DIVERSITY AND ENDOSYMBIONTS OF TICK BORNE PATHOGENS AT HUMAN-WILD LIFE LIVESTOCK INTERFACES IN COASTAL NATIONAL RESERVES, KENYA

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any University.

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DEDICATION

I dedicate this work to my family, my wife Asha Swale my daughter Zuhura Salim, Abdulrahman Salim, Ali salim and Abubakar Salim.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
ATBF	African tick bite fever
BLAST	Basic Local Alignment Search Tool
CCHF	Crimean-Congo hemorrhagic fever
DNA	Deoxyribonucleic acid
ECF	East Coast fever
ECF	East Coast fever
EID	Emerging infectious diseases
HRM	High Resolution Melting
MAFFT	Multiple Alignment using Fast Fourier Transform
MIR	Minimum infection rate
QGIS	Quantum Geographic Information System
RT-PCR	Reverse transcription polymerase chain reaction
SFG	Spotted fever group
SHNR	Shimba Hills National Reserve
TBPs	Tick-borne pathogens
TNR	Tsavo National Reserve

ABSTRACT

Ticks are vectors that can harbor pathogens like viruses, protozoa, and bacteria that can cause zoonotic diseases in humans. Human gets infected through tick bites where the pathogens are passed into the human blood. The lack of surveillance information about tick-borne pathogens and diseases has made it impossible to assess its impact on human and livestock. This study determined the diversity and endosymbionts of tick borne pathogens at human-wild life livestock interfaces in coastal national reserves, Kenya. Ticks were collected from both near Tsavo National Reserve in Taita Taveta County and Shimba hills national gane reserves in Kwale County using sterile forceps from restrained cattle and detected morphologically with the aid of morphological keys. Representative of the morphologicaly identified ticks were molecularly identified. Identity as well as characterization of pathogens carried by ticksand endosymbionts was done by *Anaplasma*, *Ehrlichia*, and *Rickettsia* specific RT-PCR product sequencing and HRM analysis.The sum of 317 (281 adult ticks and 36 nymphs) was sampled near Tsavo National Reserve, which includes seven species. *Amblyomma* was the most sampled genus with*Amblyomma gemma* being the most sampled species (n=135, 42.6%). Another *Amblyomma* species sampled was *Amblyomma Variegatum* (n= 40, 12.62%). Greatest species diversity was identified in *Rhipicephalus* genus with four species identified that includes; *Rhipicephalus appendiculatus* (n=44, 13.9%), *Rhipicephalus Averts* (n=1, 0.31%), *Rhipicephalus Decoloratus* (n=5, 1.6%), *Rhipicephalus Pulchellus* (n= 91, 28.7%). A single species of *Hyalomma* sp. was sampled. From near Shimba Hill game reserve (SHNR), a total of 240 adult ticks were sampled comprises of eight species. *Amblyomma* was the most sampled genus and again*Amblyomma gemma* was the most sampled species (n=156, 65 %). Other *Amblyomma* species sampled includes; *Amblyomma Lepidum* (n= 5, 2.1 %), *Amblyomma Variegatum* (n= 15, 6.3 %). Greatest species diversity was also identified in *Rhipicephalus* genus with four species identified that includes; *Rhipicephalus appendiculatus* (n=18, 7.5 %), *Rhipicephalus Averts* (n=6, 2.5 %), *Rhipicephalus Decoloratus* (n=4, 1.7 %), *Rhipicephalus Pulchellus* (n= 34, 14.2 %). The least sampled species was a single species of *Hyalomma* Scupense (n=2, 0.83 %). At near Tsavo National Reserve (TNR), a total of three pools of *Rhipicephaline appendiculatus* were positive for *Theileria parva*, two pools of *Rhipicephaline evertsi* for *Anaplasma platys,* and one pool of *Amblyomma variegatum* nymphs for *Rickettsia africae*. From near Shimba Hill game reserve (SHNR), , *Rickettsia africae* pathogen was detected in two pools of *Am. variegatum* and one pool of *Am. Gemma*. *Rickettsia sp.* and *Anaplasma sp*. were detected in *Am. Gemma* and *Rh. evertsi* respective. *R. aeschlimannii* was isolated in a pool of *Am. Gemma*.*Coxiella* spp.endosymbionts were detected in *Rhipicephalus* ticks in both study area. Robust vector surveillance and biological control programs against ticks should be emphasized in both Tsavo and Shimba Hills National Reserves. Biological control mechanisms for tick endosymbionts should be encouraged for employment as a tick control methods due to their ability to limit vector competency.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The ticks are external parasites that depend on meals made from blood acquired from a diverse rangeof birds, reptiles, and wild animalsto complete their life cycle (Boulanger *et al*., 2019). There are different species of ticks with extensive geographic dispersion (Mancuso *et al*., 2023). The main hypothesized causes of tick geographic spread are deforestation, sociodemographic variables, global warming, and the loss of wildlife habitats, which cause wildlife populations to migrate (Estrada-Peña *et al*., 2014; Tomassone *et al*., 2018). There are currently about 900 tick species identified globally, which are categorized into four main families: *Argasidae*, Deinocrotonidae, *Ixodidae*, and Nuttalliellidae. Among these families, approximately 700 tick species are categorized under the family *Ixodidae*, which comprises several genera, such as Haemaphysalis, Ixodes, Rhipicephalus, Dermacentor, and Hyalomma. These genera are known for transmitting various pathogens (Dantas-Torres *et al*., 2018). The Argasidae family comprises 193 different species of ticks (Hornok *et al*., 2019).

The complexities of tick-borne pathogen (TBP) dynamics within ecosystems involve intricate interactions amongdomestic animals, humans, and wildlife. These organisms serve as repositories for a range of tick-borne illnesses in addition to providing blood meals that sustain tick populations (Oundo *et al*., 2020). As carriers of illnesses that infect humans and animals, ticks come in second place, after mosquitoes. They are responsible for transmitting approximately 40% of all emerging vector-borne diseases that affect both people and animals (Swei *et al*., 2020). Tick's diseases may also spread consequently due

deforestation, warming of the planet, sociodemographic changes, along with thedecline of wildlife habitats, all of which lead wildlife populations to migrate (Tomassone *et al*., 2018). Emerging zoonotic diseases transmitted by ticks has been on the rise globally (Schwartz *et al*., 2017). Zoonotic tick-borne pathogens (TBPs) encompass protozoa, bacteria, and viruses. Notably, examples include *Rickettsia africae*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, with the virus that causes Crimean-Congo hemorrhagic fever (Chiuya *et al*., 2021). Tick-borne diseases continue to be significant livestock ailments globally, affecting both the health and productivity of livestock, leading to substantial economic losses within the livestock industry.In Kenya, the economic repercussions of these losses are particularly pronounced among small-scale, resource-poor households, as a larger portion of the population relies on livestock production for both financial stability and nutritional sustenance (Oundo *et al*., 2022). Recent climatic fluctuations, including changes in rainfall patterns and distribution, coupled with human activities such as intensified agriculture, deforestation, nomadic pastoralism, and cross-border animal trade, observed in recent years, could contribute to altering the epidemiology of tick-borne diseases (TBD) in Kenya (Githaka *et al.*, 2022). As a result, it is crucial to consistently revise and refresh the current epidemiological data concerning tick-borne diseases (TBD) in cattle (Oumdo *et al*., 2022).

Zoonotic rickettsiosis poses a significant and growing public health risk on a global scale (Chiuya *et al*., 2021). Over the last decade, several instances of zoonotic carried by ticks infections have been documented in Kenya. Koka and others 2017, conducted a study in pastoral communities in North Eastern Kenya and found that *Rickettsia aeschlimannii*

and *R. africae* were found in ticks taken from camels. *R. africae* was also found in a different investigation conducted by Chiuya *et al.* (2021) at slaughterhouses and cattle markets in western Kenya. Eighty two (82) out of the 96 pools pools containing *Am. variegatum* had the pathogen identified. Observed in Busia County's neighboring Siaya County, are high infection rates among *Amblyomma* ticks (Maina *et al*., 2014), Baringo County (Omondi *et al*., 2017), the Maasai Mara National Reserve (Oundo *et al*., 2020), and the Shimba Hills National Reserve (Mwamuye *et al*., 2017). This data goes back to when *R. africae* was initially described in Kenyan Amblyomma ticks (Macaluso *et al*., 2003).

The same study by Chiuya and others 2021, also reported detection of *Rhipicephaline* ticksand for the first in *H. suis* lice. *Rickettsia aeschlimannii,* also a zoonotic tick-borne pathogen that causes fever, throat discomfort, muscle aches, skin rashes with raised spots, and sudden liver inflammation (Tosoni *et al*., 2016). Anaplasmosis is another zoonotic tick-borne disease. Various types of Anaplasma, including *A. ovis, A. centrale, A. bovis, A. caudatum, A. platys, A. marginale, A. phagocytophilum,* have the potential to cause infection (Alhassan *et al*., 2021). People contract the disease through close contact with dogs that are infected with A. platys ticks (Maggi *et al*., 2013).

In low-resource countries like Kenya, systematic epidemiological surveillance of tick-borne illnesses is sporadic and limited in its breadth. This study aimed at addressing the gap in knowledge on the species of ticks involved in the spread of diseases carried by ticks that are zoonoticat human-wildlife-livestock interfaces near the Tsavo National

game reserve and near the National Game Reserve at Shimba Hills by surveying ticks' diversity and abundance, determining vector tick-borne pathogens carriage, and identifying the endosymbionts carriage.

1.2 Statement of the problem

Kenya has seen an increase in tick-borne infections that can lead to major cattle diseases and public health issues. (Lwande *et al*., 2014; Kiara *et al*., 2014). These illnesses have posed challenges to the health care system since they presentsymptoms resembling those of other fever illnesses liketyphoid and malaria. Due to the lack of proper diagnosis testing tools in our clinical settings, clinicians rely merely on clinical symptoms to diagnose patients' illnesses. This can lead to misdiagnosis and also underreporting of tick-borne diseases. Ignorance about the frequency of important human diseases carried by ticks hinders our preparedness during outbreaks. Our national reserves have been encroached by pastoral settlements, hence exposing themselves to zoonotic tick-borne pathogens from the wildlife.

The *Coxiella* spp endosymbionts are non-pathogenic but play a vital part in enhancing the nutritional fitness, competence, and reproductive efficiency of the vector tick (Smith *et al*., 2015; Khoo *et al*., 2016). Consequently, they can contribute to the spread of diseases carried by ticks. Moreover, there is a possibility of these endosymbionts evolving into pathogenic *Coxiella* in the future (Duron *et al*., 2015). It is crucial to screen for *C. burnetii*, which causes an important zoonotic disease for public health is Q fever (Elsa *et al*., 2015). Most studies focused on peridomestic ticks neglecting the specific role played

by ticks founds at human-wildlife-livestock interfaces. This study therefore determines tick diversity and abundance, the tick-borne pathogens, and screen for the presence of tick endosymbionts.

1.3 Justification

Wildlife has been incriminated as the reservoir of several new and resurfacing zoonotic illnesses (Meurens *et al*., 2021; Elsohaby *et al*., 2023). Encroachments into wildlife habitats by the pastoral settlement have altered the way zoonotic EIDs spread, causing outbreaks (Allen *et al*., 2017). Therefore, determining tick diversity, their tick-borne pathogens, and their endosymbionts can inform on the tick species with potential for tick-borne transmission during epidemics, pathogens circulating among the ticks, and the endosymbionts that might play a part in the nutrition, fitness for reproduction, and vector competence of ticks in this habitat.

1.4 Research Questions

i. What is the tick species diversity and their abundance at human-wildlife-livestock interface near Tsavo and Shimba Hills national game reserves?
ii. Which tick-borne pathogens are associated with ticks at human-wildlife-livestock interfaces near the Tsavo National game reserve and Shimba Hills National game reserve?
iii. Which endosymbionts species are found in ticks at human-wildlife-livestock interfaces near the Tsavo National game reserve and Shimba Hills National game reserve?

1.5 Objectives

1.5.1 General Objective

To determine diversity and endosymbionts of tick borne pathogens at human-wild life livestock interfaces in coastal national reserves, Kenya.

1.5.2 Specific Objectives

i. To determine tick species diversity and their abundance at human-wildlife-livestock interface near Tsavo and Shimba Hills national game reserves.
ii. To identify and characterize tick-borne pathogens circulating in ticks at human-wildlife-livestock interfaces near near Tsavo and Shimba Hills National game reserves.
iii. To identify the endosymbionts circulating in ticks at human-wildlife-livestock interfaces near Tsavo and Shimba Hills National game reserves.

1.6 Significance of the study

Data obtsined from this study will facilitates the adoption of suitable preventive measures and public health actions to minimize disease transmission. Tick-borne pathogens can impact livestock and domestic animals, resulting in economic setbacks in agriculture. Detecting these tick-borne pathogens enables the implementation of measures to safeguard animal health and avert disease outbreaks. Investigating the existence of tick endosymbionts and their possible involvement in transmitting pathogens offers understanding into the intricate relationships among ticks, pathogens, and symbiotic

microorganisms. This information can guide the creation of management plans for vector-borne illnesses and the creation of cutting-edge intervention techniques.

CHAPTER TWO: LITERATURE REVIEW

2.1 Tick vectors

Ticks are ectoparasites that depend on meals containing blood, time slots, obtained from a variety of birds, reptiles, and wild animals to undergo metamorphosis (in the forms ofadult, nymph, and larva) and successfully finalize their lives cycle (Boulanger *et al*., 2019). Ticks come in around 900 different species, with a broad range of geographic distribution (Mancuso *et al*., 2023). Ticks carry and transfer the most extensive range of pathogens compared to other blood-feeding arthropods, making them the primary vectors responsible for causing illnesses in both animals and humans globally (Dennis *et al*., 2014). These parasites have the ability to be moved passively over significantlengths via the mobility of their hosts, thereby playing a part of thespread diseases carried by ticksof tick-borne pathogens.

Birds, due to their remarkable mobility capabilities, are among the vertebrate hosts capable of overcoming geographical barriers and facilitating the dissemination of parasites on various scales (Léger *et al*., 2013). They contribute to highly intricate health challenges that emerge at the intersection of human-animal-environment interactions. Zoonotic cycles of tick-borne diseases encompass a range of domestic and wild animals, along with people, adding to the complexity of the issue (Boulanger *et al*., 2019). Numerous species of ticks exist, including those in the genera *Rhipicephalus*, *Hyalomma*, *Amblyomma*, and *Haemaphysalis* (Oundo *et al*., 2020; Chiuya *et al*., 2021).

2.1.1 *Amblyomma* ticks

The *Amblyomma* genus includes some of the largest tick species on the planet. The most obvious feature shared by all species is the enormous mouthparts and elaborate scutum found on both male and female individuals. In addition, they have festoons on all stages, banded legs, and flat or beady eyes. Anal plates are absent in males (Marchiondo *et al*., 2019). *Amblyomma* ticks are known vectors for*Rickettsia africae*.According to a study by Pintore and colleagues (2022), *R. africae* was found in a male *Amblyomma variegatum* tick species that had infected a sheep. Another study done in France by Cicculli and co-workers 2019, also reported isolation of *R. africae* in a male *Amblyomma variegatum* tick. According to a study conducted in 2023 in South Africa by Thekisoe and colleagues, *R. africae* was found in the tick species *Amblyomma hebraeum*. *R. africae* have also been dectected in other species of *Amblyomma* ticks. Vogel *et al*. (2018) conducted a study in Nicaragua, Central America, which revealed the molecular identification of *R. africae* in *Am. ovale* ticks.Oundo *et al*. (2020) study was able to isolate *R. africae* was found in *Am. gemma*.This demonstrates the close connection between *Amblyomma* ticks and the global *R. africae* epidemic.

2.1.2 *Hyalomma* ticks

The *Hyalomma* genus is relatively limited in size, comprising more than 25 species primarily found in the Region of the Afrotropics and certain areas within the Palearctic area (Guglielmone *et al*., 2014). Different varieties of *Hyalomma* ticks are vectors of medical and veterinary importance. Many illnesses in both medicine and animals, such as the virus that causes *H. marginatum* is reported to transmit *Rickettsia aeschlimannii* and

Crimean Congo hemorrhagic fever (Chiuya *et al*., 2021; Wallménius *et al*., 2014). Because to climate change, migratory birds, and international trade, *Hyalomma* ticks have spread to different part around the globe (Messina *et al*., 2015; Rainey *et al*., 2018; Grandi *et al*., 2020). In 2018, *H. marginatum* and *H. rufipes* speciewere detected in Germany (Chitimia-Dobler *et al*., 2019).

A study done by Grandi *et al*. (2020), recorded adult tick species in Sweden: *Hyalomma marginatum* and *Hyalomma rufipes*. A study done in Sudan by Shuaib and others 2020, reported *Hyalomma anatolicum* as the most abundant *hyalomma* ticks identified. In the same study, he reported detection of *R. africae* and *R. aeschlimannii* in *H. rufipes* obtained from camels and cattle (Shuaib *et al*., 2020). Several research done in kenya have prove presence of *Hyalomma* ticks. A study done by Jimale and co-workers 2023 were able to idenfied *Hyalomma dromedarii*, *Hyalomma truncatum* from livestock. Another study by Oundo *et al*. (2024), identified *Hyalomma rufipes* and *H. albiparmatum*from Kenyan coastal cattles.

2.1.3 *Rhipicephalus* ticks

Numerous studies conducted throughout Africa have retrieved species of ticks in the genus *Rhipicephalus* that infest sheep in additionally goats (Onyiche *et al*., 2023; Adang *et al*., 2015). Several noteworthy species have been identified thus far, such as *Rh. sanguineus sensu lato*, *Rh. simus*, *Rh. microplus*, *Rh. appendiculatus* and *Rh. e. evertsi*. Tick species *Rh. sanguineus* sensu lato mainly affects dogs. A study done by Elati *et al*. (2018) were able to collect *Rh. sanguineus* sensu lato from goats and sheeps.

