, 2020). Camel populations in the region have continued to increase despite numerous challenges brought about by climate changes (Bornstein & Younan, 2013; Watson *et al*., 2016). Some communities who did not previously keep camels have started rearing them to supplement cattle production during dry seasons (Kagunyu & Wanjohi, 2014). Due to their biological and physiological adaptations, camels are able to survive well in arid and semi-arid ecologies. Camels provide a reliable source of meat and milk even during dry seasons when production from other livestock such as goat, sheep, and cattle is insufficient (Watson *et al*., 2016). They also play role as beasts of burden for water and luggage transport. However, little is known about the vector-borne camel diseases in northern Kenya mostly because livestock belong to the marginalized poor nomadic pastoralists whose economic welfare is mainly neglected.

Ticks are important vectors of a broad range of human and animal bacterial, protozoal, and viral pathogens (Walker *et al*., 2003; Jongejan & Uilenberg, 2004; Lorusso *et al*., 2016). Additionally, ticks affect their host's well-being through irritation, skin damage, blood loss, and anorexia, which lead to reduced growth of the vertebrate host (Jongejan & Uilenberg, 2004; Bornstein & Younan, 2013; Eskezia, 2016). Due to their impact on productivity in ruminant hosts, TBPs are considered to be one of the major causes of economic burden among livestock keepers around the world.

Previously, it was assumed that camels were resistant to TBPs. However, studies conducted in Kenya, Sudan, Egypt, Saudi Arabia, and Iran have reported the possibility of anaplasmosis, babesiosis, ehrlichiosis, and theileriosis as being responsible for high morbidity and mortality rates in camels (Barghash & Amani, 2016; Moshaverinia & Moghaddas, 2015; Alanazi *et al*., 2019, 2020; Kidambasi *et al*., 2020). Camels and co-grazing domestic animals such as cattle, sheep, and goats support a large population of tick vectors that are associated with the TBPs affecting both wild and domestic ruminants. Currently, information on molecular epidemiology of the intracellular TBPs in camels in the northern Kenya and the Horn of Africa is limited. Mortality of camels due to TBPs has been reported in these areas (Younan *et al*., 2021). Investigation on TBPs affecting camels in northern Kenya is of interest to improve camel farming especially in areas where people solely rely on camels for meat and milk production. Therefore, the aim of this study was to investigate the diversity of ticks collected from camels in northern Kenya. The study was also aimed at investigating presence of *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Coxiella*, *Theileria* and *Babesia* in blood and ticks collected from Kenyan camels and co-herded sheep. These tick-borne pathogens are known to cause important economic losses in livestock production in Kenya and around the world (Hughes and Anderson, 2020). The key study findings form a critical basis for designing future interventions to prevent and control TBDs affecting camels. This will improve camel health and the livelihoods of the nomadic pastoralist communities relying on them as their major source of livelihood.

1.2 Statement of the problem

Dromedary camels are important food-producing animals and vital for the economy, food security and well-being of pastoralist communities in the arid and semi-arid lands (ASAL) of Kenya. They are kept for milk (consumption and income), meat, hides, transport, and social capital (Bornstein & Younan, 2013; Kagunyu & Wanjohi, 2014). Despite the importance and resilience of camels under harsh climatic conditions (Watson *et al*., 2016), their production is constrained by pests, vector-borne diseases and parasites. Biting flies (such as Tabanus, Stomoxys and Haematopota), the camel fly or camel ked *Hippobosca camelina* (Kidambasi *et al*., 2020; Merid *et al*., 2020; Bargul *et al*., 2021) and ticks are the common haematophagous ectoparasites of camels, especially in Marsabit County. Over the recent years, outbreaks of Acute Camel Death Syndrome (ACDS) and other emerging disease of unknown cause affecting camel herds have been reported in parts of northern Kenya, with TBPs suspected to be the main cause (Younan *et al*., 2021). Up to 100% of affected adult camels can die without treatment. Sampling sheep living in close proximity to camel herds will help determine if the suspected TBPs (such as Ehrlichia sp.) also infect small ruminants, and thus what role they play in its epidemiology (Younan *et al*., 2021). While most studies have focussed on camel flies as the mechanical vectors for trypanosomes in camels (Kidambasi *et al*., 2020; Merid *et al*., 2020), very little is known about tick-borne pathogens circulating among camels in northern Kenya. The present study was aimed at characterizing TBPs in camels and co-grazing sheep and the ticks infesting them. This information will inform development of strategies and protocols for camel disease diagnosis and control for future use by veterinarians and livestock managers. This in turn will enhance camel productivity and pastoralist food security.

1.3 Justification

The economic losses amounting to billions of dollars (Nyariki and Amwata, 2019) and the impedance of livelihood improvement solely by TBPs afflicting livestock around the world necessitate intensive studies for better control (Bornstein & Younan, 2013). Recurrent droughts in northern Kenya also make the pastoralists farmers to move to

neighbouring counties and countries in search of pasture and water. The unavoidable contact of camels from different counties and countries at grazing fields and watering points enables transmission and spread of new TBDs in the region (Anderson *et al*., 2016). Over recent years, several northern Kenya counties have reported outbreak of diseases of unknown epidemiology, with TBPs suspected to be the main cause (Younan *et al*., 2021). However, there is paucity of epidemiological information on ticks and key pathogens circulating in camel population in northern Kenya. With climate changes and increased spread of TBPs, the threat of TBD outbreaks is likely to increase and this will have further impact on camel productivity. Understanding the transmission dynamics of TBPs infecting camels is critical in designing control strategies, improving animal health, increasing food security and enhancing the quality of life of the nomadic pastoralist communities in Kenya, which will contribute to the realisation of vision 2030 and sustainable development goal II (SDG).

1.4 Research hypotheses

There are diverse species of ticks infesting camels and co-herded sheep in Marsabit County which are vectors of TBPs infectious to humans and livestock.

1.5 Research questions

1. Which tick species are infesting camels and co-herded sheep in northern Kenya?
2. Which TBPs are circulating in blood of camels and co-grazing sheep, together with their associated ticks in northern Kenya?
3. To what extent are these TBPs genetically similar or diverse?

1.6 Objectives

1.6.1 General objective

To investigate the molecular epidemiology of TBPs detected in ticks and blood samples collected from camels and co-herded sheep in northern Kenya.

1.6.2 Specific objectives

1. To characterize the species composition of ticks infesting camels and sheep in Marsabit County, northern Kenya using morphological and molecular tools
2. To detect and characterize TBPs in ticks and blood of Kenyan dromedary camels and co-herded sheep using molecular tools
3. To estimate the genetic diversity of TBPs identified from blood and ticks of camels and co-herded sheep in Marsabit County, northern Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of livestock

Livestock production is an important economic and social activity in Kenya. Livestock contributes about 10% of Kenya's Gross Domestic Product (GDP) (Behnke & Muthami, 2011; Nyariki & Amwata, 2019). Livestock-based activities support millions of people living in arid and semi-arid parts of Kenya and the Horn of Africa. They supply farmers with milk, meat, hide and skin, traction power, and fertilizer (Behnke & Muthami, 2011). Camel production is the main source of livelihood in northern Kenya and much of the semi-arid and arid areas of the Horn of Africa (Somalia, Somaliland, and Djibouti) (Watson *et al*., 2016). The latest census indicates that there are over four million camels in Kenya (FAOSTAT, 2020). The camel breeds kept in Kenya include Somali, Rendille/Gabbra, Turkana, and the Pakistani (Mburu *et al*., 2003). In northern Kenya, camels are kept for meat, milk, meat, hides and skin, transport, and social, cultural, and environmental functions. Camels are physiologically and biologically adapted to survive well in harsh arid and semi-arid regions characterised by prolonged droughts. However, camel production is constrained by pests and diseases. Over the recent years, outbreaks of severe disease in camels, characterised by sudden onset, lethargy, extreme respiratory distress, occasional nervous signs and mortality of close to 100% in adult animals in the absence of antibiotic treatment, have been recorded in northern Kenya since 2015. The disease, designated ACDS, appears to be novel; clinical and postmortem findings indicate that heartwater (*Ehrlichia ruminantium* infection) is a likely candidate for the causative agent of ACDS or at least to play a role in the syndrome (Younan *et al*., 2021). Sampling sheep living in close proximity to camel herds will help determine if the candidate *Ehrlichia* sp. (Younan *et al*., 2021) and other tick-borne pathogens also infects small ruminants, and thus what role they play in its epidemiology. Sheep are the most suitable sentinels of heartwater as they exhibit the highest level and longest duration of specific antibodies following exposure (Bell-Sakyi *et al*., 2004).

2.2 Challenges faced by camel farmers in northern Kenya

Camel farmers in northern Kenya mainly practice nomadic pastoralism. This leads to frequent competition for scarce land, pastures, and water resources amongst other communities living in northern Kenya (Kagunyu & Wanjohi, 2014). Scarcity of water and pastures drive pastoralist communities to places with water and pastures and in the process, they meet with other pastoralist communities. As the livestock interact, they create a hotspot for pathogen transmission across livestock from different geographical zones (Watson *et al*., 2016; VanderWaal *et al*., 2017). The water and pasture scarcity also create wildlife-domestic animal interfaces when the pastoralist communities drive their livestock into wildlife reserves (Oundo *et al*., 2020), and this also promotes spread of pathogens across species. Tick-borne diseases, such as anaplasmosis, present major animal productivity challenges to farmers in northern Kenya (Kidambasi *et al*., 2020). In Kenya and the Horn of Africa, camel movement is not regulated. This means that camels found in northern Kenya may have moved from Ethiopia, Somalia or even Sudan. As the camels move from one locality to the other, different TBPs can be introduced in the area. Therefore, regular epidemiological studies are required to understand the dynamics, spread and role of TBPs in camel disease in the region.

2.3 Biology of tick vectors

2.3.1 Taxonomy

Ticks belong to phylum Arthropoda, subphylum Chelicerata, class Arachnida, subclass Acari, and suborder Ixodida (Walker *et al*., 2003). While ticks consist of more than 900 species, only about 10% are known to transmit pathogens (Walker *et al*., 2003; Dantas-Torres *et al*., 2012). There are three families of ticks that can be recognized in the world today (**Figure 2.1**); (i) hard ticks (Ixodidae) consisting over 700 tick species, (ii) soft ticks (Argasidae), consisting over 200 tick species, and (iii) Nuttalliellidae, which consist of only one known tick species (Hoogstraal, 1985; Walker *et al*., 2003). The family Ixododie has 13 genera and is the largest and medically important tick family. The family Argasidae has 4 genera: *Carios*, *Argas*, *Ornithodoros*, and *Otobius*. It is only genus *Ornithodoros*

that is known to be of medical importance (Hoogstraal, 1985; Jongejan & Uilenberg, 2004). Nuttalliellidae family consists of only one species, which is not known to be of any medical importance (Hoogstraal, 1985).

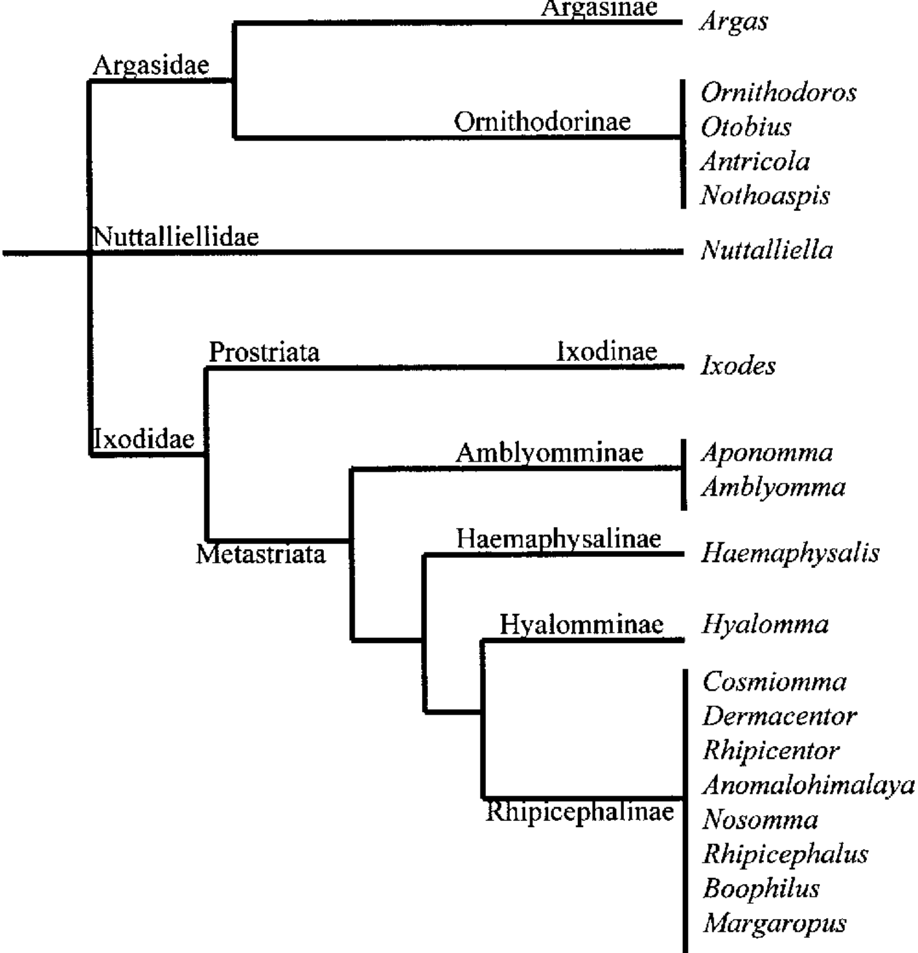

Figure 2.1: **A summary of known tick families** (Parola & Raoult, 2001)**.**

2.3.2 Morphology of hard ticks

The body of a hard tick is composed of the capitulum (mouthpart) and the idiosoma (body with protruding mouthparts) (Walker *et al.*, 2003; Service, 2012). Hard ticks have eight legs in the adult and nymphal stages (six legs in the larval stage) with six segments and a sensory organ at the tip of the first pair of legs. The legs have claws used for clinging and moving around on the host (Walker *et al.*, 2003). Tick eyes are located near the front

corners of the scutum. The mouthparts consist of hypostome, chelicerae and palps, the three structures that allow the tick to penetrate the skin of the host for a blood meal. Palps serve as sensory organs while chelicerae serve as cutting organs (**Figure 2.2**) (Jongejan & Uilenberg, 2004; Dantas-Torres *et al*., 2012).

Hard ticks are characterized by dorsoventrally flattened body when not engorged, dorsal shield (scutum) that covers nearly the entire dorsum of adult males and the anterior third or so of the adult females or immature stages of both genders, and the mouthparts directed anteriorly (prognathous) that are visible from above. The important features for genus-level identification of hard ticks include shape and location of the anal groove, presence/absence of eyes, presence/ absence of pale maculae (enamel) on the dorsal shield, presence/absence of festoons, and the length of the mouthparts in relation to the basis capituli (Walker *et al*., 2003; Service, 2012; Estrada-Peña *et al.*, 2013).

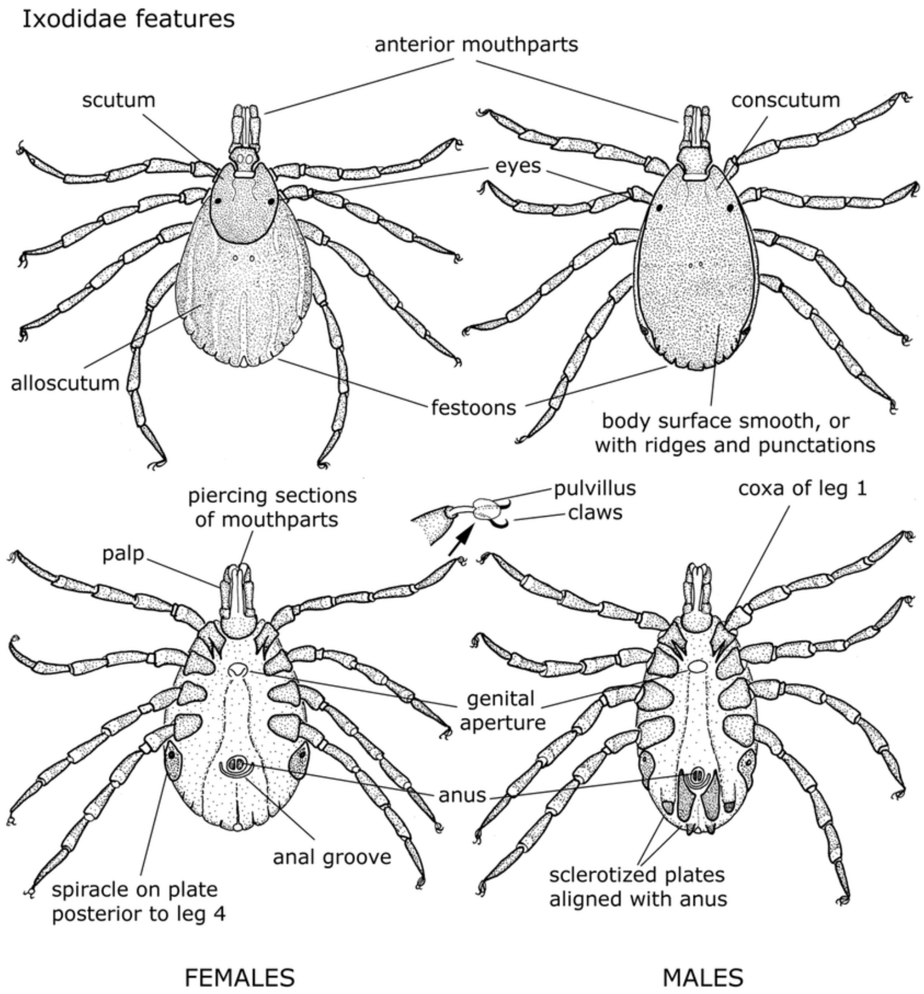

Figure 2.2. External structure of adult ixodid ticks (Walker *et al*, 2003)

2.3.3 Life cycle and feeding habit

Hard ticks have three developmental stages; larvae, nymph and adult (Walker *et al*., 2003). The hard tick species are parasites of vertebrate hosts and feed on blood of their host in all three life cycle stages (Parola & Raoult, 2001; Service, 2012). There are three life cycles in hard ticks; one-host, two-host and three-host life cycles. The first type of life cycle in hard ticks is a one-host life cycle ticks, which feed on only one host throughout all three life stages **(Figure 2.3)**. The larvae and nymphs feed and moult on the same host, until they become adults. After feeding and mating, female ticks drop off the host to lay

their batch of eggs (Walker *et al*., 2003). Example of one-host life cycle tick is *Rhipicephalus microplus*.

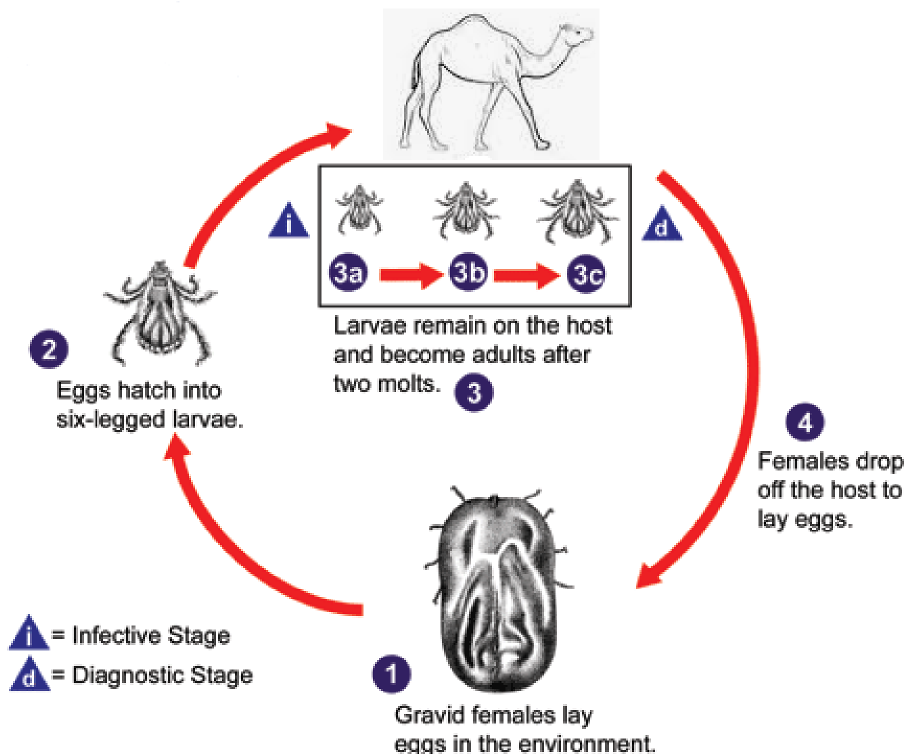

Figure 2.3: Life cycle of one-host tick.(Source: CDC website). (1) Eggs are laid in the environment (2) They hatched into larvae. (3a and 3b) Larvae attaches to a host and after two moults, it develops into adult. (4) Females drop off the host and lay eggs in the environment and the circle repeats.

The second type of life cycle in hard ticks is a two-host life cycle ticks, where the larvae and the nymph feed and moult on the same host before dropping off and moulting to adult male and female ticks (Walker *et al*., 2003). Female and male adult ticks then infest the second host, where females feed and drop off the host to oviposit after mating. Examples of two host life cycle ticks that been collected from camels include the *Hyalomma marginatum rufipes* and *Rhipicephalus evertsi* (Walker *et al*., 2003; Jongejan & Uilenberg, 2004; Service, 2012).