Rhipicephalus sanguineus spread *Anaplasma platy* to humans (Maggi *et al*., 2013). Chiuya and other 2021, were able to identify *A. platy* in *Rh. Evertsi*. *Rhipicephalus* ticks have been incriminated as the vector for African tick bite fever. A study done by Tortosa and others 2014, had detected *R. africae in Rh. appendiculatus and Rhipicephalus* (Boophilus) *microplus* ticks.

2.2 Tick-borne pathogens

The behavior of diseases carried by ticks (TBPs) within ecosystems are intricate, typically encompassing interactions among humans, domestic animals, and wildlife. These entities Not just that serve as sources of blood meals,maintaining tick numbers while serving as reservoirs as wellfor various tick-borne pathogens (Oundo *et al*., 2020). Ticks vectors contribute to 40% of all the emerging diseases spread by vectors that affect both people and animals (Swei *et al*., 2020). Given the phenomenon of globalization, Most people agree that ecological and socioeconomic risk factors will continue to interact and function as catalysts for the genesis of diseases at the interface between humans, animals, and the environment. This is accomplished by modifying the biophysical setting where dynamics areinvolving vectors, pathogens, and hosts occur, leading to heightened opportunities for interaction among Pathogens, vectors, non-human animals, and humans (Uusitalo *et al*., 2022). Most newly emerging pathogens are harbored without causing symptoms in wildlife, and vectors like ticks and mosquitoes play a vital part in transmitting those pathogens to people and animals. The past few decades have seen anoticeable global increase in the incidence of newly developing zoonotic illnesses spread by ticks. Examples include borreliosis caused by Lyme, which It affects people in

many wealthy countries (Schwartz *et al*., 2017). Sub-Saharan Africa has long been plagued by tick's diseases in cattle, including East Coast fever (ECF) (Olds *et al*., 2018).

Theileria parva, which causes East Coast fever; *Babesia bigemina*, which causes babesiosis; *Anaplasma marginale*, which causes babesiosis; *Rhipicephalus decoloratus*, which causes babesiosis; and *Ehrlichia ruminantium*, which causes heartwater, which causes *Amblyomma variegatum* are among the tick-borne diseases (TBDs) associated with livestock production challenges (Adjou Moumouni *et al*., 2015). Tick-borne pathogens (TBPs) are unquestionably important for the production of cattle, but there is mounting evidence that they may also express a worry to public health as a result of their potential for zoonotic transmission. Among these zoonotic TBPs are viruses, bacteria, and protozoa. In particular, the virus that causes Crimean-Congo haemorrhagic fever (CCHF), *Ehrlichia chaffeensis, Anaplasma phagocytophilum, and Rickettsia africae* (Chiuya *et al*., 2021).

2.2.1 *Rickettsiae*

In essence, rickettsiae are a category of gram-negative microorganisms that belong to the *Rickettsiales* order and are obligatory intracellular pathogens in the *Rickettsiaceae* family (Merhej *et al*., 2014). The *Rickettsia* genus comprises 34 species with officially recognized names (refer to www.bacterio.net/rickettsia.html) and has been tentatively categorized divided into four groups: the ancestral group, the typhus group, the spotted fever group (SFG), and the transitional group (Ebani *et al*., 2021). This classification is based on a mix of phenotypic features, clinical characteristics, and results from mouse

flirting (Diop *et al*., 2020). The spotted fever group (SFG) primarily persists and spreads through ticks, as indicated by Parola *et al*. (2013). This bunch encompasses the the greatestcount of *Rickettsia* species. In recent times, advancements in molecular techniques have resulted in a consistent rise Within the discovery and delineation of new group rickettsiae that causes spotted feve. These techniques have also facilitated the identification and understanding of human diseases caused by both familiar and previously unknown spotted fever group rickettsiae. A study done by Wang and others 2021 in China, dectected novel *Rickettsia* sp. sw (*Rickettsiales: Rickettsiaceae*) in *Amblyomma geoemydae* (Ixodida: Ixodidae) and *Rh. Microplus* ticks species. *R. monacensis* (Rickettsiales: Rickettsiaceae) is another novel SFG pathogen detected in South Korea and (Kim *et al*,. 2017 ; Portillo *et al*., 2015).

Spotted fever group rickettsiae infections can cause SFG rickettsioses in humans, primarily characterized by symptoms such as widespread lymphadenopathy, myalgia, cough, localized fever, nonspecific flu-like symptoms, and stomach ache (Sekeyová *et al*., 2019).

2.2.1.1 Rickettsia africae

Rickettsia africae is a zoonotic pathogen transmitted by ticks, producing human African tick bite fever (Thekisoe *et al*., 2023). The infectionis marked by symptoms including myalgia, rash, fever, headache, and skin lesions at the site of tick bites (Mwamuye *et al*., 2017). Due to overlapping symptoms with common febrile illnesses like malaria, it is often misdiagnosed in clinical settings. This underscores the need for vigilant

surveillance and the incorporation of *R. africae* into the diagnostic protocols of hospitals and other clinical environments.

The significance of migratory birds in disseminating ticks and the associated infections in their way as they migrate from Africa to Europeis becoming more evident. Untamed birds mayserve as hosts for various tick species, frequently carrying bacteria responsible for causing zoonotic diseases (Mancuso *et al*., 2023). In Italy, The initial instance of *R. africae* was recorded in 2018. A study done by Pintore and others 2022, reported detection of *R. africae*in a male *Amblyomma variegatum* tick specie that had infested a sheep. A phylogenetic analysis revealed the male *A. variegatum* had its origin from west Africa. The authors propose a hypothesis suggesting that the occasional identification of *A. variegatum* specimens is likely associated withbirds during their migration journey that travel from Africa through Sardinia and Corsica in the summertime(Pintore *et al*., 2021).

While this discovery may have been coincidental, it is crucial to take into account that the potential impact of warming of the globecould elevatethe possible danger of establishing colonies of these ticks, given their robust capability for widespread dissemination. During the same year, in France, the first case of *R. africae* was documented. Cicculli and others 2019, reported dectection of *R. africae* in a male *Amblyomma variegatum* tick specie. The tick specie showed a 99% nucleotide identity with a sequence of tick from Senegal. Another study done by Vogel and others, 2018 in Nicaragua in Central America reported

molecular identification of a new rickettsial strain in an *A. triste* tick and *R. africae* in *Am. ovale* ticks. This is an evidence that *R. africae* has spread in most part of the Europe.

African tick bite fever cases are commonly reported in the lowveld regions of the provinces of Mpumalanga and Limpopo in South Africa, along with the eastern areas of KwaZulu-Natal province (Mtshali *et al*., 2017). A research done by Thekisoeand co-wokers 2023 in South African reported that *R. africae* were detected in *Amblyomma hebraeum* tick species. In 2010, Ninety percent contained *R. africae*was found in*Am. variegatum* ticks (60 out of 67), 1% of *Rhipicephalus appendiculatus* ticks (1 out of 92), and 2.7% of ticks that are *Rhipicephalusmicroplus* (Boophilus) (8 out of 296) collected in the Union of the Comoros (Tortosa *et al*., 2014). Additionally, the same study done by Tortosa and others 2014, detected *R. africae* in 77.14% of *Am.variegatum* ticks (27 out of 35) collected from imported cattle. A study done by Oundo and co-workers, 2020 reported detection of *R. africae* in *A. gemma* and *A. variegatum* ticks, *A. variegatum* ticks are the reservoirs of *R. africae* but a study done by Chiuya and others, 2021 did not detect the bacteria in *Am. variegatum* ticks. These findings imply that the ticks might not effectively transmit the pathogen, and that cattle experience low and temporary levels of rickettsaemia, primarily serving as hosts for the tick (*Am. variegatum*) rather than contributing significantly to the spread of the rickettsial infection (Chiuya *et al*., 2021).

2.2.1.2 Rickettsia aeschlimannii

Rickettsia aeschlimannii, is a zoonotic tick-borne pathogen which was initially detected in Morocco from *Hyalomma marginatum* (Beati *et al*., 1997). From there it has been

identified West African countries where it was found in Rhipicephalus and Amblyomma ticks (Ehounoud *et al*., 2016; Parola *et al*., 2013). The symptoms vary and can include fever, throat discomfort, muscle aches, skin rashes with raised spots, and sudden liver inflammation (Tosoni *et al*., 2016). Migratory birds may facilitate the spread of *R. aeschlimannii* by transporting infected ticks from one area to another, potentially influencing its epidemiology. A study done by Wei and others 2015 in China, reported the detection of *R. aeschlimannii* in *Rhipicephalus turanicus* ticks. The *R. aeschlimannii* pathogen had spread to Europe from Africa. A research done in Sardinia, Italy by chisu and others 2017 had reported *R. aeschlimannii* in *Hyalomma marginatum marginatum* ticks frommouflon.Oundo *et al*. (2020) discovered Rickettsia spp. in *Rh. evertsi*and *Am. gemma.* On further characterization, the *Rickettsia spp*shown to be 97% identical to Rickettsia aeschlimannii (Oundo *et al*., 2020).

2.2.2 Anaplasmosis

Tick-borne anaplasmosis is an illness that can afflict both people and animals. It is brought on by gram-negative bacteria that damage blood cells (Ben Said *et al*., 2018). Anaplasma can induce infection in a number of ways, including *A. platys*, *A. bovis*, *A. ovis*, *A. caudatum*, *A. marginale*, *A. centrale*, and *A. phagocytophilum*. As biological vectors, Ixodidae ticks are essential to the spread and transmission of Anaplasma (Alhassan *et al*., 2021). Humans have sometimes been infected with the pathogen, which causes modest headaches, lethargy, and myalgia (Arraga-Alvarado *et al*., 2014). Humans become infected by directly interacting with dogs infested with *A. platys* ticks (Maggi *et al*., 2013).

2.2.2.1 Anaplasma platys

Anaplasma platys, is a pathogen that infects dogs and is spread by *Rhipicephalus sanguineus*, or brown dog ticks (Sainz *et al*., 2015). It has been sporadically reported to infect humans through direct contact with dogs that harbor ticks infected with *A. platys*. Additionally, the pathogen can be transmitted vertically from the mother to the newborn (Lorusso *et al*., 2016). This transmission to humans often results in symptoms such as mild headaches, fever, lymphadenopathy, anorexia, weight loss, mucous membrane paleness, nasal discharge, and bilateral uveitis (Lara *et al*., 2020).

Numerous instances of *A. Platys* had been documented in europe. A survey done in Crotia by Huber and others 2017, reported detection of *A. platys* in dogs. Another survey done in Lisbon, Portugal by Dordio *et al*., 2021, reported identification of *A. platys* in dogs. This is an evidence that the pathogen has spread to Europe. Cases of A. platys have been documented in Africa especially in West Africa. A study done in the Plateau State, Nigeria by Lorusso and others 2016, reported detection of *A. platys* in the blood ofindigenous cattles. *Anaplasma platys* wasdiscovered for the first time in Eygpt in 2018 in ticks and cattle (Al-Hosary *et al*., 2021). A research done in Eygpt by Al-Hosary and co-workers 2021, detected *A. platys* in *H. excavatum* ticks. *A. platys* has additionally been foundin cattle from Algeria (Dahmani *et al*., 2015), Morocco (Elhamiani Khatat *et al*., 2017), Kenya and Ivory coast (Matei *et al*., 2016). In Kenya, *A. platys* was found in ticks of *Rhipicephalus evertsi evertsi*, *R. pulchellus and Rhipicephalus pravus*, collected from household pets in the counties of Baringo and Homa Bay (Omondi *et al*., 2017). A study done by Omondi and others 2017, reported that in Baringo County, 100% of dogs and

19.5% of goats had *A. platys*.It was discovered in 57.1% of the dogs, 6.6% of goats, 14.3% of sheep, and 12.9% of cattle in Homa Bay County that were surveyed.

2.2.2.2 *Anaplasma phagocytophilum*

Both domestic and wild animals can contract tick-borne fever from *Anaplasma phagocytophilum*. In humans, it results in granulocytic anaplasmosis (Stuen *et al*., 2013). The main vectors of *A. phagocytophilum* transmission in Europe are Ixodes ticks, the United States, and Asia (Kolo *et al*., 2023). In the US, reservoir hosts for *A. phagocytophilum* include raccoons, chipmunks, squirrels, dusky-tailed deer, white-tailed deer and white-footed mice (Kolo *et al*., 2023). Cattle in Tunisia have been reported to contain *A. phagocytophilum* (M'ghirbi *et al*., 2016). A research done in Tunisia by Rjeibi and others 2022, indicated that *A. phagocytophilum* was separated from ticks carrying Hyalomma aegyptium that were acquired from tortoises.

2.2.3 Crimean–Congo haemorrhagic fever virus

The 1960s saw the discovery that the Congo's febrile illnesses were caused by the virus that causes Crimean-Congo hemorrhagic fever (Simpson *et al*., 1967). Since then, it has been established by serological research and documented instances in humans where CCHFV is an endemic viral hemorrhagic fever that is widespread over southern and eastern Europe, as well as the Middle East, Africa and Southeast Asia due to global trade, migratory birds and climate change (Messina *et al*., 2015; Rainey *et al*., 2018; Grandi *et al*., 2020). Studies have demonstrated that the main CCHFV vector and reservoir are ticks in the *Hyalomma* genus, while in endemic places, additional tick species might possibly

contribute to the virus's survival (Gargili *et al*., 2017). Humans get infected through tick bites, needlesticks, sexual contac, aerosol, and direct touch with infected livestock's blood or tissues (Balinandi *et al*., 2021; Pshenichnaya *et al*., 2016). In November 2011, a haemorrhagic fever, Crimean-Congoepidemic happened in the eastern Iranian county of Birjand with two reported deaths (Chinikar *et al*., 2013). A survey done in Antani Hospital, Kabul, Afghanistan by Hatami *et al*., 2019, reported that 29 of the 120 patients admitted to the CCHF ward had ELISA confirmation. A study done in spain by Moraga-Fernández *et al*. (2021), reported detection of CCHF ticks feeding on deer and wild boar. Several cases of CCHF have reported in Africa. Egypt reported the first case of CCHF in 1981 (Weidmann *et al*., 2016).

Several studies have shown the present of CCHF in Kenya. A study done by Chiuya and co-workers 2021, reported dection of Virus causing Crimean-Congo hemorrhagic fever in *Rhipicephalus* sp. and *Rh. Decoloratus* cattle ticks. Jimale et al. (2023), was also able to detect CCHF in Kenya.

2.3 Tick endosymbionts

Tick endosymbiontsare microorganisms found inside cells that are harbour by ticks to enhancethere fitnesss (Kolo *et al*., 2023). According to Smith *et al*. (2015) and Khoo *et al*. (2016), tick-borne endosymbionts may play a part in the nutrition, competence, and reproductive efficacy of the tick vector.*Coxiella* endosymbionts (CEs), *Francisella* endosymbionts (FEs), *Candidatus Midichloria mitochondrii,* and *Rickettsia* endosymbionts (REs) are some examples of these endosymbionts (Duron *et al*., 2017;

Gerhart *et al*., 2018). *Coxiella* endosymbionts are the most frequently discovered endosymbionts in ticks globally, present in both soft and hard ticks (Duron *et al*., 2017). A study done by Oundo *et al*. (2020), detected *Coxiella* sp. Endosymbionts in *Amblyomma*, *Rhipicephalus*, and *Haemaphysalis* genera. Another study by Chiuya et al. (2021), detected *Coxiella* endosymbionts in *Rhipicephalusdecoloratus* and *Amblyomma variegatum*.

2.4 Ticks control methods

Tick eradication is a method that is not highly feasible but it is preferably on islands where studies have to have been implemented successfully. However, even after successfully implementing the method, it has not fully worked on large islands and also on continents with exception of only Boophilus spp. from the USA (de la Fuente, 2018). Through employing legislative measures, it has been possible to use the eradication method since one-host ticks which have a separate favorite for livestock have contributed to a major role in bringing luck to the method. This has been successful because there have been no acaricide resistance reports during the campaign. However, some ticks have been re-establishing themselves in Mexico and it has been difficult to prevent them. Eradication of *Boophilus microplus* found in New South Wales has been failing because of Acaricide resistance (Banumathi & Benelli, 2017).

Reservoir hosts of ticks are the wild animals and thus eradicating ticks even for those living in temperature regions like *Ixodes ricinus* and which act as parasites of domestic animals will thus be an unrealistic control method to consider (Vainstein & Santi, 2020).

Tropical tick-borne diseases have been intensively controlled using acaricides, especially those ticks in areas which are highly susceptible and areas where exotic breeds or upgraded livestock. However, the use of these acaricide chemicals is also toxic because most of them contain hard metal chemicals that may affect the person spraying them. Acaricides also leave residues in meat and also cause pollution to the environment (Nwanade & Liu, 2020). In addition, acaricides are expensive since they require money to be produced and exported to other different countries. This has led to a poor livestock economy, especially in African countries which do not make acaricides and have to import them (Laing *et al*., 2018)

Moreover, most of the ticks have been resistant to these acaricides which have negatively affected livestock production (Geiger & Harrington, 2021). Ticks and tick-borne disease eradication using expensive methods such as intensive dipping and spraying has become highly unsuccessful (Kasaija *et al*., 2021). Therefore, other integrated tick control measures have been advocated for and which include the use of hosts resistant to vaccinations and tick infestations where animals and humans are being vaccinated against tick-borne disease (Stafford *et al*., 2017).

2.4.1 Chemical control

Controlling ticks by dipping animals or spraying them with acaricides has been the most conventional method used. Spraying can either be done using hand spays or a motorized spray race. There have been also some systemic acaricides which include the recently implanted and approved to be very helpful are "pour-on" formulations and ear labels and

bands loaded with acaricide (Vudriko *et al*., 2018). An example of these systemic acaricides is the pour-administrations of pyrethroids fortnightly on livestock to eradicate *A. variegatum* in the Caribbean. This method may lead to acaricide resistance in a short period than dipping and spraying methods but it was thus chosen for its excellent work in cattle management in the Caribbean (Ziegelmann & Rosenkranz, 2018).