Majority of hard ticks require three different vertebrate hosts to complete their life cycle and are, therefore, referred to us three-host ticks **(Figure 2.4).** In a three-host life cycle, questing larvae climb on vertebrate hosts, feed, and drop off to the ground where they undergo moulting into a nymph. The nymph then attaches to a second vertebrate host, feed, and then drops off to the ground where it undergoes moulting again to become an adult. Adult males and females infest the third host where they feed, mate and then female drops off to the ground (Parola & Raoult, 2001; Walker *et al*., 2003; Service, 2012). The female lays eggs (oviposition) on the ground and then dies, while the male takes several meals and mates severally before it dies. The three-host life cycle is displayed in the following hard tick species; *Hyalomma dromedarii, Amblyomma variegatum, Rhipicephalus appendiculatus* and *Rh. pulchellus* (Parola & Raoult, 2001; Guglielmone, 2010; Service, 2012)

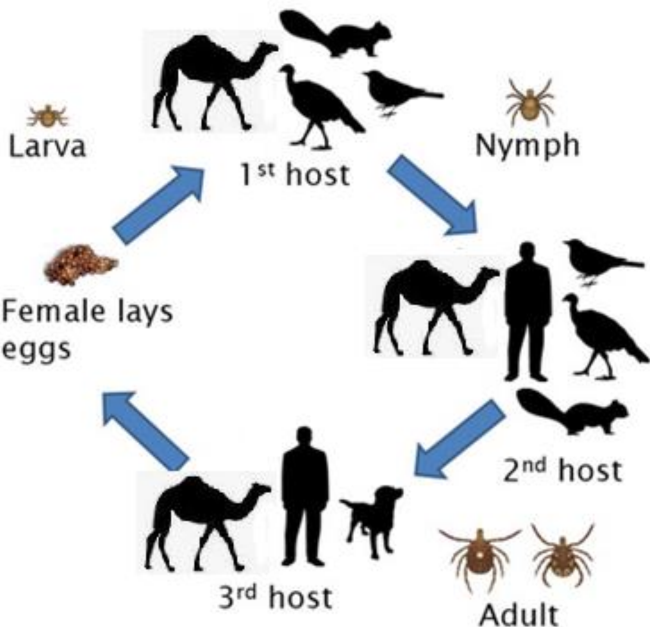

Figure 2.4: Three-host life cycle of a tick (Source: Rutgers website). The cycle starts when an adult female drops off the host and lays eggs. Eggs hatch into six-legged larvae. The larvae look for and attach to the first host, engorge and drop off and molt into nymphs. The nymphs seek out and attach to the second host and feed on the second host before dropping off and molt into adults. The adults seek out and attach to a third host. They feed and mate; females drop off and lay eggs in the environment, and the cycle repeats.

Majority of the soft ticks require many different hosts to complete their life cycles (Hoogstraal, 1985; Guglielmone, 2010). Only *Otobius megnini* completes its life cycle on one host (Hoogstraal, 1985). Larvae display different behaviours in different species; they feed very quickly in some hosts while they take days to feed in other hosts (Service, 2012). They also have different nymphal stages, which feed on multiple hosts. Unlike hard ticks that mate on their vertebrate host, soft ticks mate off hosts. After each multiple blood meal, the female soft ticks lay small batches of eggs (Hoogstraal, 1985; Walker *et al*., 2003; Service, 2012).

In Kenya, tick species falling under the genera *Rhipicephalus* spp., *Amblyomma* spp., and *Hyalomma* spp. are suspected to transmit pathogens of veterinary and public health importance that affect both humans and animals (Wesonga *et al*., 2010; Bornstein & Younan, 2013). Hylomma spp. (such as *Hyalomma dromedarii*, *Hyalomma rufipes*, and *Hyalomma impeltatum*) could be vectors for *Theileria* spp. (such as *T. annulata* and *T. ovis*), *Babesia* spp. (such as *Babesia bigemina*, *Babesia caballi*, *Babesia ovis*) and *Anaplasma* spp. (El Kady, 1998; Al-Deeb *et al*., 2015; Barghash and Hafez, 2016). *Rhipicephalus* species found in Kenya include *Rh.* (*Boophilus*) *decoloratus, Rh. Appendiculatus* (vector of *Theileria parva*)*, Rh. evertsi evertsi* (vector of *A. ovis*), and *Rh. Pulchellus* (*R. aeschlimannii*) (Wiley, 1958; Walker *et al*., 2003; Kaba, 2022). *Amblyomma* tick species found in Kenya include; *Am. lepidum*, *Am. hebraem*, *Am. variegatum* and *Am. gemma.* These ticks have the ability to transmit *E. ruminantium* to domsestic and wild ruminants (Ngumi *et al*., 1997; Kaba, 2022). Most studies conducted in Kenya have identified the following genus as being the most common in Kenya: *Amblyomma*, *Rhipicephalus*, and *Hyalomma* spp. (Wesonga *et al*., 2010; Mwamuye *et al*., 2017; Omondi *et al*., 2017; Oundo *et al*., 2020; Chiuya *et al*., 2020). Ticks belonging to *Amblyomma*, *Rhipicephalus*, and *Hyalomma* spp. have also been isolated from camels in the horn of Africa and Middle East (Dioli 2001; Alanazi et al., 2020).

2.4 Tick-host-pathogen interaction

Presence of suitable hosts determines the occurrence of ticks in a particular area (Bonnet *et al*., 2018). This host may also act as a carrier and has a potential of infecting naïve ticks. Ticks produce a feeding lesion and inhibit host haemostatic, immune, and inflammatory responses to complete feeding, as the hosts react locally and systemically to tick infestation (Fuente *et al*., 2016; Bonnet *et al*., 2018). Ticks limit pathogen infection by activating different mechanisms. Tick biological processes such as apoptosis and innate immune response are manipulated by the pathogens to facilitate infection, multiplication, and transmission (Fuente *et al*., 2016; Bonnet *et al*., 2018; Vechtova *et al*., 2018). During this process, the host immune response is inhibited by pathogens. The host reacts to pathogen infection by activating different mechanisms to control pathogen infection. Most pathogens are capable of sustaining themselves within the tick vector. Most tick-borne bacteria express surface proteins which function as adhesion molecules onto tick cells. Other pathogens have the ability to infect and multiply within tissues such as ovaries that enables successful transovarial transmission (Vechtova *et al*., 2018). Host benefit from tick infestations by increasing resistance to pathogen infection while ticks benefit from their hosts by promoting feeding. Ticks benefit from pathogen infection by increasing their fitness and survival at high and low temperatures. Tick feeding and reproduction are not affected by pathogen infection. Pathogens manipulate tick biological processes to facilitate infection (Fuente *et al*., 2016; Bonnet *et al*., 2018).

2.5 Impact of climate change on ticks and tick-borne pathogens

Ticks have to adapt to feeding on their vertebrate host and surviving on the physical environment (Gray *et al*., 2009; Nah *et al*., 2020). To maintain tick population, availability of vertebrate hosts and behaviour is important because suitable hosts are required for reproduction by adult ticks. The distribution of ticks on the host is influenced by the availability of vertebrate hosts (Gray *et al*., 2009; Nah *et al*., 2020). The physical environment is an important factor for ticks during the moulting and questing stages, which are affected by the adverse environmental conditions. As temperature rises, vector

metabolism and biting rates are accelerated. This leads to increased frequency of blood feeding, which results in enhanced egg production and vector-population sizes (Gray *et al*., 2009; Estrada-Peña *et al*., 2012; Nah *et al*., 2020). The geographical and distribution range also increases due to increase in temperature. The epidemiology of TBDs is significantly affected by the changes in precipitation patterns (Gray *et al*., 2009; Dantas-Torres *et al*., 2012; Nah *et al*., 2020). Studies in Eastern Ethiopia and northern Kenya have shown that a few of tick species are commonly found in camels, with marked seasonality abundance (Dioli, 2001; Zeleke and Bekele, 2004)

2.6 Major tick-borne pathogens

Ticks are vectors of many disease-causing pathogens, which they transmit from one host to another during blood meal (Bonnet *et al*., 2018). Some of the major diseases caused by TBPs include heartwater, rickettsioses, anaplasmosis, babesiosis, East Coast Fever, and theileriosis (Parola & Raoult, 2001; Jongejan & Uilenberg, 2004; Sadeddine *et al*., 2020). Anaplasmosis and trypanosomiasis (Surra) are the major constraints in raising productive camel herds in Kenya and the Horn of Africa (Bornstein & Younan, 2013; Watson et al., 2016). Bacterial pathogens belonging to genera *Anaplasma*, *Theileria, Babesia*, *Ehrlichia* and *Rickettsia* are commonly found in Kenya (Omondi et al., 2017, Mwamuye *et al*., 2017, Oundo *et al*., 2020; Okal *et al*., 2020). *Anaplasma* spp. and *Ehrlichia* spp. causes economically important diseases such as gall sickness and heartwater, and are found worldwide in both domestic and wild animals (Parola *et al.*, 2000; Nijhof *et al*., 2007; Kubelová *et al*., 2012; Lempereur *et al*., 2017).

2.6.1 Heartwater disease

2.6.1.1 Aetiology

Ehrlichiosis is an infection of white blood cells that affects various mammals, including mice, sheep, cattle, deer, dogs, goats, horses, and humans (Iweriebor *et al*., 2017). The genus *Ehrlichia* comprises of six recognized species: *E. chaffeensis*, *Ehrlichia canis*, *E. ewingii*, *E. ruminantium*, *E. muris*, and *E. minasensis* (Paddock & Childs, 2003; Allsopp

et al., 2007; Kubelová *et al*., 2012; Bastos *et al*., 2015). A new species of *Ehrlichia* "*candidatus* Ehrlichia regneryi" has been reported in camels without clinical signs in northern Kenya (Younan *et al*., 2021) and Saudi Arabia (Bastos *et al*., 2015). *Ehrlichia chaffeensis*, *E. ewingii* and *E. muris*-like pathogen are suspected to be the causative agents of human ehrlichiosis, a zoonotic disease (CDC, 2017). *Ehrlichia ewingii* and *E. chaffensis* are transmitted by *Amblyomma americanum* ticks (CDC, 2017). *Ehrlichia ruminantium,* formerly known as *Cowdria ruminantium,* is the causative agent of heartwater, a TBD of ruminants (Biguezoton *et al*., 2016). The rickettsial organism is transmitted by *Amblyomma* spp. *(*Ngumi *et al*., 1997). The disease has a high mortality and morbidity rates in susceptible ruminants *(*Aktas *et al*., 2011). A possible heartwater case was reported in a single camel in Sudan over 60 years ago (Karrar, 1960). Other cases have been reported in Chad with an outbreak affecting nearly 10% of 500 camels (Bechir, 2013), but diagnosis of heartwater was based on clinical signs and microscopy.

2.6.1.2. Epidemiology and transmission

Ehrlichia chaffeensis has been detected in *Am. eburneum* in Shimba Hills National Reserve in Kenya (Omondi *et al*., 2017). *Ehrlichia ruminantium* has been detected in *Am. lepidum*, *Am. gemma*, *Am. fasomarmoreum*, *Am. nuttalli*, *Am. eburneum* and *Am. variegatum* tick from Kenya (Ngumi *et al*., 1997; Mwamuye et al., 2017; Omondi *et al.*, 2017). The diseases are endemic in sub-Saharan African (SSA) countries. Outbreaks of a severe disease in camels, with clinical signs similar to those seen in domestic ruminants suffering from heartwater, were recorded in northern Kenya (Garissa, Wajir, Mandera and Marsabit Counties) in 2016 (Younan *et al*., 2016). The presence of *Amblyomma* spp. in the area was also reported (Younan *et al*., 2021). *Ehrlichia ewingii* and *E. chaffeensis* are transmitted by the lone star tick (*Am. americanum*) (Paddock & Childs, 2003). *Ehrlichia ruminantium* is transmitted by *Am. variegatum*, *Am. gemma* and *Am. lepidum* (Anifowose *et al*., 2020; Ngumi *et al*., 1997). Ehrlichiosis is transmitted through tick bites, blood transfusion and organ transplant from infected individuals (CDC, 2017).

2.6.1.3 Signs

Signs and symptoms of ehrlichiosis are usually mild or moderate and may include fever, chills, severe headache, muscle aches, confusion, rash, nausea, vomiting, diarrhoea, and loss of appetite (CDC, 2017). Rash is more common in ehrlichiosis caused by *E. chaffeensis* (Paddock & Childs, 2003; Guillemi *et al*., 2019). For heartwater, a disease caused by *E. ruminantium*, clinical characteristics include sudden high fever, nervous signs, depressed demeanour, and high mortality. Post-mortem signs of heartwater include lung oedema, hydropericardium and hydrothorax (Allsopp, 2015). Severe cases of ehrlichiosis have also been reported and the signs and symptoms include damage to the brain or nervous system, uncontrolled bleeding, organ failure, respiratory failure and death (Allsopp *et al*., 2007; Allsopp, 2015). Severe illness occurs has a result of delayed antibiotic treatment, age, and weakened immune system (Parola & Raoult, 2001; CDC, 2016). During the 2016 outbreaks of a severe disease in camels in northern Kenya, clinical signs similar to those seen in domestic ruminants suffering from heartwater that include sudden onset, aimless wandering, head pressing, lethargy, rapid breathing, extreme respiratory distress, excitability, recumbency, and mortality close to 100% in adult animals in the absence of antibiotic treatment were reported. Post-mortem examination revealed an enlarged "cooked" liver, pulmonary oedema, hydropericardium, pleural exudate, ascites, hydrothorax, and nephrosis and blood in the abomasum and intestine (Younan *et al*., 2016).

2.6.1.4 Diagnosis and treatment

Initial diagnosis of ehrlichiosis is often made based on clinical signs and symptoms, and later confirmed using parasitological and specialized laboratory tests. The common methods of laboratory tests include brain smear microscope, blood smear microscopy, polymerase chain reaction (PCR), and indirect fluorescent antibody (IFA) test (Parola & Raoult, 2001; CDC, 2016). The quantitative polymerase chain reaction (qPCR) technique a higher sensitivity in detecting ehrlichia and might provide the parasitemia monitoring

during the disease treatment. The drug of choice for treatment of ehrlichiosis is antibiotic doxycycline (CDC, 2016).

2.6.2 Spotted fever group rickettsioses

2.6.2.1 Aetiology

Rickettsia spp. are classically divided into the spotted fever group (SFG) and the typhus group (Roux & Raoult, 2000). Members of SFG *Rickettsia* include *R. aeschlimannii*, *Rickettsia australis, R. africae*, *Rickettsia japonica*, *Rickettsia conorii*, *Rickettsia helvetica*, *Rickettsia honei*, *Rickettsia massiliae*, *Rickettsia rickettsii*, *Rickettsia slovaca* and *Rickettsia sibirica* (Raoult & Roux, 1997; Roux & Raoult, 2000; Parola *et al*., 2013). The rickettsial pathogens most likely to be encountered by travellers include *R. africae* (African tick-bite fever), *R. conorii* (Mediterranean spotted fever), *A. phagocytophilum* (anaplasmosis), *R. rickettsii* (known as both Rocky Mountain spotted fever and Brazilian spotted fever), *Orientia tsutsugamushi* (scrub typhus), and *R. typhi* (murine typhus) (CDC, 2016). Spotted fever group (SFG) rickettsiae are zoonotic pathogens, which include *R. aeschlimannii* and *R. africae* (Kleinerman *et al*., 2013; Wallménius *et al.*, 2014). Camels in African and Asian arid and semi-arid regions seem to be potential hosts of rickettsial species (Kernif *et al*., 2012).

2.6.2.2 Epidemiology and transmission

Human beings are at risk of acquiring spotted fever group (SFG) rickettsial infections during travel to endemic areas (Service, 2012; Parola *et al*., 2013). An increase of SFG transmission is reported during outdoor activities as the risk of tick bite is high. Tickborne spotted fever rickettsioses are the most frequently reported travel-associated rickettsial infections. People at risk of SFG rickettsioses include local people walking in the bush, game hunters, and ecotourists to Africa (Kleinerman *et al*., 2013). Spotted fever group (SFG) rickettsioses has been reported in many African countries such as Burundi, Mali, Sudan and South Africa (Raoult *et al*., 2001). *Rickettsia africae*, *R. aeschlimanii* and *R. coronii* are the predominant species reported in these countries (Raoult & Roux, 1997;

Raoult *et al*., 2001). In Kenya, studies have reported presence of *R. africae* in *Am. variegatum*, *Am. eburneum* and *Rhipicephalus* spp. (Maina *et al*., 2014; Mwamuye *et al*., 2017; Oundo *et al*., 2020; Chiuya *et al*., 2020). Recent studies on camels in Garissa and Isiolo counties have reported presence of multiple *Rickettsia* species in *Am. gemma*, *Hy. truncatum* and *Rh. pulchellus* collected from camels (Koka *et al*., 2017). Transmission of SFG rickettsioses occurs through tick bites or inoculation of infectious fluids into the skin from ectoparasites such as fleas, lice, mites, and ticks. Infection may also result from inoculating conjunctiva or inhaling infectious materials. Transmission through transfusion of infected blood products or by organ transplantation has been reported (Raoult & Roux, 1997; Raoult *et al*., 2001).

2.6.2.3 Signs

Severity of signs and symptoms of SFG rickettsioses depends on virulence of the pathogen, immunity of the host, gender and age. The most notable signs include headache, fever, malaise, nausea and vomiting. These signs begin to appear 6-10 days after tick bite (Raoult & Roux, 1997; CDC, 2016).

2.6.2.4 Diagnosis and treatment

Clinical diagnosis of rickettsioses is difficult due to the non-specific clinical manifestation of the diseases. PCR-based detection is the primary method to detect SFG rickettsiae, especially for the early detection of infection before the development of detectable antibodies (Parola & Raoult, 2001; Chapman *et al*., 2016). PCR is known to be sensitive, and the sample type and assay used determine the success of detection. PCR can be used to detect SFG DNA from whole blood, buffy coat, serum, tissue biopsies, eschar scrapings, arthropods, and animal hosts. Other methods used for rickettsioses diagnosis include various serological tests such as enzyme-linked immuno-sorbent assay (ELISA), immuno-fluorescence assay (IFA) and complement fixation test (CFT) (Chapman *et al*., 2016). The drug of choice for treating SFG rickettsioses is antibiotic doxycycline (CDC, 2016). Self-examination after visit to tick-infested areas helps reduce the risk. The spread

of SFG rickettsioses can also be managed by wearing protective clothing, use of skin repellents or use of insecticide impregnated clothing (Piesman & Eisen, 2008; Kleinerman *et al*., 2013).

2.6.3 Q-Fever

2.6.3.1 Aetiology

Q fever is a zoonotic disease caused by *Coxiella burnetii* (Njeru *et al*., 2016), which is known to cause infections in a wide range of species, including domestic animals, birds and reptiles (Hoover *et al*., 1992). Domestic animals are known to be the main source of infections for humans (Maurin & Raoult, 1999). Ticks acquire *C. burnetii* when they feed on infected animals and are capable of transmitting it to health animals during next blood meal (Hoover *et al*., 1992; Duron *et al*., 2015). The ticks are also important vectors of *C. burnetii* and are known to maintain infection in domestic animals (Duron *et al*., 2015).

2.6.3.2 Epidemiology and transmission of Q-fever

Domestic animals such as cattle, sheep and goats act as the major reservoirs of *C. burnetii*, which can infect a large variety of animals, humans, birds, and arthropods (Porter *et al*., 2011; Duron *et al*., 2015; Neare *et al*., 2019). People get infected by breathing in dust that has been contaminated by infected animal faeces, milk, urine, and birth products that contain *C. burnetii* (Porter *et al*., 2011). People may also get infected with Q-fever by eating contaminated, unpasteurized dairy products. Professions such as veterinarians, livestock farmers, meat processing plant workers, dairy workers, and researchers at facilities housing sheep and goats are at increased exposure to *C. burnetii* (Porter *et al*., 2011). However, the epidemiology of Q fever diseases in Kenya is poorly understood due to the apparent neglect of the disease by both veterinary and medical personnel, and due to the limited capacity to enable meaningful epidemiological surveys (Njeru *et al*., 2016).

2.6.3.3 Signs and symptoms

In humans, the disease may be asymptomatic or manifest as a mild disease that is self-limiting. The incubation period averages 18 to 21 days (range 9 to 28 days). Most notable symptoms of Q-fever in humans include fatigue, chills, fever, headache, nausea, diarrhoea, stomach pain, chest pain, muscle aches, weight loss and non-productive cough (Porter *et al*., 2011). Respiratory symptoms such as a pleuritic chest pain and dry non-productive cough appear 4 to 5 days after onset of illness (CDC, 2017). Severe cases include non-specific febrile illness, pneumonia, sub-acute endocarditis, hepatitis and less commonly, granulomatous lesions in bone, soft tissues or body organs (Maurin & Raoult, 1999). Q-fever is frequently asymptomatic in domestic animals. The common clinical signs in animals include infertility, premature delivery, stillbirth, abortion, mastitis and metritis (Porter *et al*., 2011; Duron *et al*., 2015).

2.6.3.4 Diagnosis and treatment

The most commonly used diagnostic methods of Q-fever are IFA or PCR (Porter *et al*., 2011). IFA of infected tissue is the diagnostic method of choice. However, enzyme-linked immunosorbent assay (ELISA) may also be used. PCR is the most sensitive method in detecting the organism in biopsy specimens, but negative results do not rule out the diagnosis (Porter *et al*., 2011; CDC, 2015). Mild or asymptomatic cases usually resolve within a couple of weeks without any treatment. However, depending on the severity, antibiotics may be prescribed for 2 to 3 weeks (CDC, 2015). The drug of choice in treatment of Q-fever is doxycycline (Maurin & Raoult, 1999; Eldin *et al*., 2017). However, the drug is not recommended during pregnancy. The level of the disease in humans can be reduced by controlling the disease in animals. Immunization of those in high risk occupational groups such as abattoir workers is the primary preventive measure against Q fever (Maurin & Raoult, 1999; Porter *et al*., 2011; Eldin *et al*., 2017).

2.6.4 Babesiosis

2.6.4.1 Aetiology

Babesiosis, also referred to as red water, is caused by tick-borne hemoparasites of genus *Babesia*. The parasite belongs to phylum Apicomplexa, order Piroplasmida, and genus *Babesia*. The principal species of the genus *Babesia* include *Babesia bovis, Babesia bigemina, Babesia gibsoni, Babesia microti, Babesia rodhani, Babesia caballi, Babesia canis, Babesia divergens, Babesia major, Babesia ovata, Babesia occultans and Babesia jakimovi* (Kubelová *et al*., 2012; Imam *et al*., 2016; Lempereur *et al*., 2017). They infect both domestic and wild animals (Lempereur *et al*., 2017). *Babesia* spp. are transmitted into host's blood by the ticks in the genus *Rhipicephalus* spp. during feeding (Lempereur et al., 2017).

2.6.4.2 Epidemiology and transmission

All *Babesia* spp. are transmitted by ticks with a limited host range. *Rhipicephalus* spp. ticks are the principal vectors of *B. bovis* and *B. bigemina* (Lempereur *et al*., 2017). The *Rhipicephalus* spp. ticks are widespread in tropical and subtropical countries (Walker et al., 2003). The hosts for *B. bovis* and *B. bigemina* are mostly cattle, water buffalo and African buffalo. *Babesia bigemina*, which is transmitted by *Rh. decoloratus*, is the main species of *Babesia* found to infect cattle in Kenya (Wesonga *et al*., 2010). *Babesia* spp. was detected in camels showing clinical symptoms similar to those observed in other animals such as anaemia, fever, weakness, appetite loss, depression, haemoglobinuria, icterus, and gastrointestinal stasis in Saudi Arabia using parasitological tools (Swelum et al., 2014). Recent studies have also detected *Babesia bovis* in in camels in Egypt (El-Sayed et al., 2021), and *Babesia caballi* in Jordan (Qablan et al., 2012) and Iran (Mirahmadi et al., 2022) During the 2016 acute camel death syndrome (ACDS) in Marsabit and Wajir, one case of *Babesia* sp. was reported in blood samples collected from ACDS affected camels (Younan *et al*., 2021).

2.6.4.3 Signs

Common signs and symptoms of babesiosis include fever, chills, headache, sweats, body aches, nausea, loss of appetite, or fatigue (Tavassoli *et al*., 2013). *Babesia* parasites infects and destroys red blood cells, leading to haemolytic anaemia. Haemolytic anaemia causes jaundice (yellowing of the skin) and dark urine (CDC, 2015). In camels, babesiosis is characterized by anaemia, fever, weakness, appetite loss, depression, haemoglobinuria, icterus, and gastrointestinal stasis in Saudi Arabia using parasitological tools (Swelum *et al*., 2014).

2.6.4.4 Diagnosis and treatment

Blood smear microscopy can be used to detected babesia parasites in the early stages. However, this method requires significant time and expertise. Serological methods such as indirect fluorescent antibody test (IFA) (CDC, 2015) or molecular diagnostics, such as PCR are also used to detect *Babesia* parasites (Persing *et al*., 1992). Molecular diagnostics, such as PCR, are known to be sentive method of detecting *Babesia* spp. (Persing *et al*., 1992). Treatment of babesiosis requires antiparasitic drugs, such as those used for malaria. The drug of choice for mild to moderate cased is atovaquone plus azithromycin in humans and diminazene and imidocarb in livestock (CDC, 2015).

2.6.5 Theileriosis

2.6.5.1 Aetilogy

Theileriae are obligate intracellular protozoan parasites phylum Apicomplexa, order Piroplasmida, family Theileriidae, genus *Theileria* (Lempereur *et al*., 2017). They are most closely related to *Babesia*, from which they differ by having a developmental stage in leukocytes prior to infection of erythrocytes (Imam *et al*., 2016; Lempereur *et al*., 2017). *Theileria* are known to infect both domestic and wild ruminants. The most pathogenic and economically important species are *Theileria parva* (which causes East Coast fever, Corridor disease, buffalo disease, January disease, turning sickness according to the strain), *Theileria annulata* (causes Tropical theileriosis, and

Mediterranean theileriosis), and *Theileria taurotragi* (causes turning sickness (Kubelová *et al*., 2012; Lempereur *et al*., 2017; CDC, 2020). Studies have reported presence of *Theileria mutans*, *Theileria annulate*, *Theileria equi*, and *Theileria ovis* in camels worldwide (Qablan *et al*., 2012; Sazmand & Joachim, 2017)

2.6.5.2 Epidemiology and Transmission

Theileriae infect both domestic and wild Bovidae and is known to be transmitted by ixodid ticks. *Theileria* has complex life cycles in both vertebrate and invertebrate hosts (Gachohi *et al*., 2012; Bishop *et al*., 2015; Lempereur *et al*., 2017). *Theileria parva*, associated with Buffalo and cattle occurs in eastern and southern Africa (Bishop *et al*., 2015). *Theileria annulata* occurs in Sudan, Eritrea, North Africa, Mediterranean Europe, S. Russia, Near & Middle East, India, China and Central Asia. Oriental theileriosis occurs in Europe, N. America, Australia, and Asia (CDC, 2015; Gharbi *et al*., 2020). Other (benign) theilerioses are widespread in SSA and Caribbean islands (Gharbi *et al*., 2020). *Theileria mutans*, *Theileria annulate*, *Theileria equi*, and *Theileria ovis* have been reported in camels across the world (Sazmand & Joachim, 2017). However, the epidemiological role played by camels in the transmission of *Theileria* spp. is still not confirmed. In Kenya, theileriosis caused by *T. parva* (East Coast fever) has been associated with high mortalities in cattle (Gachohi *et al*., 2012). *Theileria* spp. are known to be transmitted by ixodid ticks. *Rh. appendiculatus*, *Rh. duttoni* and *Rh. zambeziensis* are the vectors of the most important pathogen *T. parva*. Ticks of *Hyalomma* genus transmit *T. annulata* (Gachohi *et al*., 2012; Lempereur *et al*., 2017; Gharbi *et al*., 2020). *Theileria* sporozoites are transmitted to susceptible animals in the saliva of the feeding tick (CDC, 2015).

2.6.5.3. Signs and symptoms

Signs and symptoms of the most economic important diseases, East Coast fever (ECF) Mediterranean Coast fever (MCF), include augmentation of lymph nodes, high fever and anorexia, drop in milk yield, nasal and ocular discharge, dyspnoea, diarrhoea, emaciation, weakness and recumbency, death after occurs after 7-10 days in 90 % of cases. Other signs

and symptoms observed include depression, tachypnoea, abortion, lethargy, decreased milk production, jaundice and death (CDC, 2015). The predominant clinical signs of theileriosis in infected camels include severe emaciation, diarrhoea in the form of intermittent bouts, ocular watery discharge, fever, and enlargement of superficial lymph nodes (El-Fayoumy et al., 2005)

2.6.5.4 Diagnosis and treatment

A definitive diagnosis is achieved by the combination of clinical examinations and appropriate laboratory testing (Gharbi *et al*., 2020). Initial diagnosis is based on clinical signs and examination of Giemsa-stained blood, lymph node and tissue impression smears. Serological methods such as an IFA or ELISA tests are preferred method of diagnosis (CDC, 2015; Lempereur *et al*., 2017). However, they are not sensitive enough to detect all infected samples. PCR test is more reliable in identifying all infected samples. It is hoped that a combination of ELISA, PCR and DNA probes will greatly enhance our present capacity to identify infected animals (CDC, 2017). In case of outbreak, the spread of the disease can be prevented by minimizing stress and movement of affected animals. Adaption of appropriate tick management practices can help prevent theileriosis and other TBDs (Kakati *et al*., 2015; Mans *et al*., 2015). Theileriosis is commonly treated using tetracycline, and buparvaquone for the treatment of clinical cases.

2.6.6 Anaplasmosis

2.6.6.1 Aetiology

Anaplasmosis, also known as gall sickness, is an arthropod-borne disease of ruminants caused by species of the genus *Anaplasma* (Aubry & Geale, 2011; Bastos *et al*., 2015). Anaplasmosis is caused by *A. marginale*, *A. centrale*, *A. ovis*, and *A. mesaeterum*, and other *Anaplasma* spp. (Aktas *et al*., 2011; Iweriebor *et al*., 2017). *Anaplasma phagocytophilum* causes human anaplasmosis while *A. marginale*, *A. ovis* and *A. bovis* causes anaplasmosis affecting domestic animals (Silveira *et al*., 2012; Stuen *et al*., 2014).

The most important vectors for anaplasmosis are one-host tick *Boophilus* spp. and *Dermacentor* spp. Anaplasmosis is distributed worldwide (Iweriebor *et al*., 2017).

2.6.6.2 Epidemiology and transmission

Anaplasmosis is the major cause of morbidity and mortality in exotic and crossbred cattle in tropics and subtropics (Aktas *et al*., 2011; Aubry & Geale, 2011). Distribution of the tick vectors and reservoir host determines the geographical distribution of the disease. The role of domestic animals on the epidemiological distribution of Anaplasmosis in Kenya is insufficient due to few studies being conducted (Aubry & Geale, 2011). Studies have shown that severity of Anaplasmosis depends of factors such as age-related host susceptibility, virulence of the strain, and breed resistance (Aubry & Geale, 2011; Brown, 2012; Renneker *et al*., 2013; Teshale *et al*., 2015). Calves are much more resistant to diseases than older cattle (CDC, 2015).
Anaplasma marginale, *Anaplasma centrale*, *Anaplasma ovis*, *Anaplasma bovis*, *Anaplasma phagocytophilum* and more recently *Anaplasma platys* are the *Anaplasma* species known to cause infections in ruminants (Aubry & Geale, 2011; Kubelová *et al*., 2012; Stuen *et al*., 2014). *Anaplasma marginale* is the most virulent of the known *Anaplasma* spp. and is characterized by a progressive hemolytic anaemia (Aubry & Geale, 2011). It is the most commonly documented *Anaplasma* spp. in Kenya (Peter *et al*., 2020; Wesonga *et al*., 2010). It is transmitted by several tick vectors including some *Rhipicephalus* (*Boophilus*) species (Aubry & Geale, 2011). In the tropical and subtropical areas, *A. marginale* is responsible for extensive economic losses. On the other hand, *A. centrale*'s clinical outbreaks are very rare. However, *A. centrale* can produce a moderate degree of anaemia (Ybañez & Inokuma, 2016). In tropical and subtropical areas, *A. centrale* is used as a live vaccine against pathogenic *A. marginale* in cattle (Aubry & Geale, 2011; Ybañez & Inokuma, 2016). The characteristics of the inclusion bodies and location in the erythrocytes can be used to distinguish *A. centrale* from *A. marginale* (Ybañez & Inokuma, 2016). *Anaplasma ovis*, *A. bovis*, and *A. platys* has been detected in livestock ticks in Homabay and Baringo counties (Omondi *et al*., 2017), *A*

phagocytophilum in Shimba Hills National Reserve (Mwamuye *et al*., 2017), *A. marginale* in Machakos, and Busia counties (Wesonga *et al*., 2010; Chiuya *et al*., 2020).

Anaplasma phagocytophilum is the major zoonotic pathogen (Stuen *et al*., 2014). *Anaplasma centrale* is capable of producing a moderate degree of anaemia, but clinical outbreaks in the field are extremely rare (Aubry & Geale, 2011). "*Candidatus* Anaplasma camelii" is a new possible *Anaplasma* species that has been reported in camels in Kenya (Kidambasi *et al*., 2020), Egypt (Naga & Barghash, 2016), Saudi Arabia (Alanazi *et al*., 2019, 2020), Iran, Tunisia and Morocco (Lbacha *et al*., 2017; Sazmand *et al*., 2019). Despite the limited number of studies undertaken on TBPs, evidence suggest that only *A. platys*-like *Anaplasma* spp., "*Candidatus* Anaplasma camelii" has been found in camels in Kenya, and in most of African and Asian countries (Bastos *et al*., 2015; Sazmand *et al*., 2019; Kidambasi *et al*., 2020). However, there is limited information on Anaplasmosis in Kenyan camels.

Biological and mechanical pathway are the two common routes through which *Anaplasma* spp. is transmitted (Aubry & Geale, 2011). Biological pathway occurs through tick bites while mechanical transmission occurs through castrating knives, reusing of needles, ear taggers, dehorners, and tattoo instruments (CDC, 2015). The organism can also be transmitted mechanically through blood-contaminated mouthparts of biting flies such as horse flies and camel keds (Dobrynin *et al*., 2009). Camel keds (*Hippobosca camelina*) can transmit "*Ca*. A. camelii" to small laboratory animals (Bargul *et al*., 2021).

2.6.6.3 Signs and symptoms

Signs and symptoms of anaplasmosis begin to appear within 1–2 weeks after the bite of an infected tick. In early days (1-5 days) after tick bite, mild or moderate signs and symptoms such as fever, chills, severe headache, muscle aches, nausea, vomiting, diarrhoea, and loss of appetite appears (CDC, 2015). Anaplasmosis can cause severe illness if treatment is delayed or there are other underlying medical conditions. Severe Anaplasmosis is characterized by respiratory failure, organ failure, bleeding problems,

and death. Older people and people with weak immunity are at a high risk of being infected (CDC, 2015; Ybañez & Inokuma, 2016).

2.6.6.4 Diagnosis and treatment

In most developing countries, microscopic examination of Giemsa stained thin blood film is the most commonly used laboratory method for the identification of the organism that causes Anaplasmosis (Aubry & Geale, 2011). However, this method cannot detect low level of rickettsiaemia as seen in infected host. Differentiating the pathogen from other pathogens with similar structures such as Heinz bodies and staining artifacts is not easy, thus rending this method unreliable. Polymerase chain reaction (PCR) is the most sensitive method in detecting the presence of low-level infection in carrier animals. PCR amplification is performed on DNA extracted from whole blood specimens (Aktas *et al*., 2011; CDC, 2017). Serological methods such as CFT, card agglutination test (CAT), capillary agglutination assay, ELISA and IFA can also be used to diagnose the infection. Anaplasmosis is treated using tetracycline antibiotics (tetracycline, chlortetracycline, oxytetracyline, doxycycline) (CDC, 2017). Studies have shown that animals that recovers from the infection either naturally or with normal therapy, remains carriers of the disease for life (Aubry & Geale, 2011). Carriers show no sign of the disease but act as sources of infection for other susceptible cattle. Anaplasmosis cases rise as tick and other biting flies increases. Therefore, control of vectors is key to preventing anaplasmosis (Aubry & Geale, 2011).

2.7 Tick control methods

2.7.1 Biological control

Biological tick control method involves controlling ticks using natural organisms that are their natural enemies (Jongejan & Uilenberg, 2004). There are three major types of organisms that are natural enemies of ticks affecting livestock: predators, parasitoids and pathogens (Ostfeld *et al*., 2006; Moyo & Masika, 2009). Birds, ants and a few mite species eat ticks attached to the host, or engorged females that have dropped off the host to the

ground. Parasitoids such as wasps deposit their eggs in ticks and the hatched larvae feed on tick tissues ultimately killing them. Pathogen such as bacteria, fungi and nematodes infect and kill ticks (Ostfeld *et al*., 2006). Studies in the United States have shown wasp to be effective in controlling *Ixodes scapularis* ticks by achieving 25 to 50% natural parasitisation rates (Hu *et al*., 1998). Another study on wasps against *Am. variegatum* in Kenya showed a parasitisation rate of about 50% (Takasu & Nakamura, 2008). Biological control of ticks is a safe, effective and cheap way of controlling ticks. Integrating chicken as natural predators of livestock tick control should be encouraged (Pegram *et al*., 2014).

2.7.2 Chemical control

This is the common method of tick control. The tick control method involves treatment of animals by dipping or spraying with acaricides (Jongejan & Uilenberg, 2004). Acaricides are synthetic chemicals used to kill ticks in the environment or on livestock. Some of the general groups of compounds used to kill ticks include arsenicals, chlorinated hydrocarbons, organophosphorous compounds, pyrethroids, carbamates and amidines (Ostfeld *et al*., 2006; Moyo & Masika, 2009; Pegram *et al*., 2014). Acaricides can successfully reduce tick populations, particularly when combined with other tick management options. There are two categories of chemical tick control methods; habitat-targeted and tick host targeted. Habitat-targeted applications of acaricides focusses primarily on tick habitat, including wooded areas around the home and grazing areas, and the borders along woodland edges, stonewalls and ornamental plantings (Ostfeld *et al.*, 2006). This method is best suited for controlling ticks in nymphal stage, but can also be effective against adult ticks. Tick-host targeted application involves treating tick hosts with an acaricide to kill any ticks which may be feeding on them. The use of chemical acaricides continues to produce resistance in ticks as some ticks such as *Rh. microplus* are highly adaptable and will probably respond to any challenge posted by a new acaricide (Eskezia, 2016). It is, therefore, imperative that the effective acaricides presently used be applied correctly and efficiently so as to prolong their usefulness as tick control agents. Water scarcity in arid and semi-arid areas hinders proper use of acaricides. Handlers are

exposed to harmful effects of acaricides if they lack protective equipment. Some acaricides are expensive for ordinary pastoral communities (Piesman & Eisen, 2008).

2.7.3 Mechanical methods

This is the traditional tick control technique which involves handpicking of ticks from restrained animals and physically killing them on the spot (Pegram *et al*., 2014). The method also involves burning infested pastures, fencing off the pasture land and firm, starving the respective ticks to death, and interfering or altering the tick's natural environment. The disadvantage with the method is that it is tedious, time consuming, and ineffective for large herds.

2.7.4 Use of ant-tick vaccines

Tick infestation can be controlled through livestock vaccination with defined protein antigens. Vaccination induces immunity against tick infestation (Vargas *et al*., 2010). This method is an alternative to mechanical and chemical method for tick infestation control as it is more environmentally friendly. Several TBPs can be effectively controlled by targeting common tick vector (Imamura *et al*., 2008; Vargas *et al*., 2010). Targeting a pathogen in the vector by blocking its transmission is an innovative and promising method to control vector-borne infections since vector-borne pathogens exploit tick proteins to establish an infection. However, the selection of suitable antigens is a major constraint on vaccine development (Vargas *et al*., 2010).

2.7.5 Use of tick resistant breeds of livestock

Tick resistance is an acquired characteristic that enables an animal to limit the establishment, growth and persistence of tick population (Bhowmick & Han, 2020). Studies have shown that indigenous breeds (*Bos indicus*) and Sanga (*Bos taurus* and *B. indicus* cross breed) are more resistant to tick infestation than exotic European (*B. taurus*) breeds of cattle (Meltzer, 1996; Hope *et al*., 2010). There are many ways through which domestic animals can manifest tick resistance; through better familiarity of indigenous

cattle of the infected zone in grazing area or by morphological differences in the host altering the chance of attachment to the ticks (Meltzer, 1996). Skin thickness also appears to play an important role on host resistance to ticks (Hope *et al*., 2010). Studies have suggested that hair density, coat type and skin secretions may play some roles in livestock tick resistance. Studies have revealed that lighter-coloured animals are more resistant to ticks than dark coloured animals (Bhowmick & Han, 2020). Females, pregnant animals and younger animals are more resistant than their counterparts (Hope *et al*., 2010; Bhowmick & Han, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Marsabit County in northern Kenya, 543.2 km north of Nairobi. The county covers an area of~66,923 km² (County Government of Marsabit CIDP, 2013). Most areas within the county lies between 300 and 900 m above sea level. It is located between longitudes 36°00' and 39°21' East and latitudes 02°45' and 04°27' North. It borders Samburu County to the South, Ethiopia to the North, Wajir and Isiolo Counties to the East, and Turkana County to the West. Temperatures in the county range from a minimum of 16℃ to a maximum of 39℃ (Siciliano *et al*., 2021). Long rains are received from March to May while short rains are received from October to December, with the annual rainfall ranging between 200 and 1,000 mm (County Government of Marsabit CIDP, 2013). Marsabit County is occupied by pastoralist farmers who solely rely on livestock for livelihood. Blood samples and ticks were collected from camel and co-grazing sheep from 12 sites: Misa, Gola, Dabel, Bori, Yabalo, and Funanyatta in Moyale subcounty, Laisamis, and Korr in Laisamis subcounty, Kamboe, Hula Hula, and Shegel in Saku subcounty, and Burgabo in North Horr subcounty (**Figure 3.1**). These areas have wells that provide drinking water for camels.