Generally, apart from applying acaricides to pastures which is a method practice that is too harmful to our environment, it is more advisable for one to kill the parasites while on the host after allowing the cattle to gather as many ticks as possible. However, while controlling soft ticks which usually remain in crevices in the shelter of animals, spraying them indoors using acaricides that have a long lasting impact is highly effective. This strategy is additionally effective for *H. anatolicumanatolicum*, *H. detritum*, and *R. sanguineus* (Benelli & Canale, 2017).

2.4.2 Host resistance to ticks

Host resistance is a biological control method that has been possibly recognized to be effective on ixodid ticks. Once the host has acquired the resistance immunity, the number of ticks attached to it reduces, followed by a decrease in engorgement weights then decreases in egg production and also the production of larvae which finally results in a reduced number in the tick population (Tabor & Jonsson, 2017). This method of tick control is thought to be influenced by the natural selection of animals exposed to ticks for a long time and it also differs by tick species and the type of breed. For example, *Bos indicus* herds in Australia possess individuals who are highly capable of getting this

struggle immunity to ticks compared to *Bos taurus* breeds in Europe (Trentelman *et al*., 2017). The ability to inherit this resistance characteristic is always high with an example being the Australian Friesian Sahiwal which has been nominated by upbringing due to its capability of effectively acquiring resistant immunity against *B. microplus*. This biological regulator of choosing tick-resistant cattle to reduce the one-host tick population, namely, the *B. microplus* has been highly applied in Australia (Rodriguez-Vivas *et al*., 2018).

However, even after using livestock that is tick-resistant in several parts of the world, the yearly production fatalities caused by ticks and the expenses used to control the ticks are still high. Furthermore, this resistant immunity is not acquired by all livestock as well is not acquired against all tick species (Mansfield *et al*., 2017). For example, after *A. variegatum* nymphs fed on goats repeatedly, there was no immunity was induced and also no immunity was induced after *A. hebraeum* nymphs repeatedly fed on sheep (Marima *et al*., 2020). Interestingly, even after adult *A. hebraeum* fed on goats and sheep for several times, its engorgement weights did not decline. The reports have suggested that the After being regularly fed to calves, the engorgement weights of *A. variegatum* increased significantly more in the fourth infection than in the first feed (Sollero & Cardoso, 2017).

2.4.3 Anti-tick vaccines

Most studies have shown pieces of evidence that are enough to prove that immunizing animals and humans with the specified protein antigen can protect them from being infested by ticks. Indeed, in humans, this immunity has been reproduced in a few

numbers of cases through vaccination of recombinant antigens which is thus a crucial step on the way to commercial vaccine production (Marina & de la Fuente, 2017). If the immunoglobulins in the blood of the host haven't been changed, they can enter the hemolymph of ticks by passing through the intestinal walls. In Australia, the anti-tick vaccine has been developed against *B. microplus* an approach that has been majorly applied to control ticks (Maruyama *et al*., 2017). Rather than using salivary antigens, this vaccine makes good use of the tick gut antigens as the targeted immune response. Allen and Humphreys successfully demonstrated this approach by inducing immunity in cattle and guinea pigs against D. andersoni. They used reproductive organs and midgut extracts of the adult ticks (Bhowmick & Han, 2020).

Extracts derived from the adult female internal organs as described by Agbede and Kemp were likely to immunize cattle against *B. microplus* (Kemp *et al*., 1986). Their conclusion showed that the antigens that are found It was found that the tick gut cells used in this kind of vaccination had plasma membranes that concealed the host immune response. These antigens seem to elicit antibodies through a specific function of host immunoglobulins that causes tick gut damage during feeding. The use of recombinant DNA technology allowed for the creation of sufficient levels of the required protein (Kopáček *et al*., 2021).

For instance, From *B. microplus*, a membrane-bound tick gut glycoprotein was extracted, then isolated and produced in *Escherichia coli* (Marina & de la Fuente, 2017). The recombinant protein was found to be capable of providing substantial levels of protection

against *B. microplus* infection in cattle. The vaccine based on this recombinant protein has recently been released on the market and shows remarkable promise. Confirmation of the vaccine's efficacy and cost sustainability would greatly improve our chances of getting comparable vaccines for use in future integrated programs against additional tick species and tick-borne disorders (Adenubi *et al*., 2018). In conclusion, the biological use of counter-tick vaccines to control ticks should be mostly preferred over the use of acaricides since it will later lead to the exploitation of cattle susceptible to ticks.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

The areas of study were Tsavo and Shimba Hills National Game Reserves, Kenya. The two selected human-wildlife-livestock interfaces were selected based on encroachment of human settlement by the pastoral community and reported cases of infections carried by ticks (Mwamuye *et al*., 2017). The Tsavo national park is a protected area in Kenya, which got its name from the Tsavo River. The park shares boundaries with Tanzania's Mkomazi wildlife reserve and Chyulu Hills National Park. The park is divided by the highway road that runs from Nairobi to Mombasa into two national parks, Tsavo West (9,065 km^2) and Tsavo East (13,747 km^2).

The two game reserves have different ecosystems that range from the flat undulating open savannahs on the East to the hilly volcanic mountains on the West. Tsavo East have many different kinds of wildlife in this national park, including red dusty Elephants (*Loxodonta*), lions (*Panthera leo)*, waterbucks (*Kobus ellipsiprymnus*), kudu (*Tragelaphus strepsiceros*), crocodiles (*Crocodylinae*), Hippopotamus (*Hippopotamus amphibious*), leopards (*Panthera pardus*), zebras (*Equus quagga*), giraffes (*Giraffa*), and gerenuk (*Litocranius walleri*). It also hosts over 500 bird species that are recorded at the park. The Shimba hills national reserve is a game-protected area in Kwale County. It is the largest forest area in east Africa and habitat to numerous wildlife species, such as the critically endangered Hippocampal sable antelope (*Hippotragus niger*), Elephant (*Loxodonta*), giraffes (*Giraffa*), leopards (*Panthera pardus*), waterbuck (*Kobus ellipsiprymnus*), bush duiker (*Sylvicapra grimmia*), buffalo (*Syncerus caffer*), bush pigs

(*Potamochoerus larvatus*), African bushbabys (*Galago moholi*), coastal black-handed titi (*Callicebus melanochir*), black and white colobus (*Colobus guereza*), Sykes' monkeys (*Cercopithecus albogularis*), greater galago black-faced vervet monkeys (*Chlorocebus pygerythrus*), serval cats (*Leptailurus serval*), warthogs (*Phacochoerus africanus*), and bushbucks (*Tragelaphus scriptus*).

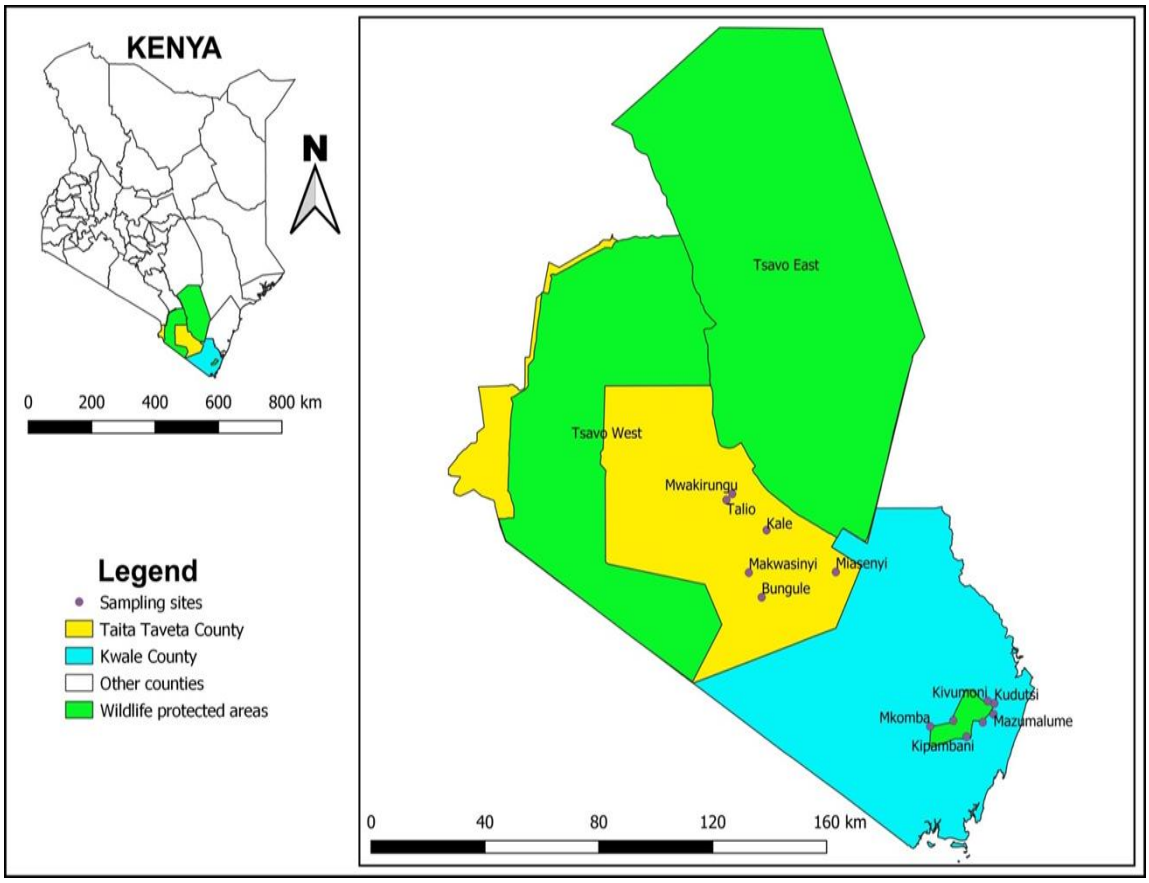

Figure 3.1: Map of ticks sampling locations near the Tsavo National Reserve in Taita Taveta and near the Shimba Hills National Reserve in Kwale Counties, Kenya (**Source:** Common-license shapefiles in QGIS software).

3.2 Study Design

A cross-sectional study design was adopted in this study.

3.3 Sample size Determination

The Slovin's Formula: n= $N/(1+N(e)^2)$ was used to calculate the sample size of the cattles to be sampled.

Number of cattle in Kwale county= 36,145 (Kenya population census, 2019)

Number of cattle in Taita/Taveta = 25,572 (Kenya population census, 2019)

N= 36,145+25572= 61,717

n= (61,717)/(1+61,717(0.05)^2)

Minimum cattle to be sampled = $\frac{400}{2}$ = 200 cattle per county of study.

3.4 Sampling Technique

The sampling was purposive in which sampling sites were deliberately chosen based on the place where livestock are found, encroaching pastoralist villages bordering the reserve, as well as documented instances of humanfebrile illnesses (Eastwood *et al*., 2017). In this way, five sampling sites including Mazimalume, Mkomba, Kipambani, Kidutsi, and Kipambani were selected in sites in the vicinity of SHNR (Shimba Hills National Reserve). In Tsavo National Reserve (TNR), six sampling sites were selected including Mwakirungu, Talio, Miasenyi, Kale, Makwasinyi, and Bungule. The map (Fig. 3.1) above shows these sampling sites were generated utilizing QGIS software and shape files with a common license.

3.5 Ticks' collection

Before sampling the ticks, the owner of the livestock gave their Informed verbal assent. Three cycles of ticks collection were done with an interval of four weeks. From the 200 cowas per county, one (1) tick was collected from each cow in each of the 3 cycles. All the ticks per cow inserted into properly labeled falcon tubes after being extracted from confined cattle using sterile forceps. The tubes were then plugged with cotton swabs and transferred to the Msambweni Division of Vector Borne Diseases Molecular Laboratory for analysis under liquid nitrogen. They were kept at -80°C until they were analyzed.

3.6 Laboratory Procedures

3.6.1 Identification of ticks

3.6.1.1 Morphological tick identification

According to the morphological keys, features of ticks were classified to the genus and/or species level according to Walker *et al*. (2003). The size of the tick, mouthparts,size and presence of the eyes, colour and patterns of the legs, scutum, pulvilli, and coxae 1 are the main morphological features used to identified the ticks.Identification was done using sterile forceps and sterile petri-dish while on sterile gloves. As stated by Oundo *et al*. (2020), ticks were pooled according to sample locations, sex, and speciesand divided into groups of 1–11 for adults and 1–20 for nymphs.

3.6.2 DNA Extraction

Ticks were homogenized in 1.5 ml sterile micro centrifuge tubes containing 750 mg of 2-mm yttria-stabilized zirconium using portable, battery-operated homogenizer oxide beads

(Glen Mills, Clifton, NJ). Following the manufacturer's instructions, utilizing the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), DNA was extracted from the homogenate (Mwamuye *et al*.,2017). Briefly, the 1.5-ml tube containing the homogenates of ticks was filled with 20 µl of proteinase K. The mixture was vortexed after ATL buffer was added. After adding two hundred microliter of pure ethanol, the mixture was vortexed. After being moved into the material was centrifuged at 8000 rpm for one minute using the spin column.

The flow-through and collection tube were discarded.After the spin-column was put into a fresh collection tube and centrifuged for a minute, Buffer AW1 (500 µl) was added. Once again, the collection tube and flow-through were thrown away. Once 500 µl of buffer AW2 has been added, the spin-column underwent centrifugation to three minutes at 13,000 rpm while being housed in a fresh collecting tube. The collection tube was kept and only the through-flow was disposed of. The spin column was centrifuged at 13,000 rpm for three minutes. The spin column was moved into a brand-new 1.5 ml Eppendorf tube, 200 µl of buffer AE was added, and it was allowed to sit at room temperature for one minute. Centrifuging the spin column was done.

3.6.3 Molecular detection and identification of tick-borne pathogens

Using genus-specific primers, specific RT-PCR products of *Anaplasma, Ehrlichia*, and *Rickettsia* were sequenced to identify tick-borne pathogens after they were screened using HRM primers (Table 3.1). For HRM, 100 ng of template DNA, 5 µl nuclease-free water, 500 nM of the matching forward and reverse primers (Table 3.1) and a final

concentration of 1x HOT FIREPol Eva-Green HRM mix (Solis BioDyne, Tartu, Estonia) were used in a 10 µl reaction volume. Nuclease-free water was used as a negative control and DNA extracts from *Rickettsia africae, Ehrlichia ruminantium* and *Anaplasma phagocytophilum* were used as positive controls. The minimal infection rate was calculated using the following formula: [number of pathogen-positive tick pools / total number of ticks of that species tested] times 1000. The minimal infection rate (MIR) makes the assumption that each pool contains a single positive tick. All the laboaratory procedures were done at Msambweni Division of Vector Borne Diseases Molecular Laboratory.

Table 3.1: Primers pairs used in the study

	Target gene	Primer name	Primer sequence (5'-3')	Amplicon size (bp)	Reference
***Rickettsia* spp.**	16S rRNA	Rick-F1 Rick-R2	GAACGCTATCGGTATGCTTAACACA CATCACTCACTCGGTATTGCTGGA	364	(Nijhof *et al*., 2007)
	ompB	ompB 2788 ompB 3599	AAACAATAATCAAGGTACTGT TACTTCCGGTTACAGCAAAGT	856	(Roux & Raoult, 2000)
***Ehrlichia* spp.**	16S rRNA	*Ehr* 16S F *Ehr* 16S R	CGTAAAGGGCACGTAGGTGGACTA CACCTCAGTGTCAGTATCGAACCA	200	(Tokarz *et al*., 2009)
		*Ehr*JV F *Ehr*JV R	GCAACCCTCATCCTTAGTTACCA TGTTACGACTTCACCCTAGTCAC	300	(Mwamuye *et al*., 2017)
***Anaplasma* spp.**	16S rRNA	*Ana* 16S F *Ana* 16S R	GGGCATGTAGGCGGTTCGGT TCAGCGTCAGTACCGGACCA	112-200	(Tokarz *et al*., 2009)
		Ana JV F *Ana* JV R	CGGTGGAGCATGTGGTTTAATTC CGRCGTTGCAACCTATTGTAGTC	300	(Mwamuye *et al*., 2017)
***Theileria and Babesia* spp.**	18S rRNA	RLB-F RLB-R	GAGGTAGTGACAAGAAATAACAATA TCTTCGATCCCCTAACTTTC	450	(Georges *et al*., 2001)

3.7 Research Permit

The National Commission for Science Technology and Innovation granted permit for the research (NACOSTI/P/23/25865). Permission to carry out the study was obtained from Kenya's Directorate of Veterinary Services (Appendix V). Informed consent was obtained from the livestock's owner before sampling the ticks.

3.8 Data Analysis

The MAFFT plugin was utilized to modify and align every nucleotide sequence using Geneious software version 8.1.9 (Kearse *et al*., 2012). Sequence identities were obtained using the GenBank database and the Basic Local Alignment Search Tool (www.ncbi.nih.gov/BLAST/). To compare the tick species and its numbers in Taita Taveta to the same species in Kwale, a t test was employed. A comparison of the species dominance in Taita Taveta to dominance in Kwale County was done using a correlation analysis. To determine thediversity of the total species of ticks in thezones, a Shannon weigner diversity index was used. To compare the number of isolates of the three pathogens obtained in the 2 counties, one-way variance analysis (ANOVA).

CHAPTER FOUR: RESULTS

4.1 Tick species diversity and their abundance at human-wildlife-livestock interface near Tsavo and Shimba hills national game reserves

In this study, a total of 557 ticks were collected for analysis. At near Tsavo National Reserve, a total of 317 (281 adult ticks and 36 nymphs) were collected, representing seven species (Table 4.1). *Amblyomma* ticks dominated the collections with *Amblyomma gemma* (OP971106, OP971111, OP971113) the most sampled species (n=135, 42.6%). Other *Amblyomma* species sampled was *Amblyomma variegatum* (OP971104, OP971105) (n= 40, 12.62%). Four species of Rhipicephalus ticks were collected including; *R. appendiculatus* (n=44, 13.9%), *R. evertsi* (OP971112) (n=1, 0.31%), *R. decoloratus* (n=5, 1.6%), and *Rhipicephalus pulchellus* (n= 91, 28.7%). A single *Hyalomma* specimen was also collected (Table 4.1).

Around the Shimba Hill wildlife reserve (SHNR), 240 mature ticks from eight different species were sampled (Table 4.1**).** *Amblyomma* was the most sampled genus and the most sampled species was once more *Amblyomma gemma* (n=156, 65 %). Other *Amblyomma* species sampled includes; *Amblyomma lepidum* (n= 5, 2.1 %), *Amblyomma variegatum* (OP971107) (n= 15, 6.3 %). Greatest species diversity was also identified in *Rhipicephalus* genus with four species identified that includes; *Rhipicephalus appendiculatus* (OP971108-OP971110) (n=18, 7.5 %), *Rhipicephalus evertsi* (OP971114 - OP971116) (n=6, 2.5 %), *Rhipicephalus Decoloratus* (n=4, 1.7 %), *Rhipicephalus pulchellus* (n= 34, 14.2 %). The least sampled species was a single species of *Hyalomma scupense*(n=2, 0.83 %). The ticks were also categorized into female and male whereby

more female were sampled in both counties with Kwale County having 97 female ticks of *A. Gemma* while in Taita Taveta 96 female ticks were sampled. In Taita Taveta, 36 Nymphs were sampled while in Kwale Nymph was not sampled (Table 4.1). Using t test to compare the tick species in Taita Taveta to the same species in Kwale, the number of tick species in Taita Taveta to those in Kwale showedthere are no noteworthy variations ($P > 0.05$), as Table 4.1 below demonstrates.

Table 4.1: Tick species diversity and their abundance at human-wildlife-livestock interface near Tsavo and Shimba hills national game reserves

Species	Near Tsavo				Near Shimba Hills				t-test	P-value
	Nymphs	Males	Females	Total %	Nymphs	Males	Females	Total %		
Am. Gemma	5	39	96	135(38%)	0	59	97	156 (%)		
Am. Lepidum	0	0	0	0(0%)	0	3	2	5(2.08%)		
Am. Variegatum	7	22	18	40(11.3%)	0	7	8	15(6.2%)		
H. scupense	0	0	0	0(0%)	0	0	2	2(0.85%)		
Hyalomma spp	0	0	1	1(0.28%)	0	0	0	0(0%)		
Rh. Appendiculatus	14	13	31	44(12.4%)	0	11	7	18(7.%)		
Rh. Averts	0	1	0	1(0.28%)	0	2	4	6(2.5)		
Rh. Decoloratus	2	3	2	5(1.4%)	0	1	3	4(1.6)		
Rh. Pulchellus	8	22	69	91(25.7%)	0	23	11	34(14.1%)		
Total	**36**	**100**	**217**	**353**	**0**	**106**	**134**	**240**	1.096	0.31

4.1.1 Distribution of tick species in each zone both near Tsavo and Shimba Hills national game reserves

In Taita Taveta, 7 tick species were recorded from the 6 zones while in Kwale County 8 tick species were recorded in the sampled 5 zones. In Taita Taveta, dominating tick species, *A. gemma* was recorded in all the six zones (100% dominance) whereas in Kwale, the same tick species (*A. gemma)* was recorded in 4 of the five zones (80% dominance). *Hyalomma scupense* was not recorded in Taita taveta whereas *Hyalomma spp* was recorded in Kwale County. A comparison of the species dominance in Taita Taveta to dominance in Kwale County, An examination of correlations revealed a strong, positive association (r = 0.750, P = 0.019). This indicated that tick species that were found in more of the zones in Taita Taveta County were similarly present in many zones in Kwale County (Table 4.2).

Table 4.2: Number of tick species identified in cattle in different zones of Taita Taveta and Kwale Counties

Species	Taita Taveta zones						Kwale county zones						
	1	**2**	**3**	**4**	**5**	**6**	**Dominance**	**7**	**8**	**9**	**10**	**11**	**Dominance**
R. pulchellus	+	+	+	+			4(66.7%)		+		+	+	3(60%)
A. gemma	+	+	+	+	+	+	6(100%)		+	+	+	+	4(80%)
A. variegatum							0(0%)	+	+	+	+		4(60%)
R. appendiculatus		+	+	+			3(50%)		+			+	2(40%)
A. lepidum	+						1(14.3%)		+		+		2(40%)
R. decoloratus				+			1(16.7%)	+		+		+	3(60%)
H. scupense							0 (0.0%)		+				1(20%)
R.averts							0(0%)	+	+				2(40%)
Hyalomma spp				+			1(14.3%)						0(0.0%)
Total	**3**	**3**	**3**	**5**	**1**	**1**	**16**	**3**	**7**	**3**	**4**	**4**	**21**

Key: 1-Kasigau, 2-Biguta Kasimenyi, 3-Miasenyi, 4-Maungu, 5-Kasigau Mbigau, 6-Talio, 7-Mazumalume, 8-Kundutsi, 9-Kivumoni, 10-Mkomba, 11-Kipambani zones. + = species identification.

In summary, in Taita Taveta County, the variety of tick species was varying between one and fivespecies. In two of the zones (Kasigau Mbigau and Talio), only one tick species (*Amblyomma gemma*) was found in the cattle. Four of the zones (Kasigau, Buguta, kasimenyi, and Miasenyi) had 3 tick species found in the cattle while 5 species were recorded in Maungu zone. Two of the zones (Mkomba and Kipambani) had 4 tick species. Mazumalume and Kivumoni zones had 3 tick species while Kundutsi had the highest number of tick species (7 tick species) as shown in Table 4.2.

Out of the 11 zones in Taita Taveta and Kwale County, tick species *Hyalomma spp* and *H. scupense* was isolated only in Maungu and Kundutsi zones respectively. *Rhipicephalus* averts was isolated in 2 zones, *A. lepidum* in 3 zones, *Rhipicephalus pulchellus* was isolated in six zones while *A. gemma* was isolated in 9 zones. The names of the tick species isolated in the respective zones were as shown in Table 4.2 above.

4.1.2 Number of tick species identified in cattle in different zones near Tsavo and Shimba hills national game reserves in Taita and Kwale counties respectively

The number of each of the tick species in each zone was determined. The highest number of tick species recorded was *A. gemma* with a total 291 species whereas the lowest number was that of *Hyalomma spp.* with 1 specie (Table 4.3).

Table 4.3: Number of tick species identified in cattle in different zones near Tsavo and Shimba Hills national game reserves in Taita Taveta and Kwale counties respectively

	Zones												
	Taita taveta County							Kwale County					
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**	**Mean**
R. pulchellus	75	6	1	9				2		7	25	125	17.86
A. gemma	4	6	21	71	19	14		3	6	146	1	291	29.10
A. variegatum							40	11	3	1		55	13.75
R. appendiculatus		5	1	38				16			2	62	12.40
A. lepidum								3		2		5	2.50
R. decoloratus	1			3			1		2		2	9	1.80
H. scupense								2				2	2.00
R.averts							1	6				7	3.50
Hyalomma spp				1								1	1.00
Total	80	17	23	122	19	14	42	43	11	156	30	557	
t– value													2.574
p value													0.033

Key: 1-Kasigau, 2-Biguta kasimenyi, 3-Miasenyi, 4-Maungu, 5-Kasigau Mbigau, 6-Talio, 7-Mazumalume, 8-Kundutsi, 9-Kivumoni, 10-Mkomba, 11-Kipambani zones.

The variation in the number of tick species isolated was established using one-sample t test. Mean number of *A. gemma* (mean 29.10) was higher than the mean number of other tick species. Using a one sample t test to compare the mean number of tick species, the result showed there was a significant difference ($t = 2.574$, $P = 0.033$).

Using t test to compare the tick species in Taita Taveta to the same species in Kwale, the number of tick species in Taita Taveta to those in Kwale showed there were no significant differences ($P > 0.05$) as shown in Table 4.4**.**

Table 4.4: Number of tick species identified in cattle in different zones of Taita Taveta and Kwale Counties

Species	Taita taveta County Mean ± SE	Kwale Count Mean ± SE	t- value	P –value
R. pulchellus	22.75± 17.49	11.33± 6.98	0.530	0.309
A. gemma	22.5 ± 10.09	39 ± 35.68	0.534	0.696
A. variegatum	-	13.75 ± 0.901	-	-
R. appendiculatus	14.67 ± 11.72	9.0 ± 0.00	0.354	0.373
A. lepidum	-	2.5 ± 0.50	-	-
R. decoloratus	2.00 ±1.00	1.67± 0.33	0.387	0.362
H. scupense	-	2.0	-	-
R.averts	-	3.5	-	-
Hyalomma spp	1	-	-	-

4.1.3 Domination of the tick species in the zones in Taita Taveta and Kwale Counties

Tick species *Amblyomma gemma,* was the most dominant species in Kasigau, Miasenyi, Maungu, Kasigau mbigau, Talio, Kivumoni, Mkomba and Kipambani zone. Tick species *Rhipicephalus pulchellus* was dominating in Kasigau and Kipambani zone. *Amblyomma variegatum* dominated in mazumalume, while *Rhipicephalus appendiculatus* was dominant in Kundutsi zone as shown in Table 4.5.

Table 4.5: Dominance of each tick species in the zones in Taita Taveta and Kwale Counties

County	Zones	Tick species	Number	Dominance index
Taita Taveta	Kasigau	*Rhipicephalus pulchellus*	75	0.9375
		Amblyomma gemma	4	
		Rhipicephalus decoloratus	1	
	Buguta kasimenyi	*Rhipicephalus pulchellus*	6	0.3529
		Rhipicephalus appendiculatus	5	
		Amblyomma gemma	6	
	Miasenyi	*Rhipicephalus pulchellus,*	1	0.9130
		Rhipicephalus appendiculatus	1	
		Amblyomma gemma	21	
	Maungu	*Rhipicephalus pulchellus*	9	0.5820
		Rhipicephalus appendiculatus	38	
		Amblyomma gemma	71	
		Rhipicephalus decoloratus	3	
		Hyalomma spp.	1	
	Kasigau mbigau	*Amblyomma gemma*	19	0.000
	Talio	*Amblyomma gemma*	14	0.000
Kwale	Mazumalume	*Amblyomma variegatum*	40	0.7143
		Rhipicephalus decoloratus	1	
		Rhipicephalus avertsi	15	
	Kundutsi	*Rhipicephalus pulchellus*	2	
		Rhipicephalus appendiculatus	16	
		Amblyomma gemma	3	0.3721
		Amblyomma lepidum	3	
		Amblyomma variegatum	11	
		Hyaloma scupense	2	
		Rhipicephalus averts	6	
	Kivumoni	*Rhipicephalus decoloratus*	2	0.5455
		Amblyomma gemma	6	
		Amblyomma variegatum	3	
	Mkomba	Rhipicephalus pulchellus	7	0.9359
		Amblyomma gemma	146	
		Amblyomma lepidum	2	
		Amblyomma variegatum	1	
	Kipambani	Rhipicephalus pulchellus	25	0.8333
		Rhipicephalus appendiculatus	2	
		Amblyomma gemma	1	
		Rhipicephalus decoloratus	2	

To establish the diversity of the total tick species in the zones, a Shannon weigner diversity index was used. The result showed a Berger-Parker Dominance Index of 0.1892 (Appendix IV). This imply that, in the 6 zones in Taita Taveta County, presence of different tick species was diverse to only 18.92%.

Shannon diversity indices of tick species in the zones were determined by the values of Berger–parker dominance index. A higher diversity index was recorded in; Kasigau zone (0.9375), Mkomba (0.9359) and Miasenyi zone (0.9130). Lower tick species diversity was recorded in Buguta Kasimenyi zone (0.3529), Kundutsi (0.3721) and Kivumoni zone (0.5455) as shown in Table 4.5 above.Dominance of tick species in Kwale to Taita Taveta County was done using two sample t test. Mean dominance in Taita Taveta (0.464 ± 0.17) was not significantly different (t = 1.02, P = 0.834) from mean tick species dominance in Kwale County (0.680 ± 0.10).

4.2 Tick pathogens identified at human-wildlife-livestock interface near Tsavo and Shimba hills national game reserves

At near Tsavo National Reserve (TNR) in Taita Taveta County, a total of three pools of *Rhipicephaline appendiculatus* were positive for *Theileria parva* (GenBank accession Number OL451869- OL451871), two pools of *Rhipicephaline evertsi* for *Anaplasma platys* (GenBank accession Number OL451873- OL451874) and one pool of *Amblyomma variegatum* nymphs for *Rickettsia africae* (GenBank accession Number OL466919) as shown in Table 4.6**.**

At Shimba Hills, *Rickettsia africae* was detected in two pools (GenBank accession Number OL466921- OL466922) of *A. variegatum* and one pool of *A. gemma*. *Rickettsia sp.* (GenBank accession Number OL466920) and *Anaplasma sp*. (GenBank accession Number OL451872) were detected in pools of *A. gemma* and *R. evertsi* respectively. *Rickettsia aeschlimannii* (GenBank accession Number OL466924) was detected in a pool of *A. gemma* (Table 4.6).

Table 4.6: Tick pathogens identified at human-wildlife-livestock interface near Tsavo and Shimba hills national game reserves

Site	***Tick-borne pathogens***	***Tick species***	***No.of pools***	***Minimum infection rate***
Near Tsavo	*Theileriaparva*	*Rh.appendiculatus*	3	9.46
National Reserve	*Anaplasmaplatys*	*Rh.evertsi*	2	6.3
	Rickettsiaafricae	*Am.variegatum* (nymphs)	1	3.15
Near Shimba Hills	*Rickettsia africae*	*Am. variegatum*	2	8.3
National Reserve		*Am. Gemma*	1	4.16
	Rickettsia sp.	*Am. Gemma*	1	4.16
	Anaplasma sp.	*Rh. averts*	1	4.16
	R. aeschlimannii	*Am. Gemma*	1	4.16

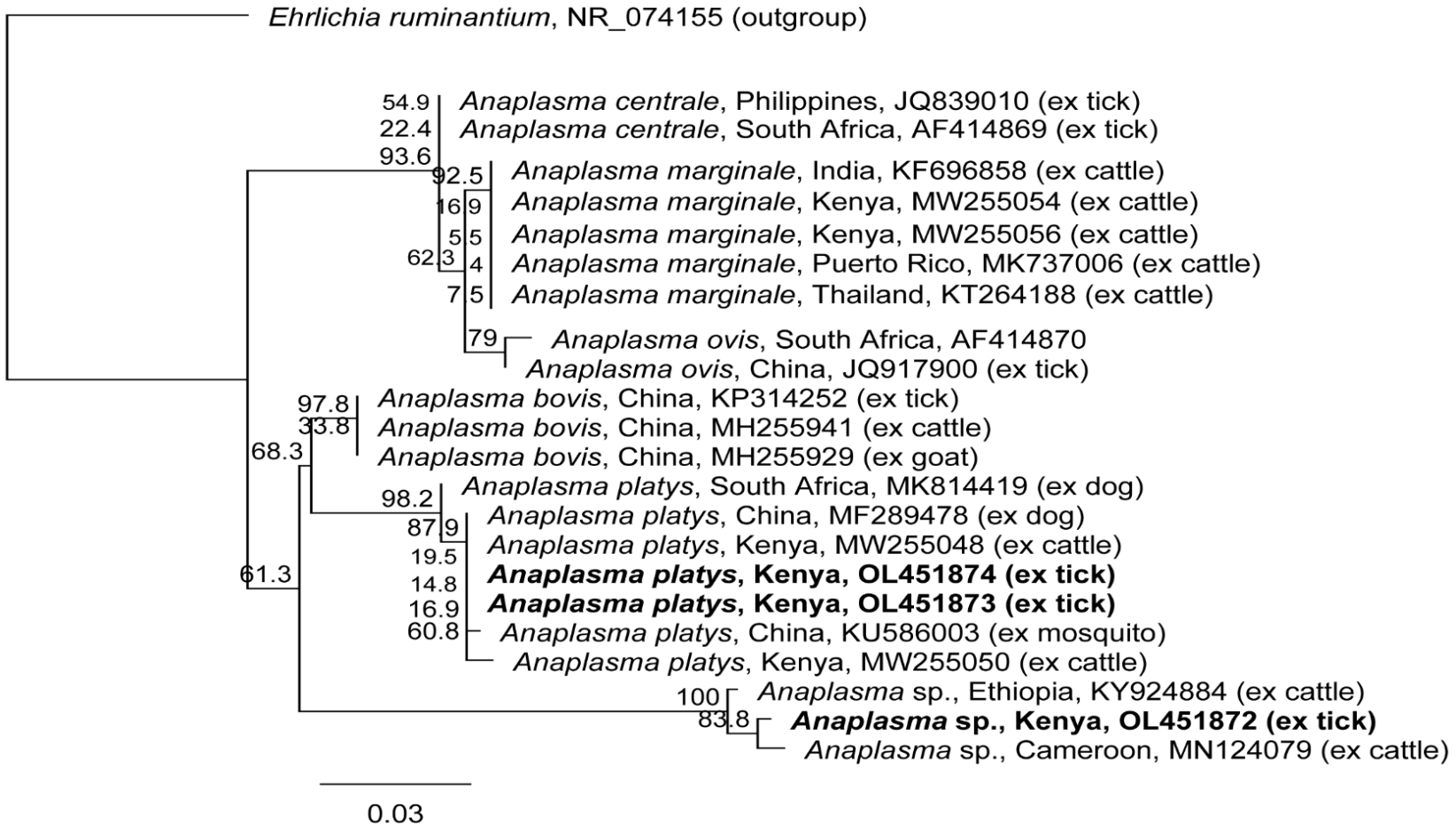

Figure 4.1: Maximum likelihood phylogenetic tree of Anaplasma 16S rRNA gene sequences (480 bp)

4.2.1 Number of tick-born pathogen isolates from the tick species in both in Taita Taveta and Kwale Counties

Most of the three pathogens were isolated from, where 25 isolates of *Ricketsia*, 25 isolates of *Ehrlichi*a and 13 isolates of *Anaplasma* were identified. No pathogen was isolated from *Hyaloma scupense* and *Hyalomma spp. Hyalomma spp.* The distribution of the tick-borne pathogens in the tick species showed that *Rhipicephalus pulchellus, Amblyomma gemma, Amblyomma variegatum* and *Amblyomma lepidum* produced the three pathogens. *Rhipicephalus appendiculatus* produced two pathogens while tick species *Rhipicephalus decoloratus* and, *Rhipicephalus averts* produced only one pathogen (*Ehrlichia*) as shown in Table 4.7.