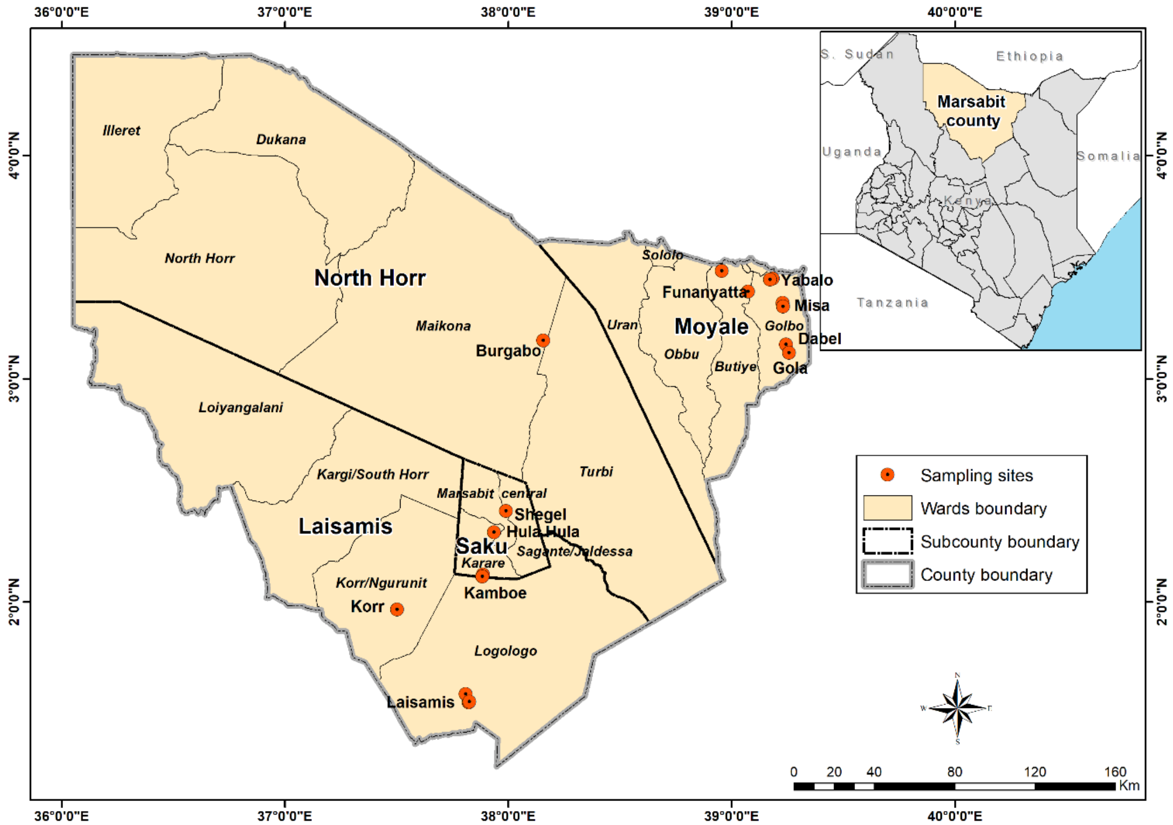

Figure 3.1: A map showing sampling locations within Marsabit County, Kenya. The maps were created using QGIS version 3.10.6 software.

3.2 Study design and sample size calculation

A cross-sectional study was employed in this study. The sample size (**N**) of camels estimated using the simple formula based on the normal approximation to the binomial distribution (Snedecor and Cochrane, 1989)

$$N = \frac{1.96^2\big(p(1-p)\big)}{d^2}$$

Where **N** is the sample size (number of herds tested), **Z** is the z-score for a given confidence level, ***p*** is the estimated true herd prevalence, and **d** is the precision around the prevalence for the 95% confidence limits. Since the prevalence of infections by

Anaplasma spp. in camels in the study area is estimated to be 68% (Kidambasi *et al*., 2020):

$$N = \frac{1.96^2(0.68(1-0.68))}{0.05^2} = \mathbf{334}$$

However, this target was not achieved as only 296 camels and 77 co-herded sheep were available for sampling.

3.3 Ethical approval

This study was undertaken in strict adherence to the experimental guidelines and procedures approved by the University of Nairobi Biosafety, Animal Use, and Ethics Committee (REF: FVM BAUEC/2019/200 and Kenya's National Commission for Science, Technology and Innovation (REF: NACOSTI/P/19/72855/27325) **(APPENDIX V; APPENDIX VI).** Camel farmers were informed about the study and sampling of blood and ticks after receiving consent from the household head. Animals were handled carefully to cause minimal discomfort. Informed oral consent was adopted to all for standardization.

3.4 Sample collection, storage, transport, and morphological identification of ticks

Sample collection took place from February to March 2020. The camels were restrained in a laying down position with the assistance of four camel handlers and four-millilitre blood samples drawn from the jugular veins using EDTA vacutainer tubes for molecular detection of TBPs. The samples stored and transported in liquid nitrogen to the Martin Lüscher Emerging Infectious Diseases (ML-EID) Laboratory at International Centre for Insect Physiology and Ecology (*icipe*) Nairobi for analysis. The ticks were held firmly over the scuta and mouthparts close to the host skin using a pair of serrated forceps before pulling out gently to avoid damage to the mouthparts. The ticks were collected into cryovials and transported in liquid nitrogen to the ML-EID Molecular biology Laboratory at *icipe* for further analysis.

Morphological identification of ticks to their respective species was done using a taxonomic key (Walker *et al*., 2003). Some of the morphological features used for identification of ticks include the colour and shape of the ticks, colour of legs, ornamentation of the scutum, and the size and distribution of punctuations and grooves. The ticks were staged under a Stemi 2000-C microscope (Zeiss, Oberkochen, Germany) and photographed using a microscope-mounted Axio-cam ERc 5s digital camera (Zeiss). The ticks were pooled into groups of between one and eight individuals based on species, host and sampling site.

3.5 DNA extraction from whole tick and blood samples

Whole ticks were frozen in liquid nitrogen and immediately homogenized in a 1.5-ml microfuge tube containing 150mg of 0.1-mm and 750mg of 2.0-mm yttria-stabilized zirconium oxide beads (Glen Mills, Clifton, New Jersey, USA) and 200 µL of 1 × PBS using a Mini-Beadbeater-16 (BioSpec, Bartlesville, OK) for 1 minute. Total genomic DNA (gDNA) was extracted from each blood and homoginized tick sample using the ISOLATE II Genomic DNA extraction kit following the manufacturer's instructions (Bioline, Meridian Bioscience inc., UK). Briefly, the tick homogenate was incubated for 1 hr. 30 minutes at 56ºC in the presence of 180 µL Lysis Buffer GL and 25 µL proteinase K solution. The samples were then lysed in 200 µl Lysis Buffer G3, vortexed and incubated at 70 ºC for 10 minutes. 200 µl absolute ethanol was added to the samples and vortexed before transferring them to spin column placed into Collection Tubes and centrifuged at 11,000 × g for 1 min to bind DNA to the column. 500 µL Wash Buffer GW1 and 600 µL Wash Buffer GW2 were used to wash the columns, and then dried by centrifugation at 11,000 × g for 1 minute. 100 µL of Elution Buffer G (pre-heated to 70 ºC) was used to elute the DNA.

3.6 Molecular identification of ticks

Representative samples from each identified adult tick species were selected for molecular identification and legs plucked for genomic DNA extraction. ISOLATE II Genomic DNA extraction kit was used to extract the tick leg DNA following manufacturer's instruction

(Bioline, UK). To confirm the morphological classification proceeded on identified tick species, fragments of cytochrome oxidase subunit 1 (CO1) from representative ticks were amplified using PCR. Fragments of 12S ribosomal (r)RNA and 16S ribosomal (r)RNA genes were also amplified from the tick DNA extracts. The PCR were performed in 10-µL reaction volumes, which included 2 µL 5× HOT FIREPol®Blend Master Mix (Solis Biodyne, Estonia), 0.5 µL of 10 µM forward and reverse primers (**Table 3.1**), and 1 µL of DNA template. The volume brought to 10 µL using nuclease-free PCR grade water. The PCR amplification conditions were set as: Initial denaturation at 95°C for 15 minutes followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C (16S rRNA and CO1) and 48°C for 12S rRNA for 30s and extension at 72°C for 1 minute. Final extension was performed at 72°C for 10 minutes. Successful amplification was determined by resolving 5 µL of the PCR products in a 1.5% (*w/v*) agarose gel electrophoresis containing 1 µg/mL ethidium bromide, and DNA fragments visualized under ultraviolet light. The remaining volume of PCR amplicons were purified using ExoSAP-IT according to manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). The samples were sequenced by Sanger sequencing platform at Macrogen Inc. (The Netherlands).

3.7 Molecular detection of TBPs by PCR-HRM analyses

PCR-HRM was used to screen the DNA samples for the presence of TBPs belonging to genera *Ehrlichia*, *Anaplasma*, *Rickettsia*, *Coxiella*, *Theileria*, and *Babesia* using genus-specific primers (**Table 3.1**). PCR-HRM reactions were done using HRM capable Rotor-Gene Q thermo cycler (QIAGEN, Hannover, Germany), Mic qPCR Cycler (Bio Molecular Systems, Australia) or Quant Studio 3 (Applied Biosystems). Samples with unique *Ehrlichia* and *Anaplasma* 16S rRNA amplicon HRM profiles were re-amplified using longer primers targeting 16S rRNA (PER1-PER2 for *Ehrlichia* and EHR16SD-1492R for *Anaplasma*). Rickettsia 16S rRNA positive samples were re-amplified using rickettsial outer membrane protein B (ompB) gene primers (120–2788 and 120–3599) (Rouxand Raoult, 2000).

PCR analyses were performed using 10-µL reaction volumes which included 2 µl 5x HOT FIREPol® EvaGreen® HRM mix (Solis BioDyne, Tartu, Estonia), 0.5 µl of 10 pmol forward and reverse primers, 25 ng of DNA template and the volume brought to 10 µL using PCR grade water. The PCR conditions used for amplification were: Initial enzyme activation at 95ºC for 15 minutes, followed by 10 cycles of denaturation at 94°C for 20 s, step-down annealing from 63.5°C to 53.5°C (decreasing by 1°C per cycle) for 25 s, extension at 72°C for 30 s followed by 25 cycles of denaturation at 94°C for 25 s, annealing at 50°C for 20 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 7 minutes. A 3-min hold at 72°C was included after PCR cycling before running HRM analysis. After PCR amplification, HRM analysis of the PCR amplicons followed. The HRM profiles were obtained by gradually increasing the temperature from 75–90˚C with increment additions of 0.1°C/2 sec (Mwamuye *et al*., 2017). Water was used as a negative control. No amplification was obtained from the negative control indicating no contaminations during preparation of the PCR reactions. Representative samples from each unique melt profile were purified with ExoSAP-IT PCR Product Cleanup kit (Affymetrix, Santa Clara, CA, USA) and submitted for sequencing by sanger method (Macrogen, Netherlands). Chromatogram files were imported into Geneious Prime software version 2020.2.2 (Biomatters, US) and used to generate consensus sequences.

Table 3.1: Primers used for molecular identification of ticks and TBPs

Primer Name	Target gene	Sequence (5’-3’)	Amplicon size (bp)	Reference
Tick COI F **Tick COI R**	COI	ATTCAACCAATCATAAAGATATTGG TAAACTTCTGGATGTCCAAAAAATCA	658	(Hebert *et al*., 2004)
SR-J-14199F **SR-N-14594R**	12S rRNA	TACTATGTTACGACTTAT AAACTAGGATTAGATACCC	430	(Simon *et al*., 1994)
Tick 16S **Tick 16S**	16S rRNA	AATTGCTGTAGTATTTTGAC TCTGAACTCAGATCAAGTAG	450	(Brahma *et al*., 2014)
Rick-F **Rick-R**	*Rickettsia* 16S rRNA	GAACGCTATCGGTATGCTTAACACA CATCACTCACTCGGTATTGCTGGA	364	(Nijhof *et al*., 2007)
120–2788 **120–3599**	*Rickettsia* ompB	AAACAATAATCAAGGTACTGT TACTTCCGGTTACAGCAAAGT	856	(Roux & Raoult, 2000)
Trans1 **Trans2**	*Coxiella* IS1111	TGGTATTCTTGCCGATGAC GATCGTAACTGCTTAATAAACCG	687	(Hoover *et al*., 1992)
Ehrlichia16S F **Ehrlichia16S R**	*Ehrlichia* 16S rRNA	CGTAAAGGGCACGTAGGTGGACTA CACCTCAGTGTCAGTATCGAACCA	200	(Tokarz *et al*., 2009)
PER1 **PER2**	*Ehrlichia* 16S rRNA	TTTATCGCTATTAGATGAGCCTATG CTCTACACTAGGAATTCCGCTAT	451	(Goodman *et al*., 1996)
EHR16SD **1492R**	*Ehrlichia* 16S rRNA	GGTACCYACAGAAGAAGTCC GGTTACCTTGTTACGACTT	1030	(Reysenbach *et al*., 1992; Parola *et al*., 2000)
AnaplasmaJV F **AnaplasmaJV R**	*Anaplasma* 16S rRNA	CGGTGGAGCATGTGGTTTAATTC CGRCGTTGCAACCTATTGTAGTC	300	(Mwamuye *et al*., 2017)
RLB F **RLB R**	Theileria/Babesia 18S rRNA	GAGGTAGTGACAAGAAATAACAATA TCTTCGATCCCCTAACTTTC	460 - 520 bp	(Gubbels *et al*., 1999)

3.8 Genetic and phylogenetic sequence analysis

Nucleotide sequences obtained in this study were edited and aligned alongside related tick or pathogen sequences available in GenBank nr database (http://www.ncbi. nlm.nih.gov/) using the MAFFT plugin in Geneious Prime software version 2020.2.2 (Biomatters) (Kearse *et al*., 2012). Nucleotide similarities were computed using PhyML v. 3.0 (Guindon et al., 2010a, b) plugin in Geneious software and phylogenetic relationships estimated using the maximum likelihood (ML) method with 1000 replications in the bootstrap test. The substitution model applied to construct phylogenetic trees was an Akaike information criterion for automatic model selection by Smart Model Selection in PhyML. Estimation of tree topologies was done using nearest neighbour interchange (NNI) improvements. Phylogenetic trees were visualized using FigTree v1.4.4.

3.9 Estimating tick infection rates using minimum infection rate (MIR)

The minimum infection rates (MIRs) of each of the obtained TBPs for each tick species were calculated as number positive pools / total number of ticks of that species tested × 100, with an assumption that only one tick is positive in a pool.

3.10 Genetic distance

Multiple sequence alignment between the ticks and TBPs was conducted on Mega 7. A comparison of estimated evolutionary divergence between the sequences obtained in this study and those of closely related published sequences from GenBank was subsequently compared by determining the number of base differences per sequences. Positions containing gaps and missing data were eliminated. Analysis was conducted using the Tajima-Nei model.

CHAPTER FOUR

RESULTS

4.1 Morphological identification of ticks

A total of 2610 adult ticks collected from camels in 12 different sites (range 0 – 20 ticks per camel and 22 - 750 ticks per site) were morphologically identified. The morphologically identified ticks were all Ixodidae and belonged to eight different species; *Hyalomma dromedarii*, *Hyalomma rufipes*, *Hyalomma impeltatum*, *Hyalomma truncatum*, *Amblyomma lepidum*, *Amblyomma gemma*, *Rhipicephalus camicasi*, and *Rhipicephalus pulchelus*. Majority of ticks collected were *Hy. dromedarii* (35.21%), followed by *Hy. rufipes* (31.03%), *Am. lepidum* (12.64%), *Hy. impeltatum* (8.47%), *Am. gemma* (4.95%), *Rh. pulchellus* (3.98%), *Rh. camicasi* (72; 2.76%), and *Hy. truncatum* (0.96%). In addition, 86 ticks belonging to five species were collected from sheep: *Rh. camicasi* (n = 45; 52.33%), *Am. gemma* (n = 15; 17.44%), *Am. lepidum* (n = 24; 27.91%), *Rh. pulchellus* (n = 1; 1.16%), and *Hy. rufipes* (n = 1; 1.16%). **Table 4.1** summarizes the number of tick species collected from camel and co-grazing sheep in Marsabit County, northern Kenya. **Figure 4.1** shows images of the tick species identified in this study.

Table 4.1: Ticks identified from camels and sheep from Marsabit County, northern Kenya

	From camels					From co-grazing sheep				
Species	**Male**	**Female**	**No. of pools**	**No. of ticks**	**Percent (%)**	**Male**	**Female**	**No. of pools**	**No. of ticks**	**Percent (%)**
Am. gemma	80	49	87	129	**4.95**	11	4	12	15	**17.44**
Am. lepidum	186	144	120	330	**12.64**	20	4	12	24	**27.91**
Hy. dromedarii	624	295	233	919	**35.21**	-	-	-	-	**-**
Hy. rufipes	557	253	251	810	**31.03**	1	-	1	1	**1.16**
Hy. truncatum	19	6	12	25	**0.96**	-	-	-	-	**-**
Hy. impeltatum	153	68	44	221	**8.47**	-	-	-	-	**-**
Rh. pulchellus	73	31	66	104	**3.98**	1	-	1	1	**1.16**
Rh. camicasi	30	42	24	72	**2.76**	22	23	22	45	**52.33**
Total	**1734**	**876**	**858**	**2610**		**55**	**31**	**48**	**86**	

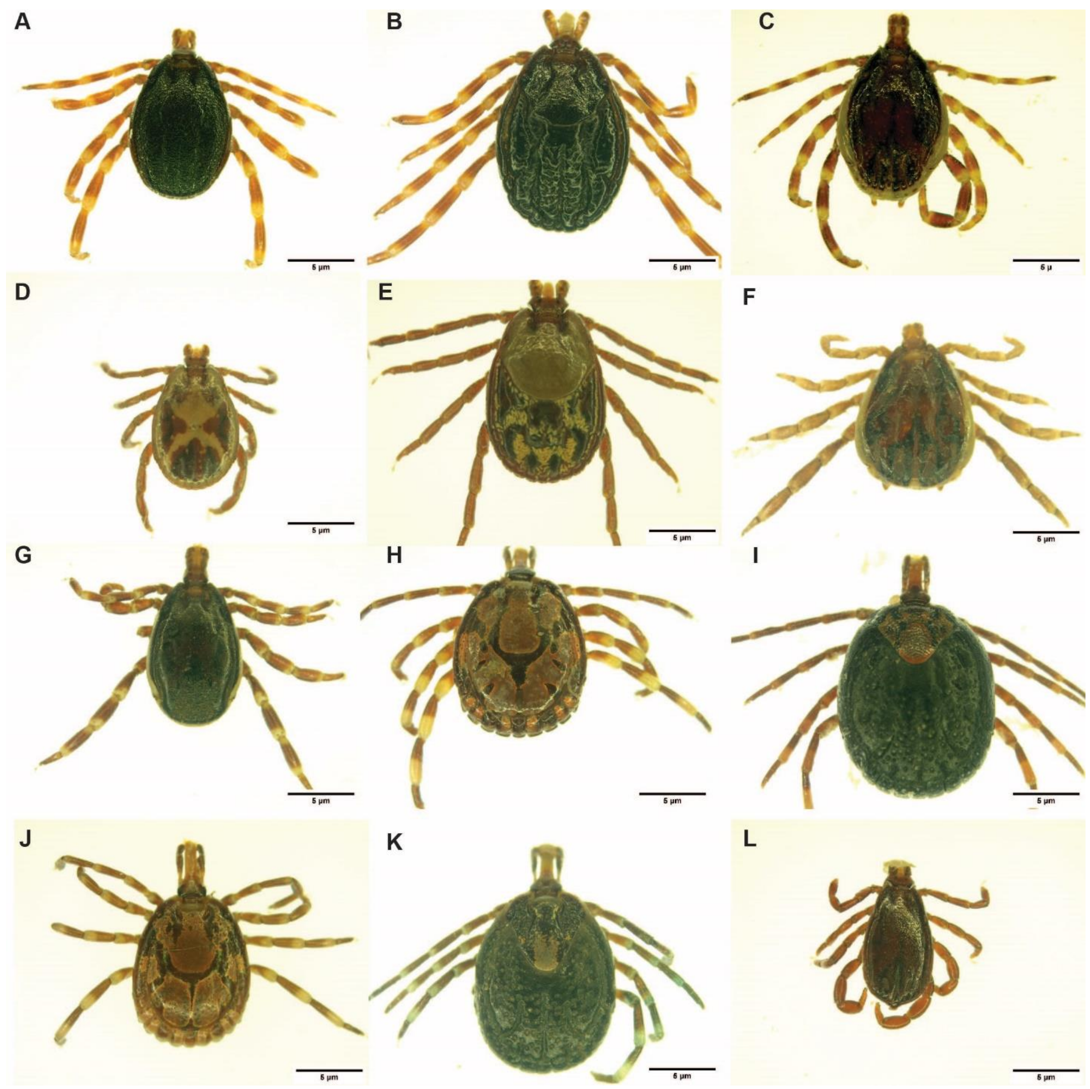

Figure 4.1: Images of tick species sampled from camel in northern Kenya. **A**. *Hyalomma rufipes* male **B**. *Hyalomma rufipes* female **C**. *Hyalomma impeltatum* male **D**. *Rhipicephalus pulchellus* male **E**. *Rhipicephalus pulchellus* female F. *Hyalomma dromedarii* male **G**. *Hyalomma truncatum* male **H**. *Amblyomma lepidum* male **I**. *Amblyomma lepidum* female **J**. *Amblyomma gemma* male **K**. *Amblyomma gemma* female **L**. *Rhipicephalus camicasi* male.