Table 4.7: Number of tick-born pathogen isolates from the tick species in both in Taveta and Kwale Counties

Tick species	Tick-born pathogen	Number of tick-born pathogen isolates
Rhipicephalus pulchellus,	*Anaplasma*	3
	Rickettsia	3
	Ehrlichia	13
Amblyomma gemma	*Anaplasma*	13
	Rickettsia	25
	Ehrlichia	25
Rhipicephalus decoloratus	*Ehrlichia*	2
Rhipicephalus	*Rickettsia*	3
appendiculatus	*Ehrlichia*	8
Amblyomma variegatum	*Anaplasma*	6
	Rickettsia	4
	Ehrlichia	7
Rhipicephalus averts	*Ehrlichia*	3
Amblyomma lepidum	*Anaplasma*	1
	Rickettsia	1
	Ehrlichia	3
Hyaloma scupense	-	-
Hyalomma spp.	-	-

4.2.1.1 Distribution of tick-born pathogens in zones in Taita Taveta and Kwale Counties

The range of diseases carried by ticks showed that in Kasigau, only one type of pathogen (*Ehrlichia*) was isolated. In Kasigau mbigau only Rickettsia was found. In Buguta Kasimenyi and in Talio, two pathogens (*Ehrlichia* and *Rickettsia*) were found. Other seven zones had all the three pathogens. The total number of pathogens in Kwale did not differ appreciably from the total number in Taita taveta ($t = 1.493$, $P = 0.915$) as shown in Table 4.8.

Table 4.8: Distribution of tick-born pathogens in zones in Taita Taveta and Kwale Counties

County	Zones	Pathogens	Number isolated	Total pathogens isolates
Taita	Kasigau	*Ehrlichia*	8	8
Taveta	Buguta	*Rickettsia*	1	3
	kasimenyi	*Ehrlichia*	2	
	Miasenyi	*Anaplasma,*	3	18
		Rickettsia	1	
		Ehrlichia	14	
	Maungu	*Anaplasma,*	1	14
		Rickettsia	10	
		Ehrlichia	3	
	Kasigau mbigau	*Rickettsia*	1	1
	Talio	*Rickettsia*	2	3
		Ehrlichia	1	
Kwale	Mazumalume	*Anaplasma,*	2	11
		Rickettsia	3	
		Ehrlichia	6	
	Kundutsi	*Anaplasma,*	6	35
		Rickettsia,	13	
		Ehrlichia	16	
	Kivumoni	*Anaplasma,*	11	17
		Rickettsia,	1	
		Ehrlichia	5	
	Mkomba	*Anaplasma,*	1	8
		Rickettsia	4	
		Ehrlichia	3	
	Kipambani	*Anaplasma*	2	9
		Rickettsia	2	
		Ehrlichia	5	
t -value				1.493
P -value				0.915

The overview of the number of pathogens in the zones, *Anaplasma* was found in; Miasenyi and Maungu zoneas in Taita taveta, and in all the 5 zones in Kwale county. *Rickettsia* was found in Buguta kasimenyi, Miasenyi, Maungu and Kasigau mbigau Zone. It was found in all the zones in Kwale County. *Ehrlichia* was recorded in 5 zones in Taita Taveta and in Kwale County (Table 4.9**).**

Table 4.9: The number of Tick-born pathogens isolated in the different zones in Taita Taveta and Kwale Counties

County	Zones	Species	Pathogens isolated from the ticks collected from cattles		
			Anaplasma	***Rickettsia***	***Ehrlichia***
Taita Taveta	Kasigau	*R. pulchellus*	0	0	8
		A. gemma	0	0	0
		R. decoloratus	0	0	0
	Buguta kasimenyi	*R. appendiculatus*	0	0	0
		R. pulchellus	0	1	1
		A. gemma	0	0	1
	Miasenyi	*R. pulchellus*	0	1	1
		R. appendiculatus	0	0	1
		A. gemma	3	0	11
	Maungu	*Hyalomma spp*	0	0	0
		R. appendiculatus	0	1	0
		R. decoloratus	0	0	0
		R. pulchelus	0	0	0
		A. gemma	1	9	3
	Kasigau mbigau	*A. gemma*	0	1	0
	Talio	*A. gemma*	0	2	1
Kwale	Mazumalume	*A. variegatum*	2	3	3
		R. decoloratus	0	0	1
		R. averts	0	0	2
	Kundutsi	*A. gemma*	3	9	5
		A. lepidum	1	0	2
		A. variegatum	1	1	2
		H. scupense	0	0	0
		R. averts	0	2	1
		R. pulchellus	1	0	0
		R. appendiculatus	0	1	6
	Kivumoni	*A. gemma*	6	1	2
		A. variegatum	3	0	2
		R. decoloratus	2	0	1
	Mkomba	*A. gemma*	0	3	2
		A. lepidum	1	1	1
		A. variegatum	0	0	0
		R. pulchellus	0	0	0
	Kipambani	*A. gemma*	0	0	0
		R. appendiculatus	0	1	2
		R. decoloratus	0	0	0
		R. pulchellus	2	1	3

4.2.1.2 Comparison of the number of pathogens in the zones of the two counties

The number of the pathogens was therefore compared in the various zones as indicated in table 4.10. There were not any notable variations in thenumber of pathogens in Kwale to the numbers in Taita Taveta County (P > 0.05).

Table 4.10: Comparison of the number of pathogens in the zones of Taita Taveta and Kwale Counties

County		**Pathogens isolated from the ticks collected from cattle**					
Taita taveta	**Zones**	***Anaplasma***	***Rickettsia***	***Ehrlichia***	**Total**	**Mean**	**SE**
	Kasigau	0	0	8	8	2.67	2.67
	Buguta kasimenyi	0	1	2	3	1.00	0.58
	Miasenyi	3	1	13	17	5.67	3.71
	Maungu	1	10	3	14	44.67	2.73
	Kasigau mbigau	0	2	0	2	0.67	0.67
	Talio	0	2	1	3	1.00	0.58
Kwale	Mazumalume	2	3	5	10	3.33	0.88
	Kundutsi	6	13	16	35	11.67	2.96
	Kivumoni	11	1	5	17	5.67	2.91
	Mkomba	1	4	3	8	2.67	0.88
	Kipambani	2	2	5	9	3.00	1.00
T- value		2.114	0.756	0.744	1.477		
P- value		0.968	0.234	0.762	0.913		

More pathogens were isolated in ticks from Kundutsi zone (35 pathogens), Miasenyi (17 pathogens) and Kivumoni (17 pathogens). Other zones had fewer pathogens. However, comparison of the mean number of pathogens show that The quantity of pathogens in the zones did not significantly differ($F = 2.20$, $P = 0.059$). Tick-born pathogen, *Ehrlichia*, was more in Kundutsi zone (16 isolates). *Rickettsia* was more in Miasenyi (13 isolates) while *Anaplasma* was more in Kivumoni zone which had 11 isolates (Table 4.10) above.

To compare the number of isolates of the three pathogens obtained in the 2 counties, one-way Analysis of variance (ANOVA) was used and it showed that the mean number of isolates of *Anaplasma* (mean 3.714), *Ehrlichia* (mean 6.300) and *Rickettsia* was a mean of 3.800. Mean comparison using One-way ANOVA showed that there was no significant difference in the number of isolates (F = 1.04, P = 0.370) as shown in Figure 4.2.

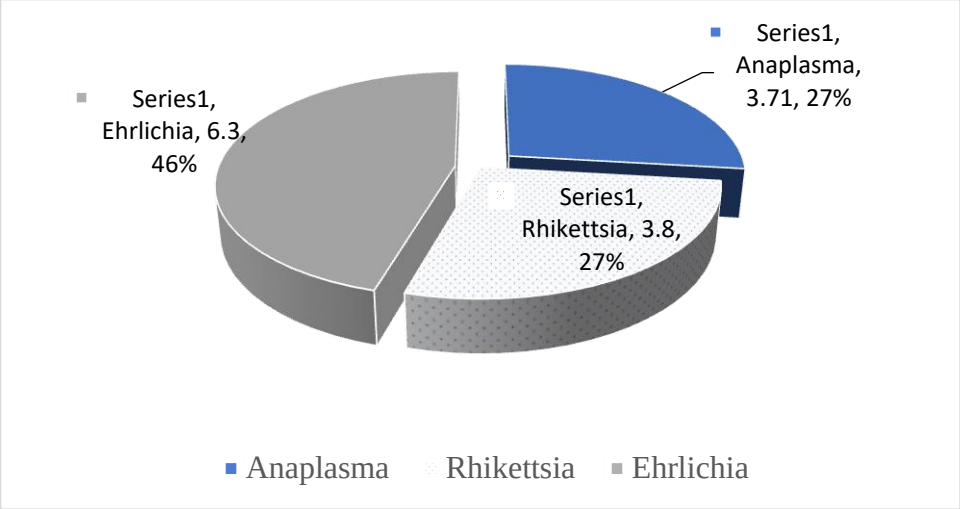

Figure 4.2: Comparison of the number of pathogen isolates obtained in Taita Taveta and Kwale Counties

To establish the most dominating pathogen in the 2 counties, a comparison of the number of pathogen isolates was carried out using One-Way Analysis of Variance. Average number of the pathogen isolates were; *Anaplasma* (mean 5.750), *Ehrlichia* (mean 8.714) and *Ricketsia* (mean 7.200). The result after statistical analysis, however indicated that there was no significant difference in the isolates (F = 0.17, P = 0.846) as shown inFigure 4.3 below.

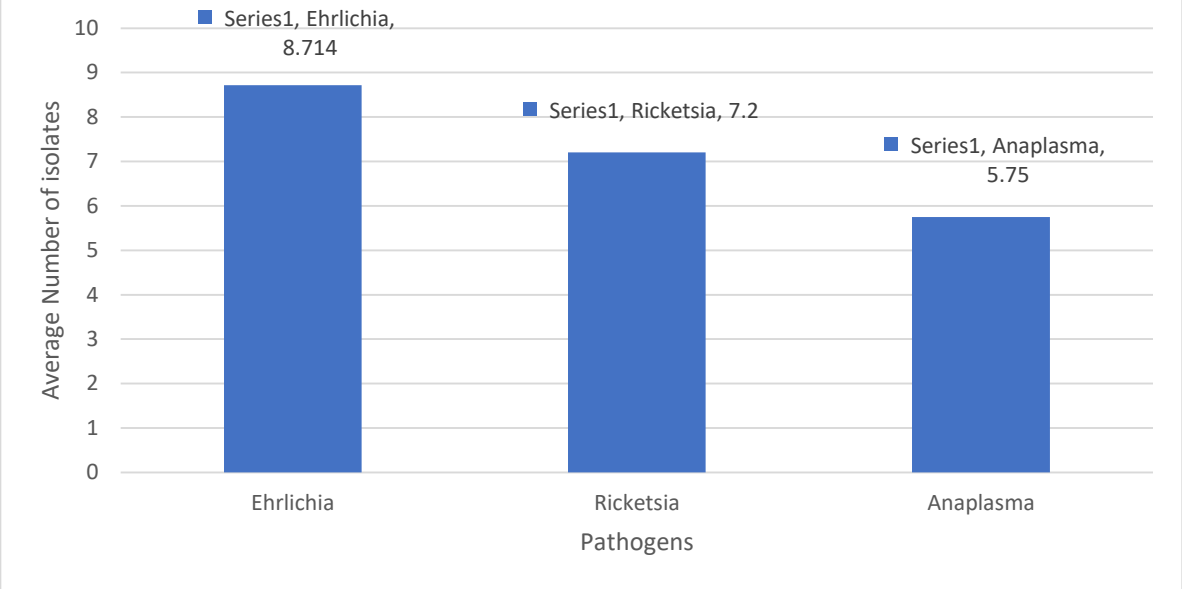

Figure 4.3: Average number of pathogen isolates in Taita Taveta and Kwale Counties

4.3 Identification of endosymbionts circulating in ticks at human-wildlife-livestock interfaces near Tsavo and Shimba Hills National game reserves

Unexpectedly, more sequence analysis of the amplicons showed that *Coxiella* sp. endosymbionts were present. In the vicinity of Tsavo National Reserve, *Coxiella* sp. endosymbionts were detected in four pools. Two pools of *Rh. appendiculatus* (OP973749, OP973750) and two pools of *Rh. evertsi* (OP973752, OP973753) contained the endosymbionts. Theileria parva co-infected the two *Rh. appendiculatus* pools, while Anaplasma platy co-infected the *Rh. evertsi* pools. Sadly, screening these pools with primers specific to C. burnetii revealed negative results.

Near Shimba Hills National Reserve, *Coxiella* sp. endosymbionts was detected in one pool of the *Rh. evertsi* (OP973751). This pool was co-infected with *Anaplasma* sp. The endosymbionts were not detected in *Amblyomma gemma* in both study sites (Table 4.11).

Table 4.11: Co-infection of *Coxiella spp.* and tick-borne pathogens identified

Site	*Tick-borne pathogens*	*Tick species*	*Endosymbiont*	*No. of pools*
Near Tsavo	*Theileriaparva*	*Rh.appendiculatus*	*Coxiella spp.*	2
National Reserve	*Anaplasmaplatys*	*Rh.evertsi*	*Coxiella spp.*	2
Near Shimba Hills	*Anaplasma sp.*	*Rh. Averts*	*Coxiella spp.*	1

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Tick diversity and abundance at human-wild-livestock interfaces near Tsavo and Shimba hills national game reserves

The identification of tick-borne diseases at the human-wildlife-livestock interfaces of the Shimba Hills National Reserve and the Tsavo National Reserve has helped to shed light on the potential for tick-borne zoonotic disease transmission from wildlife to farmed animals and humans. Both morphological and molecular identification of the ticks were in agreement. The majority of the tick species found in this study have been connected to the spread of diseases carried by ticks, which has consequences for both public health and veterinary medicine (Wikel *et al*., 2018).

Greater tick's species diversity was identified in the SHNR than in TNR, suggesting the former has a higher risk of future tick-borne pathogens outbreaks. However, the *amblyomma* spp., in particular *Am. gemma*were most abundantly sampled in both study sites. This findings are inconsitence with those of Mwamuye and co-workers 2017 who reported that only *Am. eburneum* and and *Am. tholloni* were sampled in SHNR. The high abundance of *Am. gemma* across both study areas suggests that the species is well adapted to the ecologies of both regions. Furthermore, *R. africae* was found in *Am. gemma* and *Am. variegatum* ticks in this investigation, therefore a high abundance of *Am. gemma* represents a potential public health risk for rickettsioses transmission during epidemics (Koka *et al*., 2017).

A single specimen of *Hyalomma spp.*and and least species of *Hyalomma scupense* were sampled in TNR and SHNR respectively. *Hyalomma* ticks have been incriminated as the natural Crimean-Congo haemorrhagic fever (CCHF) virus's vector and reservoir(Bonnet *et al*., 2022). This put both the study sites into the risk of a potential CCHF outbreaks in future.

5.1.1.1 Zonal distribution of tick species in Tsavo and Shimba Hills national game reserves

Among the three sampled genera; *Ambylomma*, *Rhipicephalus* and *Hyalomma*, the tick species *Ambylomma gemma* was found to be more dominant, with *Hyalomma* spp being the least dominant. *Am. gemma* was more predominant in Maungu zone of Tsavo and Mkomba zone of Shimba Hills. Tsavo National Reserve was found to have a range of 1 to 6 species of ticks parasitizing cattle in its six zones of Kasiagu, Biguta -Kasimenyi, Miasenyi, Maungu, Kasigau- Mbigau and Talio. On the other hand, SHNR was found to have a range of 1 to 8 species of ticks parasitizing cattle in its five zones of Mazalume, Kundutsi, Kivumoni, Mkomba and Kipambani. The difference in range of tick species in the two regions suggests that SHNR was more favorable ecologically for ticks to thrive as compared to Tsavo National Reserve.

In the present study, a comparative analysis of tick speciesdominance in both Tsavo and Shimba Hills national game reserves showed a positive significant relationship. This is attributable to the ecological comparability of the two zones since varied species of ticks were able adopt a common ecological niche. The most abundant species in both regions

being *Am. gemma,* followed by *Rhipicephalus* spp, wasa finding that was in line with the results of a related study done on ticks and diseases carried by ticks in Northern Kenya (Getange *et al.*, 2021).

The domination of both Tsavo and Shimba Hills National game reserve by *Ambylomma* spp and *Rhipicephalus* sppis also consistent with the knowledge that tick species from these two respective genera are epidemiologically important ticks that are commonly responsible for infestation of an array of vertebrate hosts (Getange *et al.,* 2021). These ticks transmit various pathogens like viruses, protozoa and bacteria of not only veterinary but also medical importance, more so at human-wildlife livestock interfaces (Chiuya *et al.,* 2021). Consequently, the domination of the two zones with similar tick species implies the likelihood of a common outbreak such as the tick-borne relapsing fever or East Coast fever. This can be explained by the ecological comparability of the two zones as well as heightened host similarities in ecologically similar zones (Chiuya *et al.*, 2021).