4.2 Molecular identification of ticks

Four tick samples amplified with COI gene, 15 samples with 12S rRNA and eleven samples with 16S rRNA. The homology analysis of *Rh. pulchellus, Rh. camicasi, Am. gemma, Am. lepidum, Hy. truncatum, Hy. impeltatum, Hy. dromedarii,* and *Hy. rufipes* sequences showed identities ranging from 99 to 100% with published sequences from

GenBank nr database (**Table 4.2**). Molecular identification was consistent with morphological identification across all the three amplified genes (COI, 12S rRNA and 16S rRNA). All tick sequences obtained in this study have been deposited in GenBank under accessions MT896151-MT896154 for tick CO1 gene, MT895169-MT895181 for tick 16S rRNA gene and MT895851-MT895865 for tick 12S rRNA gene. Phylogenetic analysis showing placement of the sequences to their respective species level is shown in **Figure 4.2 – 4.4**. The estimates of evolutionary diversity showing number of base substitutions per site are shown in **Table 4.3** and **Appendices I-II.**

4.2.1 Tick CO1 sequence analysis

The CO1 tick sequences obtained in this study were deposited in the GenBank database. *Hyalomma dromedarii* sequence submitted to GenBank under accession MT896151 shared 100% nucleotide identity with reference species from United Arab Emirates (MG188799 and MG188800), and Iran (KT920181). The number of nucleotide difference was 0 nucleotides). *Hyalomma impeltatum* sequence submitted to GenBank accessions MT896152 and MT896153 shared 99.2% similarity reference species from Saudi Arabia (GenBank accession KU130600). *Hy. rufipes* sequence submitted to GenBank accession MT896154 shared 99.7% similarity with *reference sequence* from Kenya (JX049282), Somalia (JX049276) and Cameroon (MK648422), differing by 2 nucleotides, 3 nucleotides, and 2 nucleotides respectively **3**). Phylogeny based on the CO1 sequences was inferred using a soft tick *Argas persicus* as the out-group (**Figure 4.2**) and the number of base substitutions per site are shown in **Table 4.2.**

Table 4.2: Morphological and molecular identification of tick samples collected from camels in Marsabit County, northern Kenya

Sample Id	Morphological ID	12S rRNA (% homology, GenBank Accession)	16S rRNA (% homology, GenBank Accession)	COI (% homology, GenBank Accession)	Consensus ID (GenBank Accession)
T1	*Hyalomma dromedarii*	*Hy. dromedarii* (99.7%, MH094484)	*Hy. dromedarii* (100%, MN960589)	*Hy. dromedarii* (100%, MT107484)	*Hy. dromedarii* (12S: MT895851; 16S: MT895169; COI: MT896151)
T108	*Hyalomma dromedarii*	*Hy. dromedarii* (100%, MH094484 and KT391030)	*Hy. dromedarii* (100%, MN960589)	-	*Hy. dromedarii* (12S: MT895852; 16S: MT895170)
T120	*Hyalomma dromedarii*	*Hy. dromedarii* (100%, MH094484 and KT391030)	-	-	*Hy. dromedarii* (12S: MT895853)
T208	*Rhipicephalus pulchellus*	*Rh. pulchellus* (100% KY676841, AF150024)	*Rh. pulchellus* (100% MK774738)	-	*Rh. pulchellus* (12S: MT895854; 16S: MT895171)
T209	*Rhipicephalus pulchellus*	*Rh. pulchellus* (100% KY676841, AF150024)	*Rh. pulchellus* (100% MK774738)	-	*Rh. pulchellus* (12S: MT895855; 16S: MT895172)
T275	*Rhipicephalus camicasi*	*Rh. camicasi* (100% FJ536556, MH094506)	-	-	*Rh. camicasi* (12S: MT895856)
T281	*Rhipicephalus camicasi*	*Rh. camicasi* (100% FJ536556, MH094506)	-	-	*Rh. camicasi* (12S: MT895857)

T303	*Hyalomma impeltatum*	*Hy. impeltatum* (100% KX132904, MN315384)	*Hy. impeltatum* (100% MN394439)	*Hy. impeltatum* (99.2% KU130599)	*Hy. impeltatum* (12S: MT895858; 16S: MT895175; COI: MT896152)
T318	*Hyalomma impeltatum*	*Hy. impeltatum* (100% KX132904, MN315384)	*Hy. impeltatum* (100% MN394439)	*Hy. impeltatum* (99.2% KU130599)	*Hy. impeltatum* (12S: MT895859; 16S: MT895176; COI: MT896153)
T362	*Hyalomma rufipes*	*Hy. rufipes* (100% MN394460)	*Hy. rufipes* (100% MK737650, MK737649)	-	*Hy. rufipes* (12S: MT895860; 16S: MT895177)
T403	*Hyalomma rufipes*	*Hy. rufipes* (100% MN394460)	*Hy. rufipes* (100% MK737650, MK737649)	*Hy. rufipes* (99.7% JX049282)	*Hy. rufipes* (12S: MT895861; 16S: MT895178; COI: MT896154)
T700	*Amblyomma lepidum*	Am. lepidum (100% MK332385)	*Am. lepidum* (100% KP987777)	-	*Am. lepidum* (12S: MT895862; 16S: MT895179)
T708	*Amblyomma lepidum*	-	*Am. lepidum* (100% MK737651)	-	*Am. lepidum* (16S: MT895180)
T710	*Amblyomma gemma*	*Am. gemma* (99.7% KX377407)	-	-	*Am. gemma* (12S: MT895863)
T749	*Amblyomma gemma*	*Am. gemma* (99.7% KX377407)	-	-	*Am. gemma* (12S: MT895864)
T852	*Hyalomma truncatum*	*Hy. truncatum* (100% KU568497)	*Hy. truncatum* (99.4% KU130475)	-	*Hy. truncatum* (12S: MT895865; 16S: MT895181)

Table 4.3: Estimates of evolutionary divergence between tick CO1 sequences. This pairwise analysis shows the number of nucleotide differences (number of nucleotides) between sequences produced in this study (in bold) and published sequences from different geographic origin. The analysis involved 18 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 538 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	AF132822 *Hy. dromedarii* (Australia)																	
2	KT920181 *Hy. dromedarii* (Iran)	2																
3	MG188799 *Hy. dromedarii* (Tunisia)	2	0															
4	**MT896151 *Hy. dromedarii* (Marsabit Kenya)**	2	0	0														
5	GQ483461 *Hy. dromedarii* (India)	4	2	2	2													
6	MG188800 *Hy. dromedarii* (UAE)	4	2	2	2	4												
7	KT906106 *Hy. detritum* (Iran)	64	64	64	64	63	64											
8	**MT896152 *Hy. impeltatum* (Marsabit Kenya)**	64	64	64	64	62	64	6										
9	**MT896153 *Hy. impeltatum* (Marsabit Kenya)**	63	63	63	63	61	63	7	1									
10	KU130600 *Hy. impeltatum* (Saudi Arabia)	64	64	64	64	63	64	6	4	5								
11	MF101817 *Hy. asiaticum* (China)	63	65	65	65	63	63	65	64	63	64							
12	AJ437099 *Hy. marginatum* (Ethiopia)	77	75	75	75	74	73	73	74	74	73	68						
13	KT989638 *Hy. turanicum* (Israel)	78	76	76	76	75	74	72	73	73	72	69	1					
14	MK648422 *Hy. rufipes* (Cameroon)	76	74	74	74	73	72	72	73	73	72	67	1	2				
15	**MT896154 *Hy. rufipes* (Marsabit Kenya)**	75	73	73	73	72	71	71	72	72	71	67	4	5	3			
16	JX049276 *Hy. rufipes* (Somalia)	77	75	75	75	74	73	73	74	74	73	66	2	3	1	2		
17	JX049282 *Hy. rufipes* (Kenya)	75	75	75	75	74	73	71	72	72	71	65	4	5	3	2	2	
18	FN394341 *Ar. persicus* (Outgroup)	143	142	142	142	142	142	130	130	129	129	134	133	134	134	135	134	134

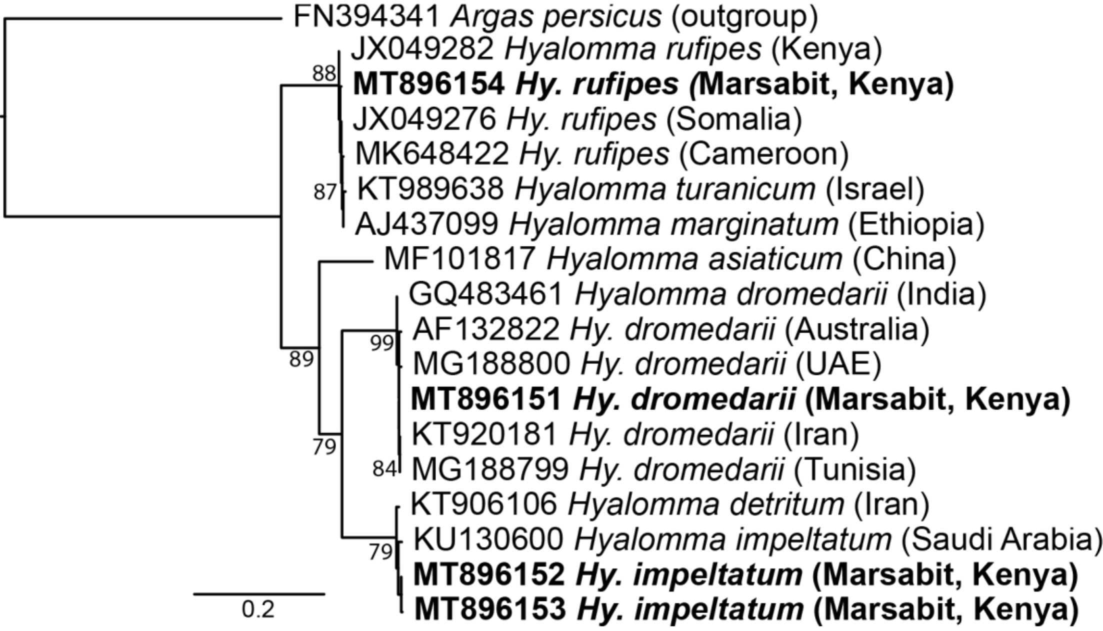

Figure 4.2. Maximum likelihood phylogenetic tree of tick CO1 mitochondrial gene sequences. The information provided include GenBank accession numbers, species identifications, and country of origin in brackets. Sequences obtained from this study are bolded. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site.

4.2.2 Tick 12S rRNA sequence analysis

The homology analysis of *Hyalomma dromedarii* sequences submitted to GenBank under accessions MT895851, MT895852 and MT895853 showed that the sequences were identical to each other and had 100% sequence identity with published *Hy. dromedarii* sequence from Egypt (KU963224) (**Figure 4.3**). *Rh. pulchellus* submitted to GenBank accessions MT895854 and MT895855 clustered closely with *Rh. pulchellus* from Tanzania (AF150024). *Rhipicephalus camicasi* sequence which was submitted to GenBank accessions MT895856 and MT895857 formed a clade with *Rh. camicasi* from Ethiopia (FJ536556). *Hyalomma impeltatum* submitted to GenBank accessions MT895858 and MT895859 formed a clade with *Hy. impeltatum* from Niger (KX132904) and Senegal (MN315384). *Hyalomma rufipes* submitted to GenBank under accessions MT895860 and MT895861 clustered with *Hy. rufipes* from Senegal (MN315383). *Amblyomma lepidum* sequence submitted to GenBank accession MT895862 clustered

closely with *Am. lepidum* from Uganda (MK332385). *Amblyomma gemma* submitted to GenBank accession MT895863 and MT895864 clustered with *Am. gemma* from Ethiopia (KX377407) and *Hy. truncatum* submitted to GenBank accession MT895865 closely clustered with *Hy. truncatum* from Guinea Bissau (KU568497). The pairwise analysis performed to determine differences between the sequences generated in this study and published sequences from GenBank is shown in **Appendix I**

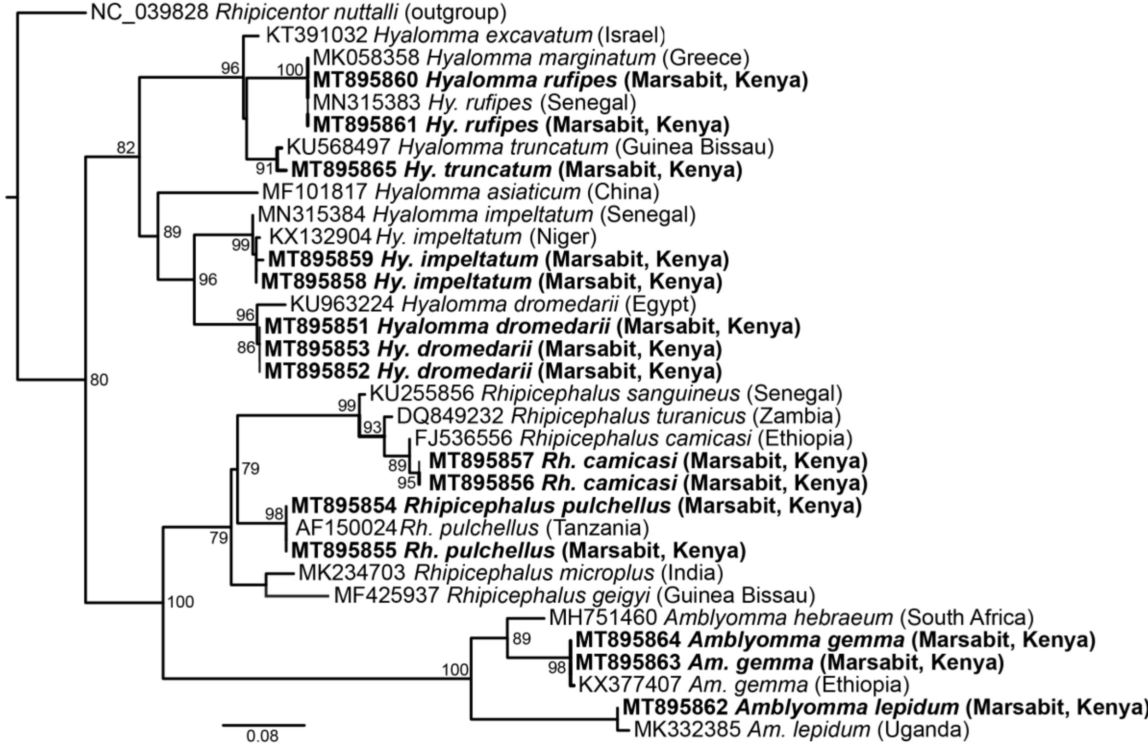

Figure 4.3. **Maximum likelihood phylogenetic tree of tick 12S rRNA gene sequences**.The information provided include GenBank accession numbers, species identifications, and country of origin in brackets. Sequences obtained from this study are bolded. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site.

4.2.3 Tick 16S rRNA sequence analysis

A *BLASTn* search analysis of *Hy. dromedarii* sequences submitted to GenBank accessions MT895169 and MT895170 showed 100% sequence identity with *Hy. dromedarii* (MN960589). *Rhipicephalus pulchellus* submitted to GenBank accessions MT895171 and MT895172 shared 100% identity with *Rh. pulchellus* from D.R. Congo (MK774738). *Hyalomma impeltatum* submitted to GenBank accession MT895175 and MT895176 shared 100% identity with *Hy. impeltatum* from Senegal (KU130436). *Hyalomma rufipes* submitted to GenBank accessions MT895177 and MT895178 shared 100% identity with *Hy. rufipes* from Egypt (MK737650). *Amblyomma lepidum* submitted to GenBank accessions MT895179 and MT895180 shared 99.7% sequence identity with *Am. lepidum* from Israel (KP987777). The homology analysis of *Hy. truncatum* sequence submitted under GenBank accession MT895181 shared 100% sequence identity with *Hy. truncatum* from Kenya (KU130475) **Figure 4.4**. The number of nucleotide differences identified are shown in **Appendix II.**

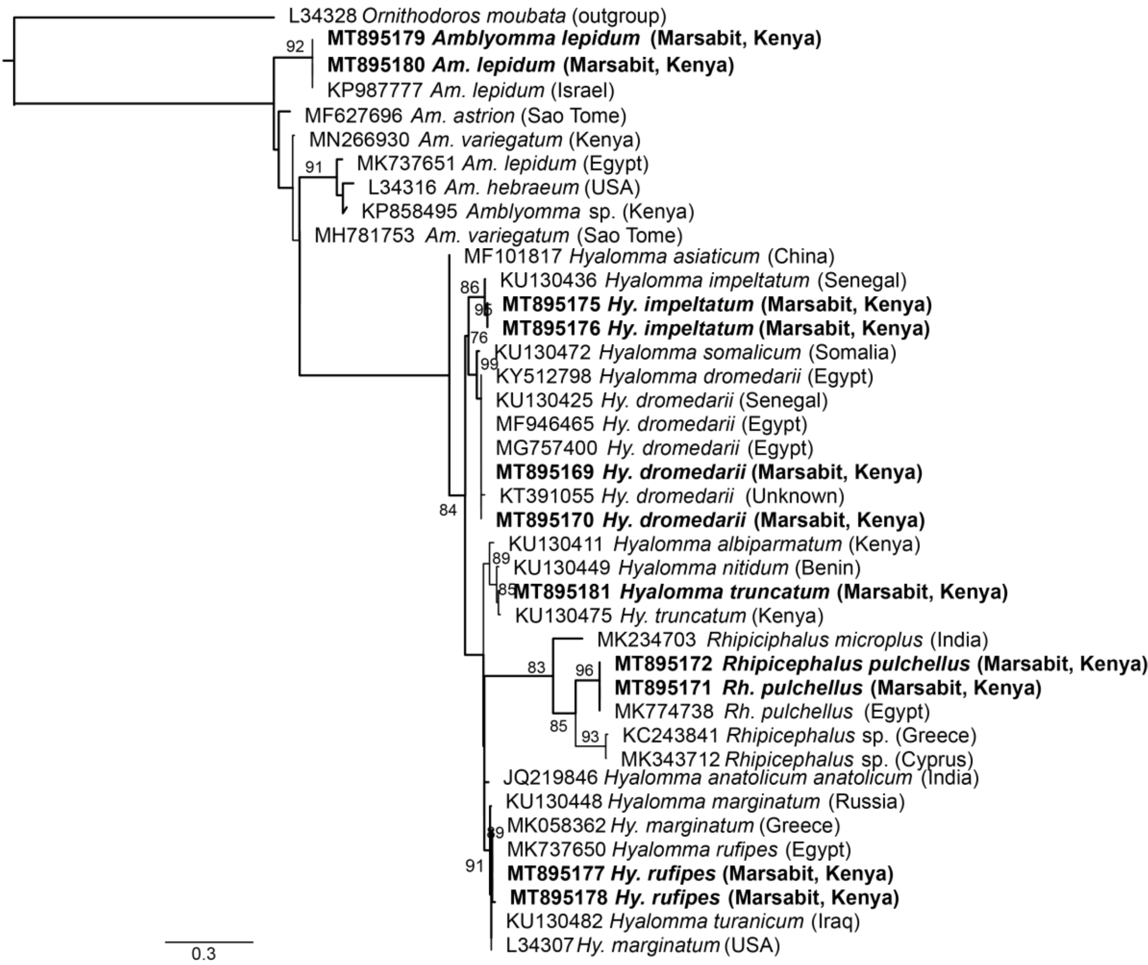

Figure 4.4. Maximum likelihood phylogenetic tree of tick 16S rRNA gene sequences. The information provided include GenBank accession numbers, species identifications, and country of origin in brackets. Sequences obtained from this study are bolded. The evolutionary relationships were inferred using the maximum likelihood method. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site.

4.3 Tick-borne pathogens detected in blood

Tick-borne pathogens with distinct HRM profiles (**Figure 4.5**), and sharing ≥ 99% sequence identity with published sequences from other regions (**Table 4.4; Table 4.5**) were detected in this study. The pathogens detected in camel blood were *Coxiella burnetii* (3.38%), "*Candidatus* Ehrlichia regneryi" (14.53%) and "*Candidatus* Anaplasma camelii" (78.72%). "*Candidatus* Anaplasma camelii" was the most prevalent TBP detected in

blood. *Erlichia ruminantium* (1.3%), *E. chaffeensis* (2.60%), *Anaplasma ovis* (88.3%), and *Theileria ovis* (88.3%) were detected in blood from co-herded sheep.

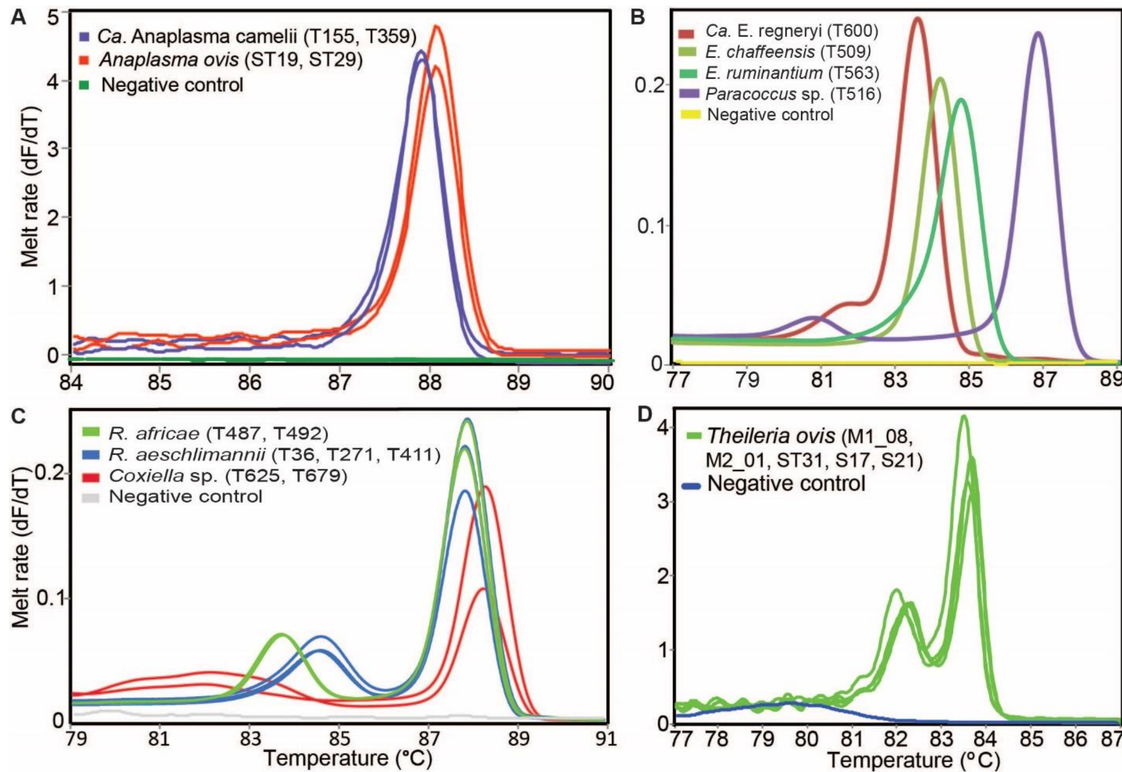

Figure 4.5: HRM Melt rate profiles showing pathogen diversity detected in blood and ticks. A. *Anaplasma* 16SrRNA amplicons, **B**. *Ehrlichia* 16S rRNA amplicons, **C**. *Rickettsia* 16S rRNA amplicons and **D**. *Theileria* 18S rRNA amplicons

Table 4.4: Minimum infection rates for TBPs identified in ticks and blood samples collected from camels in Marsabit, Kenya

Bacterial species (Target gene)	TBP Detection in Ticks – Number of Positive Pools (Minimum Infection Rate)								Camels with TBPs (Infection Rate)	GenBank Accessions		
	Hy. dromedarii	*Hy. rufipes*	*Hy. impeltatum*	*Hy. truncatum*	*Am. gemma*	*Am. lepidum*	*Rh. camicasi*	*Rh. pulchellus*		Study Sequences	Reference GenBank Accessions	Nucleotide Sequence Identity
No. of individuals	919 ticks	810 ticks	221 Ticks	25 ticks	129 ticks	330 ticks	72 ticks	104 ticks	296 camels			
Number of tick pools	254	251	44	12	87	120	24	66				
***Ehrlichia ruminantium* (16S rRNA)**	-	-	-	-	16 (12.40%)	17 (5.15%)	-	-	-	MT929193-MT929195	NR_074155, KU721071, CP001612	100%
***Ca.* Ehrlichia regneryi (16S rRNA)**	22 (2.39%)	46 (5.68%)	6 (2.72%)	-	-	-	-	-	43 (14.53%)	MT929189-MT929192	KF843826	100%
***Ehrlichia chaffeensis* (16S rRNA)**	-	-	-	-	-	2 (0.61%)	-	-	-	MT929188	NR_074500, NR_074501, CP007473-	100%

										CP007480	
***Ehrlichia* sp. (16S rRNA)**	-	1 (0.12%)	-	-	1 (0.78%)	-	3 (4.17%)	18 (17.31%)	**-**	MT929196-MT929197	MN726921, KJ410256	100%
***Candidatus* Anaplasma camelii (16S rRNA)**	25 (2.72%)	27 (3.33%)	6 (2.72%)	1 (4%)	11 (8.53%)	20 (6.06%)	6 (8.33%)	7 (6.73%)	233 (78.72%)	MT929199-MT929201, MT929169-MT929177	MT510533, MK388297	100%
***Anaplasma* sp. (16S rRNA)**	-	1 (0.12%)	-	-	-	-	-	-	-	MT929202	KJ410248, KJ410249	100%
***Rickettsia africae* (*ompB*)**	-		-	-	14 (10.85%)	31 (9.39%)	-	-	**-**	MT900495-MT900496	KU721071, KT032136, CP0011612	100%
***Rickettsia aeschlimannii* (*ompB*)**	3 (0.33%)	87 (1.07%)	6 (2.72%)	1 (4.00%)	-	-	-	5 (4.81%)	**-**	MT900489-MT900494	MK215215-MK215218	100%
***Coxiella burnetii* (IS1111)**	11 (1.20%)	12 (1.50%)	-	-	-	-	-	5 (4.81%)	10 (3.38%)	MT900497-	MT268529-MT2685	100%

									MT900501	29, KT954146	
***Coxiella* endo-symbiont (16S rRNA)**	-	-	-	-	12 (9.30%)	16 (22.22%)	-	6 (5.77%)	-	MW541904-MW541911	EU143670, JX846589, MK026405	98-100%
***Paracoccus* sp. (16S rRNA)**	2 (0.22%)	8 (1.00%)	2 (0.90%)	1 (4.00%)	1 (0.78%)	-	-	3 (2.88%)	-		KP003988	99%

Table 4.5: Minimum infection rates for TBPs identified in ticks and blood samples collected from sheep in Marsabit County, Kenya

Pathogens	Ticks					Blood	GeneBank Accessions		
	Hy. rufipes	*Am. gemma*	*Am. lepidum*	*Rh. camicasi*	*Rh. pulchellus*		Study sequences	Reference sequences	Similarity
No. of pools	1	12	12	22	1				
No. of individuals	1	14	24	45	1	**77**			
Ehrlicha ruminantium	-	2 (14.29%)	1 (4.17%)	-	-	1 (1.30%)	MW467546	MH246936 and U03776	99.7%
Ehrlichia chaffeensis	-	-	-	-	-	2 (2.60%)	-	NR_074501	100%
Anaplasma ovis	-	2 (14.29%)	2 (8.33%)	7 (15.56%)	1 (100%)	68 (88.31%)	MW467547-MW467552	MG869525	100%
***Ca.* Anaplasma camelii**	-	-	-	1 (2.22%)	-	-	MW690202	MN630836	98%
Rickettsia africae	-	2 (14.29%)	4 (16.67%)	-	-	**-**	MW478135-MW478138	KU721071	100%
Theileria ovis	-	-	-	1 (2.22%)	-	62 (80.52%)	MW467555-MW467561	MN712508, KX273858 and MG738321	100%

4.4 Pathogens and endosymbionts detected in ticks

The results obtained from screened a total of 874 tick pools from camels showed presence of a wide variety of TBPs. Positive pools for *Anaplasma*, *Ehrlichia*, *C. burnetii*, and Rickettsia species were detected. *Coxiella* endosymbionts were also detected **(Table 4.4)**. None of the tick pools was positive for *Theileria* and *Babesia* spp.

Ehrlichia ruminantium was detected in *Am. gemma* (MIR = 12.40%) and in *Am. lepidum* (MIR = 5.15%) by PCR-HRM assay. Sequencing of the PCR product and subsequent *BLASTn* search analysis of the obtained sequences indicated 100% sequence identity with *E. ruminantium* (NR_074155, MH246936, X61659 and X62432). "*Candidatus* Ehrlichia regneryi" was detected in *Hy. dromedarii* (MIR = 2.39%), *Hy. rufipes* in (MIR = 5.68%) and *Hy. impeltatum*in (MIR = 2.72%). The homology analysis of the obtained sequences showed they shared 100% sequence identity with "*Ca.* Ehrlichia regneryi" sequence from Saudi Arabia (KF843826). *Ehrlichia chaffeensis* was detected in *Am. lepidum* (MIR = 0.61%). The homology analysis of the 16S rRNA sequence obtained in this study indicated 100% sequence identity with *E. chaffeensis* (U60476, NR_074500' NR_074501 and CP007473 - CP007480). *Ehrlichia* sp. was detected in *Hy. rufipes* (MIR = 0.12%), *Am. gemma* (MIR = 0.78%), *Rh. camicasi* tick pools (MIR = 4.17%) and *Rh. pulchellus* tick pools (MIR = 17.31%). Two representative *Ehrlichia* spp. sequences (MT929196 and MT929197) could not be resolved to species level and the homology analysis indicated 100% sequence similarity with unidentified *Ehrlichia* sp. (MN726921).

Coxiella burnetii and *Coxiella* endosymbionts were detected in this study. *Coxiella burnetii* was detected in camel blood samples (3.38%) and 28 camel tick pools. The *C. burnetii* sequences obtained in this study were identical to each other and shared 100% sequence identity with *Coxiella burnetii* (MT268529 and KT954146) from other regions. *Coxiella burnetii* was detected in *Hy. dromedarii* (MIR = 1.2%), *Hy. rufipes* (MIR = 1.5%) and *Rh. pulchellus* (MIR = 4.81%). *Coxiella* endosymbionts were detected in *Am. gemma* (MIR = 10.08%), *Am. lepidum* (MIR = 5.15%) and *Rh. pulchellus* (MIR = 4.81%).

Rickettsia aeschlimannii was detected in *Hy. dromedarii* (MIR = 0.33%), *Hy. rufipes* (MIR =.74%), *Hy. impeltatum* (MIR = 2.72%), *Hy. truncatum* (MIR = 4%), and *Rh. pulchellus* (MIR = 4.81%). *BLASTn* search revealed 100% sequence identity with *R. aeschlimannii* (MK215215-MK215218). *Rickettsia africae* was detected in *Am. gemma* (MIR = 10.85%) and *Am. lepidum* (MIR = 9.39%). The *R. africae* sequences obtained in this study were identical to each other and shared 100% sequence identity with *R. africae* (KU721071, KT032136 and CP0011612)

"*Candidatus* Anaplasma camelii" was detected in *Hy. rufipes* (MIR = 3.33%), *Hy. dromedarii* (MIR = 2.72%), *Hy. impeltatum* (6/44; 2.72%), *Hy. truncatum* (MIR = %), *Rh. camicasi* (MIR = 8.33%), *Rh. pulchellus* (MIR =.73%), *Am. gemma* (MIR = 8.53%), and *A. lepidum* (MIR = 6.06%). The obtained sequences were identical to each other and shared 100% sequence identity with "*Ca.* Anaplasma camelii" (MT510533 and MK388297). However, one *Anaplasma* sp. sequence could not be resolved down to species level with *BLASTn* search analysis indicating 99.8% sequence identity with unidentified *Anaplasma* sp. from China (KJ410248 and KJ410249). The *Anaplasma* sp. was detected in *Hy. rufipes* (MIR = 0.4%).

A variety of pathogens, including zoonotic pathogens *E. ruminatium* and *R. africae*, were detected in ticks collected from camel's co-grazing sheep. *Ehrlichia. ruminantium* was detected in *Am. gemma* (MIR = 14.29%) and *Am. lepidum* (MIR = 4.17%). *Theileria ovis* was detected in *Rh. camicasi* (MIR = 2.22%) while *Anaplasma ovis* was detected in *Am. gemma* (MIR = 14.29%), *Am. lepidum* (MIR = 8.33%), *Rh. camicasi* (MIR = 15.56%), and *Rh. pulchellus* (MIR = 100%) tick species. *Anaplasma* sp. close to *Anaplasma platys* was detected in *Rh. camicasi*. *Rickettsia africae* was detected in *Am. gemma* (MIR = 14.29%) and *Am. lepidum* (MIR = 16.67%) (**Table 4.4**). The distributions of ticks and pathogens according to the sampling sites are shown in **Appendix IV**.

All sequences generated in this study have been submitted to GenBank accessions: *R. aeschlimannii* (MT900489-MT900494), and *R. africae* (MT900495-MT900496) , *C. burnetii* (MT900497- MT900501) for, *Ca*. Ehrlichia regneryi (MT929189 - MT929192),

E. ruminantium (MT929193 - MT929195 and MW467546), *E. chaffeensis* (MT929188), *Ehrlichia* spp.(MT929196- MT929197), *Ca.* Anaplasma camelii (MT929169- MT929177 and MT929199 - MT929201), *Anaplasma* sp. (MT929202), *Coxiella* endosymbionts (MW541904- MW541911), *T. ovis* (MW467547- MW467552), and *A. ovis* (MW467555-MW467561).

Estimated evolutionary divergence between the obtained gene sequences and closely related sequences from GenBank was compared through determined the number of base differences using Tajima-Nei model in MEGA 7 (**APPENDIX III**). The phylogenetic relationships between the sequences obtained in this study and related sequences previously deposited in GenBank using Maximum Likelihood techniques is shown in **Figure 4.6**.

Table 4.6: **Estimates of evolutionary divergence between *Ehrlichia* spp. and *Anaplasma* spp. 16S rRNA sequences.** This pairwise analysis shows the number of nucleotide differences (number of nucleotides) between sequences produced in this study (in bold) and published sequences from different geographic origins. All ambiguous positions were removed for each sequence pair. The analysis involved 25 nucleotide sequences. The sequences obtained from this study are bolded. There was a total of 156 positions in the final dataset. Evolutionary analyses were conducted using Mega 7 (Kumar et al., 2016).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
1. AF069758 ***Ehrlichia* (*Cowdria*) *ruminantium*** (S. Africa)																									
2. MH246936 ***E. ruminantium*** (Tanzania)	0																								
3. MT929194 *E. ruminantium* (Marsabit Kenya)	0	0																							
4. CP033454 ***E. ruminantium*** (South Africa)	1	1	1																						
5. MT929193 *E. ruminantium* (Marsabit Kenya)	1	1	1	0																					
6. KF843826 ***Candidatus*** Ehrlichia regneryi (Saudi Arabia)	4	4	4	5	5																				
7. MT929191 *Ca*. Ehrlichia regneryi (Marsabit Kenya)	4	4	4	5	5	0																			
8. MT929189 *Ca*. Ehrlichia regneryi (Marsabit Kenya)	7	7	7	8	8	3	3																		
9. MT929190 *Ca*. Ehrlichia regneryi (MarsabitKenya)	4	4	4	5	5	0	0	3																	
10. MT066094 ***Ehrlichia canis*** (Egypt)	4	4	4	5	5	0	0	3	0																
11. NR_074500 ***Ehrlichia chaffeensis*** (USA)	5	5	5	6	6	1	1	4	1	1															
12. MT929188 *E. chaffeensis* (Marsabit Kenya)	5	5	5	6	6	1	1	4	1	1	0														

13. KJ410256 ***Ehrlichia*** sp. BL157-6 (China)	2	2	2	3	3	2	2	5	2	2	3	3												
14. MT929197 *Ehrlichia* sp. (Marsabit Kenya)	2	2	2	3	3	2	2	5	2	2	3	3	0											
15. MN630836 *Anaplasma platys* (China)	18	18	18	17	17	20	20	21	20	20	19	19	18	18										
16. MH936009 ***Candidatus*** Anaplasma camelii (Kenya)	18	18	18	17	17	20	20	21	20	20	19	19	18	18	0									
17. MT929169 *Ca*. Anaplasma camelii (Marsabit Kenya)	18	18	18	17	17	20	20	21	20	20	19	19	18	18	0	0								
18. MT510533 ***Ca***. Anaplasma camelii (Kenya)	18	18	18	17	17	20	20	21	20	20	19	19	18	18	0	0	0							
19. MT929201 *Ca*. Anaplasma camelii (Marsabit_Kenya)	18	18	18	17	17	20	20	21	20	20	19	19	18	18	0	0	0	0						
20. KJ410249 ***Anaplasma*** sp. BL102-7 (China)	18	18	18	17	17	20	20	21	20	20	19	19	18	18	5	5	5	5	5					
21. MT929202 *Anaplasma* sp. (Marsabit_Kenya)	19	19	19	18	18	21	21	22	21	21	20	20	19	19	6	6	6	6	6	1				
22. MG869525 ***Anaplasma ovis*** (China)	18	18	18	17	17	18	18	19	18	18	17	17	16	16	7	7	7	7	7	8	9			
23. MW467550 *A. ovis* (Marsabit Kenya)	18	18	18	17	17	18	18	19	18	18	17	17	16	16	7	7	7	7	7	8	9	0		
24. MW467551 *A. ovis* (Marsabit Kenya) Sheep	18	18	18	17	17	18	18	19	18	18	17	17	16	16	7	7	7	7	7	8	9	0	0	
25. KP276587 ***Candidatus*** Cryptoplasma californiense (outgroup)	18	18	18	17	17	20	20	23	20	20	19	19	18	18	10	10	10	10	10	10	11	13	13	13

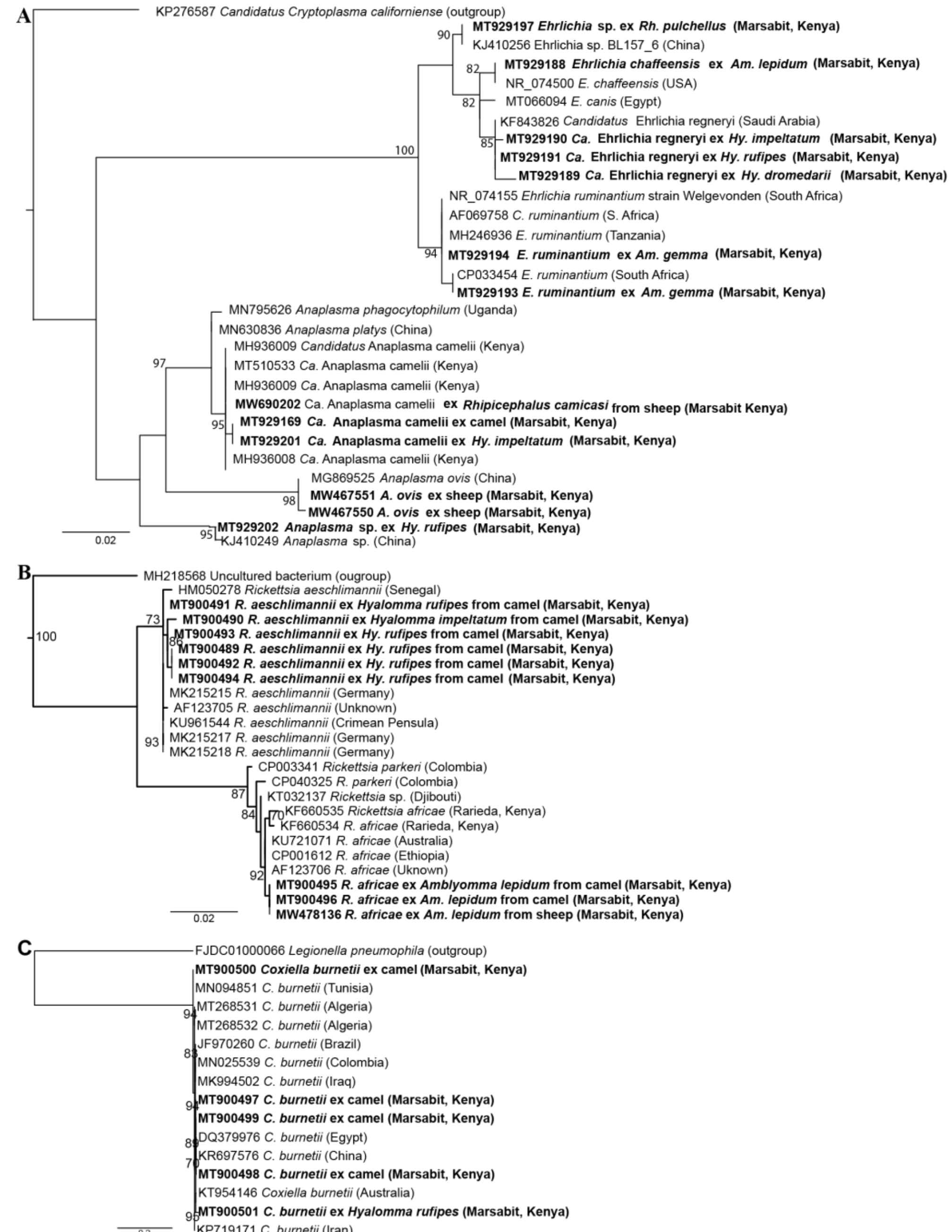

Figure 4.6. **Maximum likelihood phylogenetic tree of identified TBPs**. **A)** *Anaplasma* spp. (1030 bp) with 16S partial sequences and *Ehrlichia* spp. (451 bp) with 16S partial sequences **B)** *Rickettsia* spp. (857 bp) with ompB partial sequences, and **C)** *Coxiella burnetii* (687 bp) with IS1111 partial sequences showing the position of revealed sequences isolated from blood and ticks infesting camels and co-grazing sheep in Marsabit, Kenya. Sequences obtained from this study are bolded.

CHAPTER FIVE

DISCUSSION

This study investigated the diversity and abundance of camel and co-grazing sheep ticks and the TBPs harboured by these ticks and their animal hosts. The study reports presence of diverse tick species in camels and their associated TBPs circulating in northern Kenya, which creates a risk of disease dissemination. The study reports for the first time that *Hy. impeltatum* parasitizes camels in northern Kenya. Further, the study reports presence of zoonotic pathogens such as *E. ruminantium* (in camel ticks, sheep blood and ticks), *C. burnetii* (camel ticks and blood) and *R. africae* (in ticks from camel and sheep), and pathogens of veterinary importance such as "*Ca.* Anaplasma camelii", and "*Ca.* Ehrlichia regneryi" (in both blood and ticks from camels) in the study area. These pathogens are of major economic importance, thus represent major risks to the health and welfare of both humans and animals (Raboloko *et al*., 2020; Wikel, 2018; Jongejan & Uilenberg, 2004). The information on tick species diversity, ecology and distribution will help improve our understanding of disease dynamics (Kanduma *et al*., 2016). The information is critical in crafting effective TBD surveillance and prevention programs in northern Kenya.

5.1 Species diversity of ticks associated with camels and sheep in northern Kenya

Eight epidemiologically-important tick species from three different genera, *Hyalomma*, *Amblyomma* and *Rhipicephalus* were identified in camels and co-herded sheep using morphological tools and gene sequencing. *Hyalomma dromedarii* and *Hy. rufipes* were the most abundant tick species in camels. The camels were also infested by other tick species such as *Am. lepidum*, *Hy. impeltatum*, *Am. gemma*, *Rh. pulchellus*, *Rh. camicasi* and *Hy. truncatum*. *Rhipicephalus camicasi* was the most abundant tick species in sheep. Other tick species identified in sheep include: *Amblyomma gemma*, *Am. lepidum*, *Rh. pulchellus*, and *Rh. camicasi*.