5.1.1.2 Tick species dominance in cattle in Tsavo and Shimba Hills national game reserves

High dominance indices of tick species were identified in Kasiagu zone of TNR (0.9375), Mkomba zone of SHNR (0.9359) and Miasenyi zone of TNR (0.9130) while low dominance indices were recorded in Buguta Kasimenyi zone of TNR (0.3529), Kundutsi zone of SHNR (0.3721) and Kivumoni zone of SHNR (0.5455). Talio and Kasigau Mbigau zones of TNR were the least dominated with tikcs, both having a dominance index of 0.000. This could suggest that proper tick control measures like frequent use of acaricides are in place in these two regions.

The differences in dominance indices can be explained by various factors that are known to influence host-parasite relationships. These include the population density, immunocompetence of the host, animal translocation, behavioral and sociality characteristics of the host, sex and supplementary feeding amongst others (Halliday *et al.,* 2020). A significant difference in the species of ticks parasitizing the cattle was reported in this study, a finding that was comparable to that of a similar study on tick-borne infections at Western Kenyan slaughterhouses and animal markets (Chiuya *et al.,* 2021). It was intriguing to observe that *Hyalomma* spp was isolated only in Maungu zone, of all the eleven zones in the study. This could be explained as an occurrence of chance and not necessarily due to any statistical significance.

5.1.2 Identification of tick-borne pathogens circulating in ticks at human-wildlife-livestock near Tsavo and Shimba hills national game reserves

African tick bite fever is impacted by *Rickettsia africae*, a zoonotic tick-borne infection that induces headaches, high temperatures, rashes, myalgia, and skin lesions at the point of a tick bite (Silva-Ramos *et al*., 2021). Since it mimics most of the symptoms of other febrile disorders like malaria, it is frequently misdiagnosed by clinicians and doctors. This necessitates vigilant monitoring and the inclusion of *Rickettsia africae* testing in hospitals and other healthcare settings. *Rickettsia africae* was found in *Amblyomma gemma* and *Amblyomma variegatum* ticks in this investigation. These findings are in accordance with those that reveal *Rickettsia africae* circulates among *Amblyomma* species (Oundo *et al*., 2020; Mwamuye *et al*., 2017). Moreover, *Rickettsia africae* was also discovered in *Am. variegatum* tick nymphs. Since the nymphs had been sampled

from the cattle, we can't identify if they were infected transovarially or straight from the cattle.

Rickettsia aeschlimannii is also considered to be a zoonotic pathogen carried by ticks that has been reported on patients traveling from Africa (Parola *et al*., 2013). There has been evidence of this pathogen in *Hyalomma* ticks in Kenya (Omondi *et al*., 2017). It is believed ticks of the genus *Hyalomma* serves as reservoirs for *R. aeschlimannii* (Wallménius *et al*., 2014). However, in the current research, *R. aeschlimannii* was isolated in *Am. gemma*. To our knowledge, this is the first time *R. aeschlimannii* has been isolated in *Am. gemma*. Sometimes migratory birds will play an important involvement in epidemiology of *R. aeschlimannii* by bringing ticks contaminated with *R. aeschlimannii* from one region to another (Ebani & Mancianti 2021).

Uncharacterized *Rickettsia* spp. was detected in *Am. variegatum* ticks. Although its pathogenicity was unknown, it can be a public health threat in the future. Many known *Rickettsia* species that are of public health concern today were initially considered not to be harmful to humans. This species needs further characterization with better markers such as *ompA, sca4, and 17kDa* (Oundo *et al*., 2020).

The tick-borne pathogen *Anaplasma platy* infects dogs and is spread by brown dog ticks (*Rhipicephalus sanguineus*) (Snellgrove *et al*., 2020). Humans have sometimes been infected with the pathogen, which causes modest headaches, lethargy, and myalgia (Arraga-Alvarado *et al*., 2014). Humans become infected by directly interacting with

dogs infested with *A. platys* ticks (Maggi *et al*., 2013). *Anaplasma platy* was found in *Rh. Evertsi* in the present research. These findings were consistent with those of Chiuya and other 2021 who detected *A. platy* in other *Rhipicephalus* tick species. The *A. platy* identified in this study (GenBank accessions OL451873-OL451874) were closely related to those found in Kenya (GenBank accessions MW255048, MW255050) -MT459321, MT459326 and MT459328) and China (GenBank accession MF289478).

Cattle that contract East Coast Fever are affected by a TBP of veterinary significance called *Theileria parva* (Walker *et al*., 2014). In this current study, *Theileria parva* was isolated in *Rh. appendiculatus* ticks. These results agreed with Oundo et al.'s (2020) findings. The cattle get infected by coming into closeness to buffaloes which are the natural reservoir for *T. parva* (Morrison *et al*., 2020).

5.1.2.1 Zonal distribution oftick-borne pathogens in Tsavo and Shimba hills national game reserves

In Tsavo, Miasenyi zone had the highest number (17) of tick-borne pathogens isolated, while Talio zone had the least number of pathogens (3) isolated. On the other hand in SHNR, Kundutsi zone had the highest number (35) of tick-borne pathogens isolated, while Mkomba zone had the least number (8) of pathogens isolated. In overall, the pathogen *Ehrlichia* was the predominant isolated pathogen, while *Anaplasma* and *Rickettsia* were isolated in small numbers. Contrary to the findings of other similar studies, *Hylalomma* spp ticks in the current study were found to have no pathogens isolated from them. Despite the two regions having a domination of similar tick's

pathogens, The numbers did not differ statistically, which is attributable to the ecological comparability of the two regions, with both being along the Kenyan coast and hence many similar characteristics of the hosts (Halliday *et al.*, 2020).

These results are similar to those of a study carried out in Ecuador that found *Ehrlichia* spp as the most common tick's pathogen in ticks collected from cattle (Pesquera *et al.*, 2015). Kundutsi and Miasenyi zones, having the highest number of isolated tick-borne pathogens were at higher risks livestock losses, owing to the fact that the pathogens such as the bacteria *Ehrlichia* spp infect the hosts' neutrophils, macrophages and vascular endothelial cells, causing serious and potentially fatal bacterial infections in the host (Getange *et al.* 2021). Isolation of *Rickettsia* spp in ten of the eleven zones, more so in the most abundant *Ambylomma* ticks highlights the importance of cattle-associated *Ambylomma* ticks as important zoonotic Rickettsia africae reservoirs for additional cattle (Pesquera *et al.*, 2015).

5.1.2.2 Comparison of numbers of tick borne pathogens across Tsavo and Shimba hills national game reserves

In overall, the two game reserves' respective pathogen counts did not differ muchwhere an average p-value of 0.846 was obtained. Moreover, despite the fact that some zones had more tick-borne pathogens than others, the mean number of pathogens demonstrated that there were no appreciable variations in thesame; *Anaplasma* (mean 5.750), *Ehrlichia* (mean of 8.714) and *Rickettsia* (mean of 7.200). This notable lack of significant differences in the tick-borne pathogens isolated across the two counties can be explained

by the close morphologic and genetic similarity of the host ticks (Kasaija *et al*., 2021; Oundo et al., 2020).

5.1.3 Identification of endosymbionts circulating in ticks at human-wild-livestock near Tsavo and Shimba hills game reserves

Tick-borne endosymbionts are believed to have a potential role in the nutrition fitness, competency, and reproductive efficacy of the tick vector (Smith *et al*., 2015; Khoo *et al*., 2016). Not all ticks carried the zoonotic disease Q fever, caused by Coxiella burnetii, which is significant for public health. Our finding was in line with that of Oundo *et al.* (2020)who failed to isolate*Coxiella burnetii*at the human-wildlife-livestock interfaces. The findings were also consistent with those of Ndeereh and others (2017) who also did not detect *C. burnetii* in ticks. However, tick endosymbionts, specifically *Coxiella* spp endosymbionts were detected after sequencing amplicons produced for *Rickettsia* species utilizing the 16S rRNA primers as described by Nijhof *et al*. (2007).

In the current study, *Coxiella* spp endosymbionts were detected only in ticks of the *Rhipicephalus* genera. These findings were consistent with those of Chiuya and others, 2021 but were contrary to those of Mwamuye and others (2017) who found out that *Coxiella* spp endosymbionts were only found in a nymph of *Am. eburneum*, but not in other tick samples from the coast of Kenya. *Coxiella* spp endosymbionts are not pathogenic but they play a significant part in the nutrition fitness, competency, and reproductive efficacy of the tick vector (Smith *et al*., 2015; Khoo *et al*., 2016). In this way, they contribute intransmitting of *Theileria parva* by *Rh. appendiculatus* and

Anaplasma platy by *Rh. Evertsi.* Additionally, they may evolve into pathogenic Coxiella in future (Duron *et al.,* 2015). It is paramount to screen for *C. burnetii* but caution must be taken since there is likelihood for cross-reaction with other Coxiella-like bacteria (Elsa*et al*., 2015).

5.2 Conclusions

i. Greater tick species diversity was identified in the Shimba Hills National Reserve than in the Tsavo National Reserve, with the most abundant species being *Ambylomma gemma* across both national reserves. The least abundant species across both national reserves was *Hyalomma spp.*

ii. The predominant tick-borne pathogen identified among the numerous *ambylomma* ticks in the investigation was *Rickettsia africae*, which causes African tick bite fever.

iii. *Coxiella* spp were the only endosymbionts circulating in ticks in both Tsavo and Shimba Hills National Reserves and were only detected among ticks of the *Rhipicephalus* genera.

5.3 Recommendations

i. Robust vector surveillance and biological control programs against ticks should be emphasized in both Tsavo and Shimba Hills National Reserves.
ii. Health centers and hospitals around Tsavo and Shimba Hills National Reserves should be well equipped in diagnosing and treatment capacities for African tick bite fever, should an outbreak occur around those areas.
iii. Biological control mechanisms for tick endosymbionts should be encouraged for employment as a tick control methods due to their ability to limit vector competency.
iv. Further study to be done on human who are living within the interfaces to ascertain whether the isolated pathogens also affect humans.

5.3.1 Further studies

Further study to be done on human who are living within the interfaces to ascertain whether the isolated pathogens also affect humans.

REFERENCES

Adang, K. L., Ayuba, J., & Yoriyo, K. P. (2015). Ectoparasites of sheep (Ovis aries L.) and goats (Capra hirus L.) in Gombe, Gombe State, Nigeria.

Adenubi, O. T., McGaw, L. J., Eloff, J. N., & Naidoo, V. (2018). In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: A critical review. Veterinary parasitology, 254, 160-171.

Adjou Moumouni, P. F., Aboge, G. O., Terkawi, M. A., Masatani, T., Cao, S., Kamyingkird, K., Jirapattharasate, C., Zhou, M., Wang, G., Liu, M., Iguchi, A., Vudriko, P., Ybanez, A. P., Inokuma, H., Shirafuji-Umemiya, R., Suzuki, H., & Xuan, X. (2015). Molecular detection and characterization of Babesia bovis, Babesia bigemina, Theileria species and Anaplasma marginale isolated from cattle in Kenya. Parasites & vectors, 8, 496.

Alhassan, A., Hove, P., Sharma, B., Matthew-Belmar, V., Karasek, I., Lanza-Perea, M., Werners, A. H., Wilkerson, M. J., & Ganta, R. R. (2021). Molecular detection and characterization of Anaplasma platys and Ehrlichia canis in dogs from the Caribbean. Ticks and tick-borne diseases, 12(4), 101727.

Al-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, A. M., & Silaghi, C. (2021). Tick species identification and molecular detection of tick-borne pathogens in blood and ticks collected from cattle in Egypt. Ticks and tick-borne diseases, 12(3), 101676.

Allen, T., Murray, K. A., Zambrana-Torrelio, C., Morse, S. S., Rondinini, C., Di Marco, M., Breit, N., Olival, K. J., & Daszak, P. (2017). Global hotspots and correlates of emerging zoonotic diseases. Nature communications, 8(1), 1124.

Arraga-Alvarado, C. M., Qurollo, B. A., Parra, O. C., Berrueta, M. A., Hegarty, B. C., & Breitschwerdt, E. B. (2014). Case report: Molecular evidence of Anaplasma platys infection in two women from Venezuela. The American journal of tropical medicine and hygiene, 91(6), 1161–1165.

Balinandi, S., Whitmer, S., Mulei, S., Nyakarahuka, L., Tumusiime, A., Kyondo, J., Baluku, J., Mutyaba, J., Mugisha, L., Malmberg, M., Lutwama, J., Shoemaker, T. R., & Klena, J. D. (2021). Clinical and Molecular Epidemiology of Crimean-Congo Hemorrhagic Fever in Humans in Uganda, 2013-2019. The American journal of tropical medicine and hygiene, 106(1), 88–98.

Banumathi, B., Murugan, K., & Benelli, G. (2017). Exploitation of chemical, herbal and nanoformulated acaricides to control the cattle tick, Rhipicephalus (Boophilus) microplus–A review. Veterinary parasitology, 244, 102-110.

Beati, L., Meskini, M., Thiers, B., & Raoult, D. (1997). Rickettsia aeschlimannii sp. nov., a new spotted fever group rickettsia associated with Hyalomma marginatum ticks. International journal of systematic bacteriology, 47(2), 548–554.

Ben Said, M., Belkahia, H., & Messadi, L. (2018). Anaplasma spp. in North Africa: A review on molecular epidemiology, associated risk factors and genetic characteristics. Ticks and tick-borne diseases, 9(3), 543–555.

Benelli, G., & Canale, A. (2017). Nanoparticles as effective acaricides against ticks—a review. Ticks and tick-borne diseases, 8(6), 821-826.

Bhowmick, B., & Han, Q. (2020). Understanding tick biology and its implications in anti-tick and transmission-blocking vaccines against tick-borne pathogens. Frontiers in Veterinary Science, 7, 319.

Bonnet, S. I., Vourc'h, G., Raffetin, A., Falchi, A., Figoni, J., Fite, J., Hoch, T., Moutailler, S., & Quillery, E. (2022). The control of Hyalomma ticks, vectors of the Crimean-Congo hemorrhagic fever virus: Where are we now and where are we going?. PLoS neglected tropical diseases, 16(11), e0010846.

Boulanger, N., Boyer, P., Talagrand-Reboul, E., & Hansmann, Y. (2019). Ticks and tick-borne diseases. Medecine et maladies infectieuses, 49(2), 87-97.

Chinikar, S., Shayesteh, M., Khakifirouz, S., Jalali, T., Rasi Varaie, F. S., Rafigh, M., Mostafavi, E., & Shah-Hosseini, N. (2013). Nosocomial infection of Crimean-Congo haemorrhagic fever in eastern Iran: case report. Travel medicine and infectious disease, 11(4), 252–255.

Chisu, V., Leulmi, H., Masala, G., Piredda, M., Foxi, C., & Parola, P. (2017). Detection of Rickettsia hoogstraalii, Rickettsia helvetica, Rickettsia massiliae, Rickettsia slovaca and Rickettsia aeschlimannii in ticks from Sardinia, Italy. Ticks and tick-borne diseases, 8(3), 347–352.

Chitimia-Dobler, L., Schaper, S., Rieß, R., Bitterwolf, K., Frangoulidis, D., Bestehorn, M., Springer, A., Oehme, R., Drehmann, M., Lindau, A., Mackenstedt, U., Strube, C., & Dobler, G. (2019). Imported Hyalomma ticks in Germany in 2018. Parasites & vectors, 12(1), 134.

Chiuya, T., Masiga, D. K., Falzon, L. C., Bastos, A. D. S., Fèvre, E. M., & Villinger, J. (2021). Tick-borne pathogens, including Crimean-Congo haemorrhagic fever virus, at livestock markets and slaughterhouses in western Kenya. Transboundary and emerging diseases, 68(4), 2429–2445.

Chiuya, T., Villinger, J., Masiga, D. K., Ondifu, D. O., Murungi, M. K., Wambua, L., Bastos, A. D. S., Fèvre, E. M., & Falzon, L. C. (2021). Molecular prevalence and risk factors associated with tick-borne pathogens in cattle in western Kenya. BMC veterinary research, 17(1), 363.

Cicculli, V., de Lamballerie, X., Charrel, R., & Falchi, A. (2019). First molecular detection of Rickettsia africae in a tropical bont tick, Amblyomma variegatum, collected in Corsica, France. Experimental & applied acarology, 77(2), 207–214.

Dahmani, M., Davoust, B., Benterki, M. S., Fenollar, F., Raoult, D., & Mediannikov, O. (2015). Development of a new PCR-based assay to detect Anaplasmataceae and the first report of Anaplasma phagocytophilum and Anaplasma platys in cattle from Algeria. Comparative immunology, microbiology and infectious diseases, 39, 39–45.

Dantas-Torres F. (2018). Species Concepts: What about Ticks?. Trends in parasitology, 34(12), 1017–1026. https://doi.org/10.1016/j.pt.2018.09.009

de la Fuente, J. (2018). Controlling ticks and tick-borne diseases… looking forward. Ticks and tick-borne diseases, 9(5), 1354-1357.

Dennis, D.T., & Piesman, J.F. (2014). Overview of tick-borne infections of humans. In J.L. Goodman, D.T. Dennis, & D.E. Sonenshine (Eds.), Tick-Borne Diseases of Humans (pp. 1–11). ASM Press; Washington, DC, USA.

Diop, A., El Karkouri, K., Raoult, D., & Fournier, P. E. (2020). Genome sequence-based criteria for demarcation and definition of species in the genus Rickettsia. International Journal of Systematic and Evolutionary Microbiology, 70, 1738–1750.