The finding of *Hy. dromedarii* and *Hy. rufipes* as the most prevalent tick species sampled from camels agrees with studies from Saudi Arabia, Sudan, Egypt, Iran, and Tunisia where

Hy. dromedarii is considered to be the main tick species parasitizing dromedary camels (Elghali & Hassan, 2009; Moshaverinia & Moghaddas, 2015; Ghoneim *et al*., 2017; Selmi *et al*., 2019; Alanazi *et al*., 2019, 2020). *Hyalomma dromedarii* can pose a significant threat to animals' health since it has been implicated with transmission of emerging and re-emerging diseases like viruses such as Crimean-Congo haemorrhagic fever virus (CCHFV), rickettsiosis (Kleinerman *et al*., 2013; Wallménius *et al*., 2014), *Francisella* spp. (Ghoneim *et al*., 2017), and *C. burnetii* (responsible for zoonotic Q fever) (Bellabidi *et al*., 2020). *Hyalomma rufipes* are known to be a vector of CCHFV, *Babesia occultans, Anaplasma marginale, R. aeschlimannii,* and *Rickettsia conorii* (Kamani *et al*., 2013; Omondi *et al*., 2017; Chitimia-Dobler et al., 2019). Other *Hyalomma* spp. ticks found on camels in northern Kenya are *Hy. impeltatum* and *Hy truncatum*. Though *Hy. impeltatum* is known to infest a wide range of hosts such as sheep and cattle (Alanazi *et al*., 2019), the tick species is strongly associated with camels and has previously been found on dromedary camels in northern Sudan and Iran (Elghali & Hassan, 2009; Shemshad *et al*., 2012; Moshaverinia & Moghaddas, 2015). *Hyalomma impeltatum* has been implicated as the probable vector of Sindbis, Dhori (causing human febrile illnesses), CCHF viruses and *Theleria hirci* to sheep in Saudi Arabia (Alanazi *et al*., 2018).

The study found *Am. gemma* and *Am. lepidum* in camels and sheep. The *Amblyomma* spp. are of great economic importance (Jongejan & Uilenberg, 2004) since they are known to be efficient vectors of *E. ruminantium* in SSA (Allsopp, 2015). *Amblyomma* spp. ticks also serve as vector for emerging and remerging human and animal's pathogenic bacteria such as *C. burnetii*, new *Borrelia* spp. and SFG *Rickettsia* (Kumsa *et al*., 2015). Immature tick stages of *Ambylomma* ticks are known to often bite humans, whereby they act as vectors of tick-bite fever caused by *R. africae*. Rhipicephaline ticks, *Rh. camicasi* and *Rh. pulchellus* were detected in camels and sheep. *Rhipicephalus camicasi* has been implicated as probable vector of *A. platys* in dogs in Kenya and Ivory Coast (Matei et al., 2016). *Rhipicephalus pulchellus* has been implicated previously as a probable vector of Nairobi sheep diseases (Edelsten, 1975). The findings of *Rh. camicasi* in sheep and camels in northern Kenya extend knowledge about the geographic range and dynamics of this tick

species in Kenya. As most of these tick species are competent vectors for diseases, camels in the region may be exposed to a variety of TBDs.

The findings presented in this study provide important information on the distribution and abundance of ticks in northern Kenya. The diverse species of ticks found parasitizing camels in the study area indicates the important role domestic animals play in distribution and abundance of *Hyalomma*, *Amblyomma* and *Rhipicephalus* spp. ticks in northern Kenya. The Ministry of Livestock should encourage use of tick control methods such as strategic application of acaricides especially at the beginning of the short and long major rainy season to help lower tick infestation.

5.2 Tick-borne bacteria and endosymbionts identified in ticks and blood from camels and sheep

This study reports the occurrence of *Ehrlichia ruminantium*, *E. chaffeensis*, "*Ca*. Ehrlichia regneryi", *C. burnetii*, "*Ca*. anaplasma camelii", *R. africae*, *R. aeschlimannii*, *C. burnetii*, “*Ca*. Ehrlichia regneryi”, and "*Ca*. anaplasma camelii" in camel and co-herded sheep in northern Kenya. The study also reports presence of *Coxiella* endosymbionts and *Paracoccus* sp. in ticks collected from camel.

5.3 *Ehrlichia* spp.

The current study reports presence of a range of *Ehrlichia* spp. circulating in camels and co-herded sheep in northern Kenya.

This study provides molecular evidence showing presence of *E. ruminantium* in *Am. gemma* and *Am. lepidum* ticks sampled from camels and co-grazing sheep and in sheep blood, but not camel blood samples. *Ehrlichia ruminantium* is the causative agent of heartwater, an economically important rickettsial disease in Caribbean islands and SSA (Walker & Olwage, 1987; Dumler et al., 2001; Jongejan & Uilenberg, 2004). The bacterium, mainly transmitted by *Amblyomma* ticks, is known to parasitize vascular endothelial cells, macrophages, and neutrophils of the mammalian hosts (Allsopp, 2015). *Ehrlichia ruminantium* has previously been detected in *Am. variegatum*, *Am. lepidum*, *Am. hebraem*, and *Am. gemma* ticks in Kenya (Ngumi *et al*., 1997; Omondi *et al*., 2017;

Mwamuye *et al*., 2017), and *Am. variegetam* and *Am. lepidum* in neighbouring Ethiopia (Teshale *et al*., 2015; Hailemariam *et al*., 2017). During sample collection for this project, cases of sick or death animals were not encountered to enable collection of samples for detection of *E. ruminantium* inclusion bodies in endothelial cells of capillaries in the brain. Most of the affected camels in the 2016 diseases outbreak (Younan *et al*., 2021) were indigenous breeds (Rendille, Gabra, and Somali), suggesting that local breeds may be susceptible to heartwater than initially thought. The cross-border movement and trade of camels may also jeopardize establishment of robust immunity against heartwater. Detection of *E. ruminantium* in *Am. gemma* and *Am. lepidum* in this study agrees with previous studies that have suggested the pathogen is transmitted by *Amblyomma* spp., and is a major cause of livestock loss in SSA (Ngumi *et al*., 1997; Allsopp, 2015). The findings of *E. ruminantium* in *Amblyomma* spp. further confirms recent reports on their potential impact on SS African camel populations (Bechir *et al*., nd; Younan *et al*., 2021). Absence of *E. ruminantium* in camel blood may suggest that the number of pathogens circulating in blood may be very low and that the pathogen has a predilection for endothelial cells and can only be periodically found in bloodstream (Lorusso *et al*., 2016). These findings provide evidence of circulation of *E. ruminantium* in northern Kenya, which poses a threat for domestic animals and human health. Though the study reports presence of the pathogen in ticks collected from camels, the possible role of camels as important reservoir hosts deserves further investigation.

This study serves as the first report of the molecular detection of the *E. chaffeensis* organisms in *Am. lepidum* ticks collected from dromedary camels. *Ehrlichia chaffeensis* was also detected in blood from sheep, but not ticks from sheep. *Ehrlichia chaffeensis*, an emerging TBP, is known to cause illness in humans (Paddock & Childs, 2003). The first human monocytic ehrlichiosis due to *E. chaffeensis* was reported in 1987 in the United States but became a reportable disease only in 1999 (Biggs *et al*., 2016). Since then, evidence of *E. chaffeensis* has been reported in more than 30 states in the United States, and in Asia, Latin America, and Africa. *Ehrlichia chaffeensis* has previously been found in *Dermacentor variabilis*, *Ixodes pacificus*, *Am. maculatum*, and *Am. americanum*, in

North America (Paddock and Yabsley, 2007), *Rh. microplus* in Argentina (Guillemi *et al*., 2019), in *Haemaphysalis leachi* ticks collected from dogs in Uganda (Proboste et al., 2015), *Rhipicephalus sanguineus* from dogs in Cameroon (Ndip *et al*., 2010), and in *Am. hebraeum* collected from both cattle and sheep in South Africa (Iweriebor *et al*., 2017). In Kenya, *Ehrlichia chaffeensis* has been reported in *Am. eburneum* ticks (Mwamuye *et al*., 2017). Combined, these findings suggest that diverse *Amblyomma* tick species may be vectoring this pathogen, however, further studies are needed to appraise *E. chaffeensis* vector competence.

"*Candidatus* Ehrlichia regneryi" was detected in blood and in *Hy. dromedarii*, *Hy. rufipes*, and *Hy. impeltatum* ticks collected from camels. Presence of "*Candidatus* Ehrlichia regneryi" *Hyalomma* spp. is interesting as it may suggest the importance of the tick species as vectors of the pathogen. "*Candidatus* Ehrlichia regneryi" is a novel *Ehrlichia* sp. first described in Saudi Arabia (Bastos et al., 2015), which has recently also been found in blood from camels in a recent outbreak in northern Kenya (Younan *et al*., 2021). "*Candidatus* Ehrlichia regneryi" is phylogenetically closely related to *E. canis* (Bastos *et al*., 2015). The findings from this study suggested that camels may play a role as reservoir hosts for "*Ca*. Ehrlichia regneryi". Further investigations are needed to identify vector competence, and zoonotic potential of "*Ca*. Ehrlichia regneryi".

5.4 *Coxiella* spp.

Coxiella burnetii is a zoonotic pathogen responsible for Q (Query) fever in both domestic ruminants and humans worldwide (Duron et al., 2015; Njeru *et al*., 2016). Q fever is one of the most widespread neglected zoonosis worldwide with the highest seroprevalence rates recorded in female camels with a history of abortion (Duron *et al*., 2015). A study from Chad well documents the association between camel exposure, seroprevalence in camels and human Q fever infections (Schelling *et al*., 2003). Further studies have shown that *C. burnetii* is transmitted through inhalation of contaminated aerosols, birth products, infected faeces, through tick bite or even sexually through infected sperm cells (Maurin & Raoult, 1999; Njeru *et al*., 2016; Eldin *et al*., 2017). In Laikipia, Kenya, just south of

this study's geographic focus, 18.6% of camels have been found to have been exposed to *C. burnetii* by seropositivity (Browne *et al*., 2017). The current study detected *C. burnetii* in blood and *Hy. rufipes*, *Hy. dromedarii* and *Rh. pulchellus* ticks from dromedary camels. These findings agree with previous studies that have reported presence of *C. burnetii* in *Hyalomma* spp. ticks in Algeria, *Hy. dromedarii* and *Hy. impeltatum* ticks from camels in Tunisia, and in *Rhipicephalus* and *Hyalomma* spp. in Senegal (Mediannikov *et al*., 2010) and Kenya (Knobel *et al*., 2013; Ndeereh *et al*., 2017; Koka *et al*., 2018). Cases of Q fever outbreak in Kenya have been reported among local communities living in Baringo and among international travellers. Several studies on seroprevalence surveillance reports in Kenya have found antibodies against *C. burnetii* in domestic animals and humans (Knobel *et al*., 2013). Domestic animals are known to be the main reservoirs of infections for humans (Njeru *et al*., 2016). Q fever outbreaks have been reported in the United States mainly from occupational exposures involving livestock farmers, meat processing plant workers, veterinarians, dairy workers, and researchers at facilities housing sheep. Ticks are known to be important vectors of *C. burnetii* and are known to maintain infection in domestic animals (Duron *et al*., 2015). They acquire the pathogen during blood meal. This show that camels and their associated ticks in northern Kenya are probably an important epidemiological reservoir of the pathogen, which increases human exposure to these zoonotic pathogens, and that veterinarians, farmers and abattoir workers in northern Kenya are at risk. Therefore, effort should be made to increase awareness of Q fever in public, veterinary health authorities, animal handlers, and decision makers. Further studies are needed to better understand the role of camels in the epidemiology of Q fever.

Coxiella endosymbionts were detected in *Am. gemma*, *Am. lepidum*, and *Rh. pulchellus* ticks from Rickettsia 16S rRNA primer amplicons. These results are consistent with reports identifying *Coxiella* endosymbionts in ticks collected from coastal region (Mwamuye *et al*., 2017), the Maasai Mara National Reserve (Oundo *et al*., 2020), and Busia (Chiuya *et al*., 2020). Previous studies have suggested that *Coxiella* endosymbionts help in blood meal processing and egg production by supplementing the host with essential micronutrients and macronutrients (Zhong *et al*., 2007; Ben-Yosef *et al*., 2020).

Their elimination with antibiotic treatment was shown to negatively impact the fitness of the lone star tick *Amblyomma americanum* (Zhong *et al*., 2007). However, their role in ticks is still not clear and needs further investigation.

5.5 *Rickettsia* spp.

The SFG rickettsiae are obligate intracellular bacteria transmitted by ticks that cause an emerging disease affecting humans (Raoult & Roux, 1997). *Rickettsia africae* was detected in *Am. lepidum* and *Am. gemma* removed from clinically healthy camels and sheep, but not in camel or sheep blood. *Rickettsia africae* is the causative agent of African tick-bite fever (ATBF) in humans (Raoult & Roux, 1997; Raoult *et al*., 2001). *Rickettsia africae* is known to be endemic in places with the abundance of *Amblyomma* ticks across the African continent (Mediannikov *et al*., 2010). In the current study, *R. africae* was not detected in *Hyalomma* and *Rhipicephalus* tick species collected from camels or co-herded sheep. These findings suggest that the animal and human populations in northern Kenya and other travellers visiting the region may be at a greater risk of rickettsiosis since ticks feeding on humans are highly anticipated.

Rickettsia aeschlimannii was detected in *Rh. pulchellus*, *Hy. truncatum*, *Hy. dromedarii*, *Hy. rufipes,* and *Hy. impeltatum* ticks collected from clinically healthy camels. This study correlates well with other studies that have predominantly detected *R. aeschlimannii* in *Hyalomma* spp. and *Rh. pulchellus*. *Rickettsia aeschlimannii* has previously been isolated from *Hy. truncatum* parasitizing camels in the Kano area of Nigeria (Kamani *et al*., 2013), *Hy. truncatum, Hy. marginatum rufipes* and *Rh. pulchellus* ticks in Kenya (Koka *et al.*, 2017; Omondi *et al*., 2017). It is important to note that *R. aeschlimannii* were only detected in ticks, but not in blood samples. The presence of *R. aeschilimanii* in ticks collected from camels is significant as it may highlight the importance of these animals as reservoirs of SFG *Rickettsia* spp.

5.6 *Anaplasma* spp.

The present study reports presence of "*Ca*. Anaplasma camelii" in blood and tick sampled from clinically healthy camels, indicating persistent infection with the pathogen. Pathogen

persistence in the host is an important strategy for successful pathogen transmission to ticks and for developing resistance against reinfection of hosts (Brown, 2012). The present study corroborates previous findings of "Ca. Anaplasma camelii" in blood from clinically healthy camels in Kenya (Kidambasi *et al*., 2020; Younan *et al*., 2021) and in other dromedary camel populations (Belkahia *et al*., 2015; Azmat *et al*., 2018; Bahrami *et al*., 2018; Selmi, 2019). The relatively high prevalence of the pathogen in clinically healthy camels may be an indication of endemic stability or infection by non-pathogenic "*Ca.* Anaplasma camelii". However, further investigations are needed to prove this hypothesis. In ticks collected from camels, "*Ca.* Anaplasma camelii" was detected in *Hy. rufipes, Hy. dromedarii, Hy. impeltatum, Hy. truncatum, Rh. camicasi, Rh. pulchellus, Am. gemma*, and *Am. lepidum*. Our phylogenetic analyses revealed "*Ca.* Anaplasma camelii" to be genetically close to *A. platys*, which is known to cause cyclic thrombocytopenia in dogs. Presence of the pathogen in blood and ticks collected from these camels suggests the potential role of ticks as vectors of "*Ca.* Anaplasma camelii". However, a study by Kidambasi and colleagues found *Anaplasma* spp. similar to "*Ca.* Anaplasma camelii" in blood and hippoboscid flies (*H. camelina*) collected from Camels in Laisamis, Kenya (Kidambasi *et al*., 2020). These flies can transmit "*Ca*. Anaplasma camelii" to small laboratory animals (Bargul *et al*., 2021), indicating that hippoboscids might play a role in the transmission and evolution of *Anaplasma* sp. Further studies such as molecular screening of the tick salivary glands are needed to investigate the vectoral role of ticks in the transmission of this pathogen in camels. Investigations to assess the zoonotic potential of "*Ca.* Anaplasma camelii" strain is also needed since this has not been established.

Sheep anaplasmosis due to *Anaplasma ovis* infection is a subclinical infection with little economic importance (Friedhoff, 1997). The disease is frequently misdiagnosed due to the absence of specific symptoms and consequently its confusion with other diseases. Acute Anaplasmosis disease due to *A. ovis* tends to be associated with stress factors such as co-infection, hot weather, heavy tick burden, deworming, vaccination, long distance transportation and animal movement (Renneker *et al*., 2013). The disease is reported to be transmitted by ticks (Friedhoff, 1997), and transmission by sheep hippoboscid flies

(keds) has been suspected (Hornok *et al*., 2011). *Anaplasma ovis* was detected in blood and tick samples from sheep in northern Kenya. *Anaplsma ovis* was detected in *Am. gemma*, *Am. lepidum*, *Rh. camicasi* and *Rh. pulchellus*. It is important to note that *A. ovis* was detected in clinically healthy sheep. However, as for other *Anaplasma* spp., *A. ovis* infection could be more severe in stressful situations or in the presence of co-infections (Yasini *et al*., 2012). No *A. ovis* infection was detected in camels, whereas most of the sheep were infected. Presence of these pathogens in clinically healthy sheep and their associated ticks suggests that sheep in northern Kenya may serve as a reservoir for these pathogens. Further studies are needed to evaluate the pathogenic potential of *A. ovis* for sheep and the possibility of transmission of these pathogens from sheep to camels and other livestock and humans in northern Kenya.

5.7 *Theileria/Babesia* sp.

No *Theileria* or *Babesia* spp. DNA was detected in camel blood or their associated tick samples. However, *T. ovis* in blood (80.52%) and *Rh. camicasi* tick samples from sheep. The infected sheep did not show any clinical signs during sampling suggesting they may be important reservoirs of the pathogens. This may also indicate the benign nature of this parasite for native sheep. Similar high prevalence of *T. ovis* has previously been reported in Ethiopia (91.9%) (Gebrekidan et al., 2014) and Sudan (88.6%) (Imam et al., 2016). Though it is not known to cause serious illness in big ruminants (Ringo, 2019), presence of *T. ovis* in sheep blood and ticks presents a potential risk of theileriosis infection in camels as they are co-herded. While *T. ovis* has not been implicated with disease outbreaks in Kenya and worldwide, there is need to assess its significance, the risk it poses to animals and humans, and factors underlying infection and transmission dynamics of the pathogens by tick vectors.

5.8 *Paracoccus* sp.

Paracoccus spp. are coccobacillary bacterium that are typically present in a wide range of ecosystems. A *Paracoccus* sap. was detected in *Ambylomma*, *Hyalomma* and *Rhipicephalus* spp. collected from camels in northern Kenya. This raises the possibility of

these bacteria being transmitted or harboured by ticks or by another invertebrate organism parasitising ticks. Previous studies have reported presence of these bacteria in ticks feeding on horses at a single site in Brazil (Machado-Ferreira *et al*., 2012), *Amblyomma* spp. ticks collected from livestock and tortoises at a single sample site, questing *Haemaphysalis concinna* ticks at two sites in Hungary (Egyed & Makrai, 2014) and *Rhipicephalus microplus* ticks removed from a collared peccary in Peru (Rojas-Jaimes *et al*., 2021). Whether the detected *Paracoccus* sp. represents another group of pathogenic Rhodobacteraceae, pose any risk to animal or human health or plays a role in physiology of ticks remains unknown.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The study shows that hard ticks (ixodid ticks) are widespread and most significant ticks infesting camels in northern Kenya, with *Hy. dromedarii* and *Hy. rufipes* being the most prevalent tick species. Furthermore, the study reports the presence of *E. ruminantium, Ca.* Ehrlichia regneryi, *E. chaffeensis, "Ca*. Anaplasma camelii", *R. aeschilimannii, R. africae*, *C. burnetii* and *Coxiella* endosymbionts circulating in camel blood and ticks in northern Kenya. Presence of *Coxiella* endosymbionts in ticks raises exciting questions on the role they play in pathogen transmission. Our findings expand the knowledge about the TBPs that are present in blood and ticks from camel in northern Kenya and highlight the risk of human infection with zoonotic pathogens, such as *E. ruminantium, R. aeschilimannii, R. africae*, and *C. burnetii*. These findings are important in formulating a strategic framework for research and develop tick control techniques, which are needed in preventing ongoing and new threats posed by TBDs.

6.2 Recommendations

Based on the findings from this study, the following recommendations are put forward:

1. Further molecular studies focussing on ticks and TBPs in other livestock in the study area to identify carrier animals for treatment.
2. Further investigations, including molecular screening of the tick salivary glands to obtain more information on the vectorial role of the *Hyalomma* spp. ticks in transmission of "*Ca*. Anaplasma camelii" and "*Ca*. Ehrlichia regneryi".
3. Further investigation to elucidate the relationship between *Paracoccus* bacteria and ticks and whether they pose any risk to animal or human health.
4. Screening of human population in Marsabit for presence of zoonotic TBPs
5. Extensive research involving a large number of camels is needed in northern Kenya to confirm the presence of *E. ruminantium* and *E. chaffeensis* in camels in Kenya.

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APPENDICES

Appendix I: Estimates of evolutionary divergence between Tick 12S rRNA sequences using the Tajima-Neil Model.