Dordio, A. M., Beck, R., Nunes, T., Pereira da Fonseca, I., & Gomes, J. (2021). Molecular survey of vector-borne diseases in two groups of domestic dogs from Lisbon, Portugal. Parasites & vectors, 14(1), 163.

Duron, O., Binetruy, F., Noël, V., Cremaschi, J., McCoy, K. D., Arnathau, C., Plantard, O., Goolsby, J., Pérez de León, A. A., Heylen, D. J. A., Van Oosten, A. R., Gottlieb, Y., Baneth, G., Guglielmone, A. A., Estrada-Peña, A., Opara, M. N., Zenner, L., Vavre, F., & Chevillon, C. (2017). Evolutionary changes in symbiont community structure in ticks. Molecular ecology, 26(11), 2905–2921.

Duron, O., Noël, V., McCoy, K. D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A. S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., & Chevillon, C. (2015). The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, Coxiella burnetii. PLoS Pathogens, 11(5), e1004892.

Eastwood, G., Sang, R. C., Guerbois, M., Taracha, E. L. N., & Weaver, S. C. (2017). Enzootic circulation of chikungunya virus in East Africa: Serological evidence in non-human Kenyan primates. American Journal of Tropical Medicine and Hygiene, 97(5), 1399–1404.

Ebani, V. V., & Mancianti, F. (2021). Potential Role of Avian Populations in the Epidemiology of Rickettsia spp. and Babesia spp. Veterinary sciences, 8(12), 334.

Ehounoud, C. B., Yao, K. P., Dahmani, M., Achi, Y. L., Amanzougaghene, N., Kacou N'Douba, A., N'Guessan, J. D., Raoult, D., Fenollar, F., & Mediannikov, O. (2016). Multiple Pathogens Including Potential New Species in Tick Vectors in Côte d'Ivoire. PLoS neglected tropical diseases, 10(1), e0004367.

Elati, K., Hamdi, D., Jdidi, M., Rekik, M., & Gharbi, M. (2018). Differences in tick infestation of Tunisian sheep breeds. Veterinary Parasitology: Regional Studies and Reports, 13, 50-54.

Elhamiani Khatat, S., Daminet, S., Kachani, M., Leutenegger, C. M., Duchateau, L., El Amri, H., Hing, M., Azrib, R., & Sahibi, H. (2017). Anaplasma spp. in dogs and owners in north-western Morocco. Parasites & vectors, 10(1), 202.

Elsa, J., Duron, O., Séverine, B., González-Acuña, D., & Sidi-Boumedine, K. (2015). Molecular methods routinely used to detect Coxiella burnetii in ticks cross-react with Coxiella-like bacteria. Infection ecology & epidemiology, 5, 29230.

Elsohaby, I., & Villa, L. (2023). Zoonotic diseases: Understanding the risks and mitigating the threats. BMC Veterinary Research, 19(1), 186.

Estrada-Peña, A., & de la Fuente, J. (2014). The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral research, 108, 104–128.

Gargili, A., Estrada-Peña, A., Spengler, J. R., Lukashev, A., Nuttall, P. A., & Bente, D. A. (2017). The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies. Antiviral research, 144, 93–119. https://doi.org/10.1016/j.antiviral.2017.05.010

Geiger, A., & Harrington, L. C., (2021). A survey of tick surveillance and control practices in the United States. Journal of medical entomology, 58(4), 1503-1512.

Georges, K., Loria, G. R., Riili, S., Greco, A., Caracappa, S., Jongejan, F., & Sparagano, O. (2001). Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Veterinary parasitology, 99(4), 273-286.

Gerhart, J. G., Auguste Dutcher, H., Brenner, A. E., Moses, A. S., Grubhoffer, L., & Raghavan, R. (2018). Multiple Acquisitions of Pathogen-Derived Francisella Endosymbionts in Soft Ticks. Genome biology and evolution, 10(2), 607–615.

Getange, D., Bargul, J.L., Kanduma, E., Collins, M., Bodha, B., Denge, D., Chiuya, T., Githaka, N., Younan, M., Fèvre, E.M. and Bell-Sakyi, L., (2021). Ticks and tick-borne pathogens associated with dromedary camels (Camelus dromedarius) in northern Kenya. Microorganisms, 9(7), p.1414.

Githaka, N. W., Bishop, R. P., Šlapeta, J., Emery, D., Nguu, E. K.,Kanduma, E. G. (2022). Molecular survey of babesia parasites in kenya: first detailed report on occurrence of babesia bovis in cattle. Parasites & Vectors, 15(1).

Grandi, G., Chitimia-Dobler, L., Choklikitumnuey, P., Strube, C., Springer, A., Albihn, A., Jaenson, T. G. T., & Omazic, A. (2020). First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks and tick-borne diseases, 11(3), 101403.

Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A., & Horak, I. G. (2014). The hard ticks of the world. Springer, Dordrecht. doi, 10, 978-994.

Halliday, F.W., Heckman, R.W., Wilfahrt, P.A. and Mitchell, C.E., (2020). Eutrophication, biodiversity loss, and species invasions modify the relationship between host and parasite richness during host community assembly. Global change biology, 26(9), pp.4854-4867.

Hatami, H., Qaderi, S., & Omid, A. M. (2019). Investigation of Crimean-Congo hemorrhagic Fever in Patients Admitted in Antani Hospital, Kabul, Afghanistan, 2017-2018. International journal of preventive medicine, 10, 117.

Hornok, S., Szőke, K., Meli, M. L., Sándor, A. D., Görföl, T., Estók, P., ... & Hofmann-Lehmann, R. (2019). Molecular detection of vector-borne bacteria in bat ticks (Acari: Ixodidae, Argasidae) from eight countries of the Old and New Worlds. Parasites & vectors, 12(1), 1-7.

Hornok, S., Szőke, K., Meli, M. L., Sándor, A. D., Görföl, T., Estók, P., Wang, Y., Tu, V. T., Kováts, D., Boldogh, S. A., Corduneanu, A., Sulyok, K. M., Gyuranecz, M., Kontschán, J., Takács, N., Halajian, A., Epis, S., & Hofmann-Lehmann, R. (2019). Molecular detection of vector-borne bacteria in bat ticks (Acari: Ixodidae, Argasidae) from eight countries of the Old and New Worlds. Parasites & vectors, 12(1), 50.

Huber, D., Reil, I., Duvnjak, S., Jurković, D., Lukačević, D., Pilat, M., Beck, A., Mihaljević, Ž., Vojta, L., Polkinghorne, A., & Beck, R. (2017). Molecular detection of Anaplasma platys, Anaplasma phagocytophilum and Wolbachia sp. but not Ehrlichia canis in Croatian dogs. Parasitology research, 116(11), 3019–3026.

Jimale, K. A., Wall, R., & Otranto, D. (2023). Ticks and tick-borne pathogens of domestic animals in Somalia and neighbouring regions of Ethiopia and Kenya. Acta tropica, 243, 106944.

Kasaija, P. D., Estrada-Peña, A., Contreras, M., Kirunda, H., & de la Fuente, J. (2021). Cattle ticks and tick-borne diseases: a review of Uganda's situation. Ticks and tick-borne diseases, 12(5), 101756.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Alexei, D. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(4), 1647–1649.

Kemp, D. H., Agbede, R. I., Johnston, L. A., & Gough, J. M. (1986). Immunization of cattle against Boophilus microplus using extracts derived from adult female ticks: feeding and survival of the parasite on vaccinated cattle. International journal for parasitology, 16(2), 115–120.

Khoo, J. J., Lim, F. S., Chen, F., Phoon, W. H., Khor, C. S., Pike, B. L., Chang, L. Y., & AbuBakar, S. (2016). Coxiella Detection in Ticks from Wildlife and Livestock in Malaysia. Vector borne and zoonotic diseases (Larchmont, N.Y.), 16(12), 744–751.

Kiara, H., Jennings, A., Bronsvoort, B. M., Handel, I. G., Mwangi, S. T., Mbole-Kariuki, M., Van Wyk, I. C., Poole, E. J., Hanotte, O., Coetzer, J. A., Woolhouse, M. E., & Toye, P. G. (2014). A longitudinal assessment of the serological response to Theileria parva and other tick-borne parasites from birth to one year in a cohort of indigenous calves in western Kenya. Parasitology, 141(10), 1289–1298.

Kim, Y. S., Choi, Y. J., Lee, K. M., Ahn, K. J., Kim, H. C., Klein, T., Jiang, J., Richards, A., Park, K. H., & Jang, W. J. (2017). First isolation of Rickettsia monacensis from a patient in South Korea. Microbiology and Immunology, 61, 258–263.

Koka, H., Sang, R., Kutima, H. L., & Musila, L. (2017). The detection of spotted fever group Rickettsia DNA in tick samples from pastoral communities in Kenya. Journal of medical entomology, 54(3), 774–780.

Kolo A. (2023). Anaplasma Species in Africa-A Century of Discovery: A Review on Molecular Epidemiology, Genetic Diversity, and Control. Pathogens (Basel, Switzerland), 12(5), 702.

Kolo, A. O., & Raghavan, R. (2023). Impact of endosymbionts on tick physiology and fitness. Parasitology, 150(10), 859–865.

Kopáček, P., Šíma, R., & Perner, J. (2021). An mRNA-based anti-tick vaccine catches ticks red-handed. Science translational medicine, 13(620), eabm2504.

Laing, G., Aragrande, M., Canali, M., Savic, S., & De Meneghi, D. (2018). Control of Cattle Ticks and Tick-Borne Diseases by Acaricide in Southern Province of Zambia: A Retrospective Evaluation of Animal Health Measures According to Current One Health Concepts. Frontiers in public health, 6, 45.

Lara, B., Conan, A., Thrall, M. A., Ketzis, J. K., Branford, G. C., & Rajeev, S. (2020). Serologic and Molecular Diagnosis of Anaplasma platys and Ehrlichia canis Infection in Dogs in an Endemic Region. Pathogens (Basel, Switzerland), 9(6), 488.

Léger, E., Vourc'h, G., Vial, L., Chevillon, C., & McCoy, K. D. (2013). Changing distributions of ticks: causes and consequences. Experimental & applied acarology, 59(1-2), 219–244.

Lorusso, V., Wijnveld, M., Majekodunmi, A. O., Dongkum, C., Fajinmi, A., Dogo, A. G., Thrusfield, M., Mugenyi, A., Vaumourin, E., Igweh, A. C., Jongejan, F., Welburn, S. C., & Picozzi, K. (2016). Tick-borne pathogens of zoonotic and veterinary importance in Nigerian cattle. Parasites & vectors, 9, 217.

Lwande, O. W., Venter, M., Lutomiah, J., Michuki, G., Rumberia, C., Gakuya, F., Obanda, V., Tigoi, C., Odhiambo, C., Nindo, F., Symekher, S., & Sang, R. (2014). Whole genome phylogenetic investigation of a West Nile virus strain isolated from a tick sampled from livestock in north eastern Kenya. Parasites & vectors, 7, 542

Macaluso, K. R., Davis, J., Alam, U., Korman, A., Rutherford, J. S., Rosenberg, R., & Azad, A. F. (2003). Spotted fever group rickettsiae in ticks from the Masai Mara region of Kenya. The American journal of tropical medicine and hygiene, 68(5), 551–553.

Maggi, R. G., Mascarelli, P. E., Havenga, L. N., Naidoo, V., & Breitschwerdt, E. B. (2013). Co-infection with Anaplasma platys, Bartonella henselae and Candidatus Mycoplasma haematoparvum in a veterinarian. Parasites & vectors, 6, 103.

Maina, A. N., Jiang, J., Omulo, S. A., Cutler, S. J., Ade, F., Ogola, E., Feikin, D. R., Njenga, M. K., Cleaveland, S., Mpoke, S., Ng'ang'a, Z., Breiman, R. F., Knobel, D. L., & Richards, A. L. (2014). High prevalence of Rickettsia africae variants in Amblyomma variegatum ticks from domestic mammals in rural western Kenya: implications for human health. Vector borne and zoonotic diseases (Larchmont, N.Y.), 14(10), 693–702.

Mancuso, E., Di Domenico, M., Di Gialleonardo, L., Menegon, M., Toma, L., Di Luca, M., Casale, F., Di Donato, G., D'Onofrio, L., De Rosa, A., Riello, S., Ferri, A., Serra, L., & Monaco, F. (2023). Tick Species Diversity and Molecular Identification of Spotted Fever Group Rickettsiae Collected from Migratory Birds Arriving from Africa. Microorganisms, 11(8), 2036.

Mansfield, K. L., Cook, C., Ellis, R. J., Bell-Sakyi, L., Johnson, N., Alberdi, P., de la Fuente, J., & Fooks, A. R. (2017). Tick-borne pathogens induce differential expression of genes promoting cell survival and host resistance in Ixodes ricinus cells. Parasites & vectors, 10(1), 81.

Marchiondo, A. A., Cruthers, L. R., & Fourie, J. J. (2019). Chapter 4 - Arachnida. In A. A. Marchiondo, L. R. Cruthers, & J. J. Fourie (Eds.), Parasiticide Screening, Volume 1 (pp. 257-377). Academic Press. ISBN 9780128138908.

Marima, J. K., Nel, C. L., Marufu, M. C., Jonsson, N. N., Dube, B., & Dzama, K. (2020). A genetic and immunological comparison of tick-resistance in beef cattle following artificial infestation with Rhipicephalus ticks. Experimental and Applied Acarology, 80(4), 569-590.

Marina, A., & de la Fuente, J. (2017). Applying proteomics to tick vaccine development: where are we? Expert Review of Proteomics, 14(3), 211-221.

Maruyama, S. R., Garcia, G. R., Teixeira, F. R., Brandão, L. G., Anderson, J. M., Ribeiro, J. M. C., Valenzuela, J. G., Horackova, J., Veríssimo, C. J., Katiki, L. M., Banin, T. M., Zangirolamo, A. F., Gardinassi, L. G., Ferreira, B. R., & de Miranda-Santos, I. K. F. (2017). Mining a differential sialotranscriptome of Rhipicephalus microplus guides antigen discovery to formulate a vaccine that reduces tick infestations. Parasites & vectors, 10(1), 206.

Matei, I. A., D'Amico, G., Yao, P. K., Ionică, A. M., Kanyari, P. W., Daskalaki, A. A., Dumitrache, M. O., Sándor, A. D., Gherman, C. M., Qablan, M., Modrý, D., & Mihalca, A. D. (2016). Molecular detection of Anaplasma platys infection in free-roaming dogs and ticks from Kenya and Ivory Coast. Parasites & vectors, 9, 157.

Merhej, V., Angelakis, E., Socolovschi, C., & Raoult, D. (2014). Genotyping, evolution and epidemiological findings of Rickettsia species. Infection, Genetics and Evolution, 25, 122–137.

Messina, J. P., Pigott, D. M., Golding, N., Duda, K. A., Brownstein, J. S., Weiss, D. J., Gibson, H., Robinson, T. P., Gilbert, M., William Wint, G. R., Nuttall, P. A., Gething, P. W., Myers, M. F., George, D. B., & Hay, S. I. (2015). The global distribution of Crimean-Congo hemorrhagic fever. Transactions of the Royal Society of Tropical Medicine and Hygiene, 109(8), 503–513.

Meurens, F., Dunoyer, C., Fourichon, C., Gerdts, V., Haddad, N., Kortekaas, J., Lewandowska, M., Monchatre-Leroy, E., Summerfield, A., Wichgers Schreur, P. J., van der Poel, W. H. M., & Zhu, J. (2021). Animal board invited review: Risks of zoonotic disease emergence at the interface of wildlife and livestock systems. Animal: an international journal of animal bioscience, 15(6), 100241.

M'ghirbi, Y., Bèji, M., Oporto, B., Khrouf, F., Hurtado, A., & Bouattour, A. (2016). Anaplasma marginale and A. phagocytophilum in cattle inTunisia. Parasites & vectors, 9(1), 556.

Moraga-Fernández, A., Ruiz-Fons, F., Habela, M. A., Royo-Hernández, L., Calero-Bernal, R., Gortazar, C., de la Fuente, J., & Fernández de Mera, I. G. (2021). Detection of new Crimean-Congo haemorrhagic fever virus genotypes in ticks feeding on deer and wild boar, Spain. Transboundary and emerging diseases, 68(3), 993–1000.

Morrison, W. I., Hemmink, J. D., & Toye, P. G. (2020). Theileria parva: a parasite of African buffalo, which has adapted to infect and undergo transmission in cattle. International journal for parasitology, 50(5), 403–412.

Mtshali, K., Nakao, R., Sugimoto, C., & Thekisoe, O. (2017). Occurrence of Coxiella burnetii, Ehrlichia canis, Rickettsia species and Anaplasma phagocytophilum-like bacterium in ticks collected from dogs and cats in South Africa. Journal of the South African Veterinary Association, 88(0), e1–e6.

Mwamuye, M. M., Kariuki, E., Omondi, D., Kabii, J., Odongo, D., Masiga, D., & Villinger, J. (2017). Novel Rickettsia and emergent tick-borne pathogens: A molecular survey of ticks and tick-borne pathogens in Shimba Hills National Reserve, Kenya. Ticks and tick-borne diseases, 8(2), 208–218.

Ndeereh, D., Muchemi, G., Thaiyah, A., Otiende, M., Angelone-Alasaad, S., & Jowers, M. J. (2017). Molecular survey of Coxiella burnetii in wildlife and ticks at wildlife-livestock interfaces in Kenya. Experimental & applied acarology, 72(3), 277–289.

Nijhof, A. M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Franssen, L., Jebbink, F., & Jongejan, F. (2007). Ticks and associated pathogens collected from domestic animals in the Netherlands. Vector borne and zoonotic diseases (Larchmont, N.Y.), 7(4), 585–595.

Nwanade, C. F., Wang, M., Wang, T., Yu, Z., & Liu, J. (2020). Botanical acaricides and repellents in tick control: current status and future directions. Experimental and Applied Acarology, 81(1), 1-35.

Olds, C. L., Mason, K. L., & Scoles, G. A. (2018). Rhipicephalus appendiculatus ticks transmit Theileria parva from persistently infected cattle in the absence of detectable parasitemia: implications for East Coast fever epidemiology. Parasites & vectors, 11(1), 126.

Omondi, D., Masiga, D. K., Fielding, B. C., Kariuki, E., Ajamma, Y. U., Mwamuye, M. M., Ouso, D. O., & Villinger, J. (2017). Molecular Detection of Tick-Borne Pathogen Diversities in Ticks from Livestock and Reptiles along the Shores and Adjacent Islands of Lake Victoria and Lake Baringo, Kenya. Frontiers in veterinary science, 4, 73.

Omondi, D., Masiga, D. K., Fielding, B. C., Kariuki, E., Ajamma, Y. U., Mwamuye, M. M., Ouso, D. O., & Villinger, J. (2017). Molecular Detection of Tick-Borne Pathogen Diversities in Ticks from Livestock and Reptiles along the Shores and Adjacent Islands of Lake Victoria and Lake Baringo, Kenya. Frontiers in veterinary science, 4, 73.

Onyiche, T. E., & MacLeod, E. T. (2023). Hard ticks (Acari: Ixodidae) and tick-borne diseases of sheep and goats in Africa: A review. Ticks and tick-borne diseases, 14(6), 102232.

Oundo, J. W., Kalayou, S., Bosch, Q. T., Villinger, J., Koenraadt, C. J. M., & Masiga, D. (2024). Ticks (Acari: Ixodidae) infesting cattle in coastal Kenya harbor a diverse array of tick-borne pathogens. Ticks and tick-borne diseases, 15(1), 102266.

Oundo, J. W., Masiga, D., Ten Bosch, Q., Villinger, J., Koenraadt, C. J. M., & Kalayou, S. (2022). Epidemiology of tick-borne pathogens of cattle and tick control practices in coastal Kenya. Preventive veterinary medicine, 209, 105777.

Oundo, J. W., Villinger, J., Jeneby, M., Ong'amo, G., Otiende, M. Y., Makhulu, E. E., Musa, A. A., Ouso, D. O., & Wambua, L. (2020). Pathogens, endosymbionts, and blood-meal sources of host-seeking ticks in the fast-changing Maasai Mara wildlife ecosystem. PloS one, 15(8), e0228366.

Parola, P., Paddock, C. D., Socolovschi, C., Labruna, M. B., Mediannikov, O., Kernif, T., Abdad, M. Y., Stenos, J., Bitam, I., Fournier, P. E., & Raoult, D. (2013). Update on tick-borne rickettsioses around the world: a geographic approach. Clinical microbiology reviews, 26(4), 657–702.

Pesquera, C., Portillo, A., Palomar, A.M. and Oteo, J.A., (2015). Investigation of tick-borne bacteria (Rickettsia spp., Anaplasma spp., Ehrlichia spp. and Borrelia spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador. Parasites & vectors, 8(1), pp.1-10.

Pintore, E., Olivieri, E., Floriano, A. M., Sassera, D., Sanna, N., & Garippa, G. (2021). First detection of Amblyomma variegatum and molecular finding of Rickettsia africae in Sardinia, Italy. Ticks and tick-borne diseases, 12(1), 101561.

Portillo, A., Santibáñez, S., García-Álvarez, L., Palomar, A. M., & Oteo, J. A. (2015). Rickettsioses in Europe. Microbes and Infection, 17, 834–838.

Pshenichnaya, N. Y., Sydenko, I. S., Klinovaya, E. P., Romanova, E. B., & Zhuravlev, A. S. (2016). Possible sexual transmission of Crimean-Congo hemorrhagic fever. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 45, 109–111.

Rainey, T., Occi, J. L., Robbins, R. G., & Egizi, A. (2018). Discovery of Haemaphysalis longicornis (Ixodida: Ixodidae) Parasitizing a Sheep in New Jersey, United States. Journal of medical entomology, 55(3), 757–759.

Rjeibi, M. R., Amairia, S., Mhadhbi, M., Rekik, M., & Gharbi, M. (2022). Detection and molecular identification of Anaplasma phagocytophilum and Babesia spp. infections in Hyalomma aegyptium ticks in Tunisia. Archives of microbiology, 204(7), 385.

Rodriguez-Vivas, R. I., Jonsson, N. N., & Bhushan, C. (2018). Strategies for the control of Rhipicephalus microplus ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology research, 117(1), 3-29.

Roux, V., & Raoult, D. (2000). Phylogenetic analysis of members of the genus Rickettsia using the gene encoding the outer-membrane protein rOmpB (ompB). International journal of systematic and evolutionary microbiology, 50(4), 1449-1455.

Sainz, Á., Roura, X., Miró, G., Estrada-Peña, A., Kohn, B., Harrus, S., & Solano-Gallego, L. (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasites & vectors, 8, 75.

Schwartz, A. M., Hinckley, A. F., Mead, P. S., Hook, S. A., & Kugeler, K. J. (2017). Surveillance for Lyme Disease - United States, 2008-2015. Morbidity and mortality weekly report. Surveillance summaries (Washington, D.C. : 2002), 66(22), 1–12.

Sekeyová, Z., Danchenko, M., Filipčík, P., & Fournier, P. E. (2019). Rickettsial infections of the central nervous system. PLoS neglected tropical diseases, 13(8), e0007469.

Shuaib, Y. A., Elhag, A. M. W., Brima, Y. A., Abdalla, M. A., Bakiet, A. O., Mohmed-Noor, S. E., Lemhöfer, G., Bestehorn, M., Poppert, S., Schaper, S., Dobler, G., Bakkes, D. K., & Chitimia-Dobler, L. (2020). Ixodid tick species and two tick-borne pathogens in three areas in the Sudan. Parasitology research, 119(2), 385–394.

Silva-Ramos, C. R., & Faccini-Martínez, Á. A. (2021). Clinical, epidemiological, and laboratory features of Rickettsia africae infection, African tick-bite fever: A systematic review. Le infezioni in medicina, 29(3), 366–377.

Simpson, D. I., Knight, E. M., Courtois, G., Williams, M. C., Weinbren, M. P., & Kibukamusoke, J. W. (1967). Congo virus: a hitherto undescribed virus occurring in Africa. I. Human isolations--clinical notes. East African medical journal, 44(2), 86–92.

Smith TA, Driscoll T, Gillespie JJ, Raghavan R. A (2015). Coxiella-like endosymbiont is a potential vitamin source for the Lone Star tick. Genome Biology and Evolution. 2015 Mar; 7(3):831–838.

Snellgrove, A. N., Krapiunaya, I., Ford, S. L., Stanley, H. M., Wickson, A. G., Hartzer, K. L., & Levin, M. L. (2020). Vector competence of Rhipicephalus sanguineus sensu stricto for Anaplasma platys. Ticks and tick-borne diseases, 11(6), 101517.

Sollero, B. P., & Cardoso, F. F. (2017). Tag SNP selection for prediction of tick resistance in Brazilian Braford and Hereford cattle breeds using Bayesian methods. Genetics Selection Evolution, 49(1), 1-15.

Stafford III, K. C., Williams, S. C., & Molaei, G. (2017). Integrated pest management in controlling ticks and tick-associated diseases. Journal of Integrated Pest Management, 8(1), 28.

Stuen, S., Granquist, E. G., & Silaghi, C. (2013). Anaplasma phagocytophilum--a widespread multi-host pathogen with highly adaptive strategies. Frontiers in cellular and infection microbiology, 3, 31.

Swei, A., Couper, L. I., Coffey, L. L., Kapan, D., & Bennett, S. (2020). Patterns, Drivers, and Challenges of Vector-Borne Disease Emergence. Vector borne and zoonotic diseases (Larchmont, N.Y.), 20(3), 159–170.

Tabor, A. E., & Jonsson, N. N. (2017). Cattle tick Rhipicephalus microplus-host interface: a review of resistant and susceptible host responses. Frontiers in Cellular and Infection Microbiology, 7, 506.

Thekisoe, O., Ramatla, T., Ringo, A., Mnisi, S., Mphuthi, N., Mofokeng, L., Lekota, K., & Xuan, X. (2023). Molecular detection of rickettsia africae from amblyomma hebraeum ticks in mafikeng city of north west province, south africa. Research in Veterinary Science, 164, 105027–105027.

Tokarz, R., Kapoor, V., Samuel, J. E., Bouyer, D. H., Briese, T., & Lipkin, W. I. (2009). Detection of tick-borne pathogens by MassTag polymerase chain reaction. Vector-Borne and Zoonotic Diseases, 9(2), 147-152.

Tomassone, L., Berriatua, E., De Sousa, R., Duscher, G. G., Mihalca, A. D., Silaghi, C., Sprong, H., & Zintl, A. (2018). Neglected vector-borne zoonoses in Europe: Into the wild. Veterinary parasitology, 251, 17–26.

Tomassone, L., Portillo, A., Nováková, M., De Sousa, R., & Oteo, J. A. (2018). Neglected aspects of tick-borne rickettsioses. Parasites & vectors, 11(1), 1-11.

Tortosa, P., Socolovschi, C., Yssouf, A., Parola, P., Kernif, T., Lagadec, E., & Temmam, S. (2014). First molecular detection of rickettsia africae in ticks from the union of the comoros. Parasites & Vectors, 7(1), 1–6.

Tosoni, A., Mirijello, A., Ciervo, A., Mancini, F., Rezza, G., Damiano, F., Cauda, R., Gasbarrini, A., Addolorato, G., & Internal Medicine Sepsis Study Group (2016). Human Rickettsia aeschlimannii infection: first case with acute hepatitis and review of the literature. European review for medical and pharmacological sciences, 20(12), 2630–2633.

Trentelman, J. J., van de Crommert, J., & Schetters, T. P. (2017). A new method for in vitro feeding of Rhipicephalus australis (formerly Rhipicephalus microplus) larvae: a valuable tool for tick vaccine development. Parasites & vectors, 10(1), 1-9.

Uusitalo, R., Siljander, M., Lindén, A., Sormunen, J. J., Aalto, J., Hendrickx, G., Kallio, E., Vajda, A., Gregow, H., Henttonen, H., Marsboom, C., Korhonen, E. M., Sironen, T., Pellikka, P., & Vapalahti, O. (2022). Predicting habitat suitability for Ixodes ricinus and Ixodes persulcatus ticks in Finland. Parasites & vectors, 15(1), 310.

Vainstein, M. H., Schrank, A., & Santi, L. (2020). Updating the application of Metarhizium anisopliae to control cattle tick Rhipicephalus microplus (Acari: Ixodidae). Experimental Parasitology, 208, 107812.

Vogel, H., Foley, J.,Fiorello, C. V. (2018). Rickettsia africae and novel rickettsial strain in amblyomma spp. ticks, nicaragua, 2013. Emerging Infectious Diseases, 24(2), 385–387.

Vudriko, P., Okwee-Acai, R., & Suzuki, H. (2018). Chemical tick control practices in southwestern and northwestern Uganda. Ticks and tick-borne diseases, 9(4), 945-955.

Walker AR, Bouattour A, Camicas J-L, Preston PM (2014). Ticks of Domestic Animals in Africa: a guide to identification of species. Bioscience Reports, Edinburgh; 2014. Available:

Walker, A. R. (2003). Ticks of domestic animals in Africa: a guide to identification of species (Vol. 74). Edinburgh: Bioscience Reports.

Wallménius, K., Barboutis, C., Fransson, T., Jaenson, T. G., Lindgren, P. E., Nyström, F., Olsen, B., Salaneck, E., & Nilsson, K. (2014). Spotted fever Rickettsia species in Hyalomma and Ixodes ticks infesting migratory birds in the European Mediterranean area. Parasites & vectors, 7, 1-20.

Wang, Q., Guo, W.-B., Pan, Y.-S., Jiang, B.-G., Du, C.-H., Que, T.-C., Zhan, L., Wu, J.-H., Yu, M.-H., Cui, X.-M., Zhao, L., Xu, D.-L., Xia, L.-Y., Ye, R.-Z., Li, J., Li, L.-F., Wei, W., Zhou, Y.-H., Jiang, J.-F., Jia, N., Cao, W.-C., (2021). Detection of Novel Spotted Fever Group Rickettsiae (Rickettsiales: Rickettsiaceae) in Ticks (Acari: Ixodidae) in Southwestern China. Journal of Medical Entomology, 58(3), 1363–1369.

Wei, Q. Q., Guo, L. P., Wang, A. D., Mu, L. M., Zhang, K., Chen, C. F., Zhang, W. J., & Wang, Y. Z. (2015). The first detection of Rickettsia aeschlimannii and Rickettsia massiliae in Rhipicephalus turanicus ticks, in northwest China. Parasites & vectors, 8, 631.

Weidmann, M., Avsic-Zupanc, T., Bino, S., Bouloy, M., Burt, F., Chinikar, S., Christova, I., Dedushaj, I., El-Sanousi, A., Elaldi, N., Hewson, R., Hufert, F. T., Humolli, I., Jansen van Vuren, P., Koçak Tufan, Z., Korukluoglu, G., Lyssen, P., Mirazimi, A., Neyts, J., Niedrig, M., Ozkul, A., Papa, A., Paweska, J., Sall, A. A., Schmaljohn, C. S., Swanepoel, R., Uyar, Y., Weber, F., & Zeller, H. (2016). Biosafety standards for working with Crimean-Congo hemorrhagic fever virus. Journal of General Virology, 97(11), 2897–2907.

Wikel S. K. (2018). Ticks and Tick-Borne Infections: Complex Ecology, Agents, and Host Interactions. Veterinary sciences, 5(2), 60.

Ziegelmann, B., & Rosenkranz, P. (2018). Lithium chloride effectively kills the honey bee.

APPENDICES

Appendix I: Kenyatta University Research Proposal Approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 15th October, 2018

TO: Salim Kobo Godani
C/o Medical Laboratory Sciences
Department.

REF: P150/CE/26196/2014

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 19th September, 2018 approved your Research Proposal for the M.Sc Degree Entitled, "Determination of Tick – Borne Pathogens, their Genetic Diversity in Cattle in Taita Taveta County, Kenya".

You may now proceed with data collection, subject to clearance with the Director General, Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking forms per semester. The form has been developed to replace the progress report forms. The supervision Tracking Forms are available at the University's website under Graduate School webpage downloads.

Thank you.

JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Medical Laboratory Sciences

Supervisors:

1. Dr. Nelson Chengo Menza
C/o Medical Laboratory Sciences
Kenyatta University

2. Dr. Margaret Muturi
C/o Department of Medical Laboratory Sciences
Kenyatta University

Appendix II: Research Authorization from Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P150/CE/26196/2014

DATE: 15th October, 2018

Director General,
National Commission for Science
& Innovation,
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR SALIM KOBO GODANI – REG. NO. P150/CE/26196/2014

I write to introduce Mr. Salim Kobo Godani who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the Department of Medical Laboratory Sciences.

Mr. Godani intends to conduct research for an M.Sc Proposal entitled, "Determination of Tick – Borne Pathogens, their Genetic Diversity in Cattle in Taita Taveta County, Kenya".

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. PAUL OKEMO
FOR: DEAN, GRADUATE SCHOOL

POO/rwm

Appendix III: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **276195**

Date of Issue: **15/September/2022**

RESEARCH LICENSE

This is to Certify that Mr.. SALIM KOB0 GODANI of Kenyatta University, has been licensed to conduct research in Taita-Taveta on the topic: DETERMINATION OF TICK- BORNE PATHOGENS, THEIR GENETIC DIVERSITY IN CATTLE IN TAITA TAVETA COUNTY, KENYA for the period ending : 15/September/2023.

License No: **NACOSTI/P/22/20114**

276195

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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Appendix IV: Publication

Scientific Research Publishing

Advances in Entomology, 2023, 11, 1-9
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Zoonotic Pathogens Detected in Ticks in Kenyan Game Reserves

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Abstract

Little is known on tick-borne pathogens and their role in disease in game reserves in Kenya. Ticks were collected by sterile forceps from restrained cattle hide and placed into labeled falcon tubes. Ticks were screened for pathogens by High Resolution Melting (HRM) analysis and sequencing of specific RT-PCR products of *Anaplasma*, *Ehrlichia*, and *Rickettsia* species. A total of 317 ticks (281 adult ticks and 36 nymphs) comprising seven species were collected around the Tsavo National Reserve (TNR) in Taita Taveta County with *Amblyomma gemma* being the most commonly collected species (n = 135, 42.6%). From near Shimba Hill game reserve (SHNR), a total of 240 adult's ticks were sampled, representing eight species, with again *Amblyomma*

Appendix V: Veterinary Introductory Letter

COUNTY COVERNMENT OF TAITA TAVETA

Telephone: 0725777031
Email: livesfish.taty@gmail.com
oyindomartin@gmail.com

P.O. BOX 504-80300
VOI

DEPARTMENT OF AGRICULTURE, LIVESTOCK AND FISHERIES IRRIGATION

Office Of The County Director Of Livestock Production

CDLP/TT/ADM/12 18th December 2018

RE: LETTER OF INTRODUCTION MR SALIM KOBO GODANI-MSC RESEARCH STUDENT
REGISTRATION NO:P150/CE/26196/2014

The above named student is from Kenyatta University Medical Laboratory Sciences Department is on a field research mission for Masters Degree entitled – Determination of Tick' borne pathogens and their genetic diversity in cattle in Taita Taveta County – Kenya. He will conduct his research within Voi Sub-County visiting firms to collect data and information.

The purpose of this letter is to serve as an introduction letter to allow him do the research. I therefore request you to accord him necessary support.
From the research findings we expect him to give a feedback and recommendations from the report.

Regards

Oyando Martin Luther
AG. COUNTY DIRECTOR OF LIVESTOCK PRODUCTION