The number of base differences per sequence from between sequences are shown. All ambiguous positions were removed for each sequence pair. The analysis involved 33 nucleotide sequences. The sequences obtained from this study are highlighted in red. There was a total of 288 positions in the final dataset. Evolutionary analyses were conducted using MEGA7.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
1. MK332385 *Am. lepidum* (Uganda)																																	
2. MT895862 *Am. lepidum* (Marsabit Kenya)	4																																
3. MH751460 *Am. hebraeum* (S. Africa)	31	31																															
4. MT895864 *Am. gemma* (Marsabit Kenya)	36	36	17																														
5. KX377407 *Am. gemma* (Ethiopia)	38	38	19	2																													
6. MT895863 *Am. gemma* (Marsabit Kenya)	37	37	18	1	1																												
7. MT895856 *Rh. camicasi* (Marsabit Kenya)	56	54	52	60	60	59																											
8. KU255856 *Rh. sanguineus* (Senegal)	53	51	53	58	59	58	11																										
9. DQ849232 *Rh. turanicus* (Zambia)	56	54	53	62	62	61	11	6																									
10. FJ536556 *Rh. camicasi* (Ethiopia)	56	54	52	60	60	59	3	10	8																								
11. MT895857 *Rh. camicasi* (Marsabit Kenya)	56	54	52	60	60	59	0	11	11	3																							
12. AF150024 *Rh. pulchellus* (Tanzania)	52	50	46	49	51	50	32	31	32	29	32																						
13. MT895854 *Rh. pulchellus* (Marsabit Kenya)	52	50	46	49	51	50	32	31	32	29	32	0																					
14. MT895855 *Rh. pulchellus* (Marsabit Kenya)	52	50	46	49	51	50	32	31	32	29	32	0	0																				
15 MF425937 *Rh. geigyi* (Guinea Bissau)	54	52	51	54	54	53	32	31	32	31	32	21	21	21																			
16. MK234703 *Rh. microplus* (India)	57	57	45	47	47	46	31	32	34	30	31	21	21	21	18																		

17. NC 039828 *Rhipicentor nuttalli* (USA)	56	58	54	59	59	58	53	55	54	51	53	41	41	41	46	39																
18. KU963224 *Hy. dromedarii* (Egypt)	57	55	57	60	62	61	51	48	50	50	51	41	41	41	46	46	38															
19. MT895852 *Hy. dromedarii* (Marsabit Kenya)	56	54	56	59	61	60	50	47	49	49	50	40	40	40	45	45	37	1														
20. MT895853 Hy. dromedarii (Marsabit Kenya)	56	54	56	59	61	60	50	47	49	49	50	40	40	40	45	45	37	1	0													
21. MT895851 *Hy. dromedarii* (Marsabit Kenya)	56	54	56	59	61	60	50	47	49	49	50	40	40	40	45	45	37	1	0	0												
22. MF101817 *Hy. asiaticum* (China)	69	67	63	62	62	61	56	55	56	55	56	46	46	46	46	46	40	33	32	32	32											
23. MT895859 *Hy. impeltatum* (Marsabit Kenya)	60	58	56	57	59	58	55	55	58	54	55	39	39	39	46	44	39	23	22	22	22	31										
24. MT895858 *Hy. impeltatum* (Marsabit Kenya)	59	57	55	56	58	57	54	54	57	53	54	38	38	38	45	43	38	22	21	21	21	30	1									
25. MN315384 *Hy. impeltatum* (Senegal)	59	57	55	56	58	57	54	54	57	53	54	38	38	38	45	43	38	22	21	21	21	30	2	1								
26. KX132904 *Hy. impeltatum* (Niger)	59	57	55	56	58	57	54	54	57	53	54	38	38	38	45	43	38	22	21	21	21	30	1	0	1							
27. MK058358 *Hy. marginatum* (Greece)	60	60	56	55	55	54	52	50	52	51	52	40	40	40	42	41	43	39	38	38	38	34	39	38	37	38						
28. MT895860 *Hy. rufipes* (Marsabit Kenya)	60	60	56	55	55	54	52	50	52	51	52	40	40	40	42	41	43	39	38	38	38	34	39	38	37	38	0					
29. MT895861 *Hy. rufipes* (Marsabit Kenya)	60	60	56	55	55	54	52	50	52	51	52	40	40	40	42	41	43	39	38	38	38	34	39	38	37	38	0	0				
30. MN315383 *Hy. rufipes* (Senegal)	60	60	56	55	55	54	52	50	52	51	52	40	40	40	42	41	43	39	38	38	38	34	39	38	37	38	0	0	0			
31. KU568497 *Hy. truncatum* (Guinea Bissau)	59	57	55	51	51	50	50	48	50	49	50	42	42	42	43	36	38	36	35	35	35	33	36	35	34	35	18	18	18	18		
32. MT895865 *Hy. truncatum* (Marsabit Kenya)	59	59	55	52	52	51	50	48	50	49	50	43	43	43	44	37	39	35	34	34	34	34	36	35	34	35	19	19	19	19	3	
33. KT391032 *Hy. excavatum* (Israel)	60	60	57	58	58	57	51	49	51	50	51	38	38	38	40	36	38	35	34	34	34	33	36	35	34	35	17	17	17	17	12	13

Appendix II: Estimates of evolutionary divergence between Tick 16S rRNA sequences using the Tajima-Neil Model. The pairwise analysis shows the number of nucleotide differences (number of nucleotides) between sequences produced in this study and published sequences from different geographic origin. All ambiguous positions were removed for each sequence pair. The analysis involved 40 nucleotide sequences. There were a total of 135 positions in the final dataset. Evolutionary analyses were conducted using MEGA7.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1. L34328 Ornithodoros moubata																																				
2. MT895179 A. lepidum Marsabit Kenya	42																																			
3. MT895180 A. lepidum Marsabit Kenya	42	0																																		
4. KP987777 A. lepidum Israel	42	0	0																																	
5. L34316 A. hebraeum USA	42	14	14	14																																
6. KP858495 Amblyomma sp. Kenya	41	14	14	14	3																															
7. MK737651 A. lepidum Egypt	42	11	11	11	5	5																														
8. MF627696 A. astrion Sao Tome	43	11	11	11	14	14	15																													
9. MH781753 A. variegatum Sao Tome	43	10	10	10	12	12	9	10																												
10. MN266930 A. variegatum Kenya	45	12	12	12	13	14	12	11	5																											
11. KU130411 Hy. albiparmatum Kenya	44	42	42	42	33	35	35	37	36	34																										
12. KU130449 Hy. nitidum Benin	44	40	40	40	33	35	35	38	37	35	5																									
13. KU130475 Hy. truncatum Kenya	43	40	40	40	31	33	33	37	35	34	5	2																								
14. MT895181 Hy. truncatum Marsabit Kenya	43	40	40	40	31	33	33	37	35	34	5	2	0																							
15. KU130448 Hy. marginatum Russia	45	39	39	39	30	32	32	35	35	33	7	7	7	7																						
16. MT895178 Hy. rufipes Marsabit Kenya	46	38	38	38	29	31	31	34	34	32	7	8	8	8	2																					
17. KU130482 Hy. turanicum Iraq	45	39	39	39	30	32	32	35	35	33	7	7	7	7	0	2																				
18. L34307 Hy. marginatum USA	45	39	39	39	30	32	32	35	35	33	7	7	7	7	0	2	0																			
19. MK058362 Hy. marginatum Greece	45	39	39	39	30	32	32	35	35	33	6	7	7	7	1	1	1	1																		
20. MK737650 Hy. rufipes Egypt	45	39	39	39	30	32	32	35	35	33	6	7	7	7	1	1	1	1	0																	
21. MT895177 Hy. rufipes Marsabit Kenya	45	39	39	39	30	32	32	35	35	33	6	7	7	7	1	1	1	1	0	0																
22. JQ219846 Hy. anatolicum anatolicum India	44	38	38	38	30	30	31	34	34	33	6	7	7	7	4	5	4	4	4	4	4															
23. MF101817 Hy. asiaticum China	45	35	35	35	31	32	30	33	29	28	15	14	12	12	11	12	11	11	11	11	11	13														
24. KU130436 Hy. impeltatum Senegal	45	35	35	35	32	33	33	33	33	32	13	14	14	14	14	14	14	14	13	13	13	13	13													
25. MT895175 Hy. impeltatum Marsabit Kenya	46	35	35	35	33	34	34	33	33	32	14	15	15	15	15	15	15	15	14	14	14	14	14	2												
26. MT895176 Hy. impeltatum Marsabit Kenya	46	35	35	35	33	34	34	33	33	32	14	15	15	15	15	15	15	15	14	14	14	14	14	2	0											
27. KT391055 Hy. dromedarii Unknown	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11										
28. KU130472 Hy. somalicum Somalia	43	43	43	43	35	37	37	39	36	38	11	14	12	12	13	13	13	13	12	12	12	12	14	12	13	13	2									
29. KU130425 Hy. dromedarii Senegal	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11	0	2								
30. MF946465 Hy. dromedarii Egypt	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11	0	2	0							
31. MT895169 Hy. dromedarii Marsabit Kenya	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11	0	2	0	0						
32. MT895170 Hy. dromedarii Marsabit Kenya	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11	0	2	0	0	0					
33. MG757400 Hy. dromedarii Egypt	42	42	42	42	34	35	35	38	36	36	10	13	11	11	12	12	12	12	11	11	11	11	12	10	11	11	0	2	0	0	0	0				

Appendix III: Estimates of Evolutionary Divergence between outer membrane Protein B (OmPB) Sequences using the Tajima-Neil Model. The number of base differences per sequence from between sequences are shown. The analysis involved 24 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 609 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
1. HM050278 *R. aeschlimannii* (Senegal)	38																						
2. MT900489 *R. aeschlimannii* Marsabit Kenya	38	2																					
3. MT900492 *R. aeschlimannii* (Marsabit Kenya)	38	2	0																				
4. MT900494 *R. aeschlimannii* (Marsabit Kenya)	38	2	0	0																			
5. MT900493 *R. aeschlimannii* (Marsabit Kenya)	38	2	0	0	0																		
6. MT900491 *R. aeschlimannii* (Marsabit Kenya)	38	2	0	0	0	0																	
7. MK215215 *R. aeschlimannii* (Germany)	38	2	0	0	0	0	0																
8. MK215218 *R. aeschlimannii* (Germany)	38	2	0	0	0	0	0	0															
9. KU961544 *R. aeschlimannii* (Crimean Pensula)	38	2	0	0	0	0	0	0	0														
10. MK215217 *R. aeschlimannii* (Germany)	38	2	0	0	0	0	0	0	0	0													
11. AF123705 *R. aeschlimannii* (Unknown)	39	3	1	1	1	1	1	1	1	1	1												
12. MT900490 *R. aeschlimannii* (Marsabit Kenya)	38	2	2	2	2	2	2	2	2	2	2	3											
13. MW478136 *R. africae* (Marsabit Kenya)	50	30	28	28	28	28	28	28	28	28	28	29	30										
14. MT900495 *R. africae* (Marsabit Kenya)	50	30	28	28	28	28	28	28	28	28	28	29	30	0									
15. MT900496 *R. africae* (Marsabit Kenya)	50	30	28	28	28	28	28	28	28	28	28	29	30	0	0								
16. CP001612 *R. africae* (Ethiopia)	50	30	28	28	28	28	28	28	28	28	28	29	30	0	0	0							
17. AF123706 *R. africae* (Uknown)	50	30	28	28	28	28	28	28	28	28	28	29	30	0	0	0	0						
18. KU721071 *R. Africae* (Australia)	50	30	28	28	28	28	28	28	28	28	28	29	30	0	0	0	0	0					
19. KT032137 *Rickettsia* sp. (Djibouti)	49	29	27	27	27	27	27	27	27	27	27	28	29	1	1	1	1	1	1				

20. CP040325 *Rickettsia parkeri* (Colombia)	52	30	28	28	28	28	28	28	28	28	28	29	30	4	4	4	4	4	4	3			
21. CP003341 *Rickettsia parkeri* (Colombia)	50	26	24	24	24	24	24	24	24	24	24	25	26	4	4	4	4	4	4	3	4		
22. KF660534 *R. africae* (Rarieda Kenya)	51	31	29	29	29	29	29	29	29	29	29	30	31	1	1	1	1	1	1	2	5	5	
23. KF660535 *R. africae* (Rarieda Kenya)	52	30	28	28	28	28	28	28	28	28	28	29	30	2	2	2	2	2	2	3	6	6	3

Appendix IV: Minimum infection rates for TBPs identified in ticks and blood samples according to sampling sites

Location	Host	No. of individuals	No. of pools	*C. burnetii*	C. endosymbionts	*Ca.* A. camelii	*Anaplasma* sp.	*Ca.* E. regneryi	*E. ruminantium*	*E. chaffensis*	*Ehrlichia* spp.	*R. Africae*	*R. aeschlimanii*
Laisamis	*Hy. dromedarii*	320	76	4 (1.4%)		9 (2.8%)		8 (2.5%)					1 (0.3%)
	Hy. rufipes	364	96	6 (1.6%)		14 (3.8%)		13 (3.6%)					23 (6.3%)
	Hy. impeltatum	39	14			1 (2.6%)		2 (5.1%)					
	Hy. truncatum	3	2										
	Rh. camicasi	23	8			2 (8.7%)							
	Camel Blood	56		1 (1.8%)		52 (92.9%)		9 (16.1%)					
Kamboe	*Hy. dromedarii*	17	7			2 (11.8%)		1 (5.9)					
	Hy. rufipes	13	8			2 (15.4%)		2 (15.4%)					
	Am. gemma	19	12		1 (5.3%)	2 (10.5%)			2 (10.5%)			1 (5.3%)	
	Rh pulchelus	19	11	1 (5.3%)		3 (15.8%)					9 (47.4%)		1 (5.3%)
	Hy. truncatum	2	1										
	Camel blood	16				16 (100%)		1 (6.3%)					
Shegel	*Hy. dromedarii*	179	34	5 (2.8%)		2 (1.1%)		2 (1.1%)					
	Hy. impeltatum	145	36			5 (3.4%)		3 (2.1%)					5 (3.4%)
	Hy. rufipes	87	27	3 (3.4%)		4 (4.6%)		5 (5.7%)					9 (10.3%)
	Hy. truncatum	13	4			1 (7.7%)							
	Rh. camicasi	38	10			3 (7.9%)							
	Am. gemma	3	3										
	Am. lepidum	3	3						1 (33.3%)				

	Camel Blood	24		1 (4.2%)		20 (83.3%)	3 (12.5%)				
Korr	*Hy. dromedarii*	163	40	2 (1.2%)			3 (7.5%)				1 (0.6%)
	Hy. impeltatum	37	13				1 (0.7%)				1 (2.7%)
	Hy. rufipes	145	43	3 (2,1%)		1 (0.7%)	9 (20.9%)		1 (0.7%)		22 (15.2%)
	Rh. pulchelus	2	1								
	Camel Blood	56		7 (12.5%)		49 (87.5%)	13 (23.2%)				
Dabel	*Hy. dromedarii*	124	25			6 (4.8%)	3 (2.4%)				
	Hy. rufipes	39	15			2 (5.1%)	5 (12.8%)				8 (20.5%)
	Am. gemma	8	8		1 (12.5%)	1 (12.5%)		4 (50%)		2 (25%)	
	Am. lepidum	41	20		3 (7.3%)	2 (4.9%)		4 (9.8%)		3 (7.3%)	
	Rh. pulchelus	28	17		2 (7.1%)	3 (10.7%)			5 (17.9%)		
	Camel Blood	24				15 (62.5%)	4 (16.7%)				
Yaballo	*Hy. dromedarii*	37	13			3 (8.1%)	1 (2.7%)				1 (2.7%)
	Hy. rufipes	22	10			2 (9.1%)	7 (31.8%)				3 (13.6%)
	Am. gemma	26	16		1 (3.8%)	2 (7.7%)		4 (15.4%)		6 (23.1%)	
	Am. lepidum	29	15		1 (3.4%)	5 (17.2%)		3 (10.3%)	1 (3.4%)	3 (10.3%)	
	Rh. pulchelus	11	8			1 (9.1%)			3 (27.3%)		
	Rh. camicasi	3	3			1 (33.3%)					1 (33.3%)
	Camel Blood	24				14 (58.3%)					
Bori	*Hy. dromedarii*	15	10			1 (6.7%)	1 (6.7%)				
	Hy. rufipes	25	11				6 (24.0%)				6 (24.0%)

	Am. gemma	29	16		5 (17.2%)	2 (6.9%)		2 (6.9%)			1 (3.4%)	
	Am. lepidum	7	5		1 (14.3%)	1 (14.3%)					1 (14.3%)	
	Rh. pulchelus	21	12							2 (9.5%)		2 (9.5%)
	Camel Blood	24				16 (66.7%)	3 (12.5%)					
Gola	*Hy. dromedarii*	18	10			1 (5.6%)	2 (11.2%)					
	Am. gemma	2	2		1 (50%)							
	Am. lepidum	65	28		3 (4.6%)	4 (6.2%)		6 (9.2%)			9 (13.8%)	
	Hy. rufipes	26	16			1 (3.8%)						5 (31.3%)
	Rh. pulchelus	5	4		1 (20.0%)							1 (20.0%)
	Camel Blood	24				13 (54.2%)	3 (12.5%)					
Misa	*Hy. dromedarii*	13	4									
	Hy. rufipes	6	4			1 (16.7%)						
	Hy. truncatum	4	2									1 (25%)
	Am. gemma	26	17		2 (7.7%)	1 (3.8%)		4 (15.4%)			2 (7.7%)	
	Am. lepidum	183	48		8 (4.4%)	8 (4.4%)		3 (1.6%)	2 (1.1%)		15 (8.2%)	
	Rh. pulchelus	4	4		2 (50%)							
	Camel Blood	24				18 (75%)	2 (8.3%)					
Hula Hula	*Rh. camicasi*	8	3			2 (25%)						
	Am. gemma	4	4			3 (75%)					1 (35%)	
	Hy. dromedarii	1	1									
	Hy. rufipes	2	1									
	Rh. pulchelus	7	5	4 (57.1%)								

	Camel Blood	8		1 (12.5%)		8 (100%)		3 (37.5%)			
Funanyatta	*Rh. pulchelus*	4	4		1 (25.0%)				1 (25.0%)		
	Hy. dromedarii	7	4			1 (14.3%)					
	Am. gemma	12	7		1 (8.3%)				1 (8.3%)	1 (8.3%)	
	Hy. rufipes	21	8								5 (23.8%)
	Am. lepidum	2	1								
	Hy. truncatum	2	2								
	Camel Blood	8				5 (62.5%)		1 (12.5%)			
Burgabo	*Hy. rufipes*	60	15			1 (1.7%)	1 (1.7%)	1 (1.7%)			6 (10.0%)
	Hy. dromedarii	25	9								
	Hy. truncatum	1	1								
	Rh. pulchelus	3	1								
	Camel Blood	8				7 (87.5%)					

Appendix V: Ethical approval letter

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Tel: 4449004/4442014/ 6
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Direct Line. 4448648

Dr. Esther Kanduma
University of Nairobi
Dept of Biochemistry

REF: FVM BAUEC/2019/200

07/03/2019

Dear Dr. Kanduma,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

The role of heartwater (E. rimunantium) and other tick-borne infections in Acute Camel Death Syndrome.

We refer to your ethical clearance letter dated 28th February 2019.

We have reviewed your proposal and are satisfied that the proposed handling and management of the animals meets acceptable standards for animal welfare.

We have also noted that registered veterinary surgeons will supervise the work. We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Appendix VI: Research permit letter

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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NACOSTI, Upper Kabete
Off Waiyaki Way
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NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/72855/27325**

Date: **21st February, 2019**

Dr. Esther Gathoni Kanduma
University of Nairobi
P. O. Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"The role of heartwater (Ehrlichia Ruminantium Infection) and other tick-borne pathogens in acute camel death syndrome in Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **selected Counties** for the period ending **21st February, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education of the selected Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioners
Selected Counties.

The County Directors of Education
Selected Counties.

National Commission for Science, Technology and Innovation is ISO9001:2008 Certified

Appendix VII: Publication in a peer review journal

Article

Ticks and Tick-Borne Pathogens Associated with Dromedary Camels (*Camelus dromedarius*) in Northern Kenya

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Abstract: Ticks and tick-borne pathogens (TBPs) are major constraints to camel health and production, yet epidemiological data on their diversity and impact on dromedary camels remain limited. We surveyed the diversity of ticks and TBPs associated with camels and co-grazing sheep at 12 sites in Marsabit County, northern Kenya. We screened blood and ticks (858 pools) from 296 camels and 77 sheep for bacterial and protozoan TBPs by high-resolution melting analysis and sequencing of PCR products. *Hyalomma* (75.7%), *Amblyomma* (17.6%) and *Rhipicephalus* (6.7%) spp. ticks were morphologically identified and confirmed by molecular analyses. We detected TBP DNA in 80.1% of blood samples from 296 healthy camels. "*Candidatus* Anaplasma camelii", "*Candidatus* Ehrlichia regneryi" and *Coxiella burnetii* were detected in both camels and associated ticks, and *Ehrlichia chaffeensis*, *Rickettsia africae*, *Rickettsia aeschlimannii* and *Coxiella* endosymbionts were detected in camel ticks. We also detected *Ehrlichia ruminantium*, which is responsible for heartwater disease in ruminants, in *Amblyomma* ticks infesting camels and sheep and in sheep blood, indicating its endemicity in Marsabit. Our findings also suggest that camels and/or the ticks infesting them are disease reservoirs of zoonotic Q fever (*C. burnetii*), ehrlichiosis (*E. chaffeensis*) and rickettsiosis (*R. africae*), which pose public health threats to pastoralist communities.

Keywords: dromedary camels; ticks; heartwater; zoonosis; tick-borne pathogens; *Anaplasma*; *Coxiella*; *Ehrlichia*; *Rickettsia*

1. Introduction

Kenya is home to over 3 million camels, representing about 6% of Africa's camel