MOLECULAR EPIDEMIOLOGY OF SPOTTED FEVER GROUP RICKETTSIOSES AND Q FEVER AT THE WILDLIFE-LIVESTOCK INTERFACE IN MAASAI MARA AND LAIKIPIA ECOSYSTEMS, KENYA

A THESIS SUBMITTED IN FULFILMENT OF REQUIREMENTS FOR DOCTOR OF PHILOSOPHY DEGREE OF THE UNIVERSITY OF NAIROBI (VETERINARY MEDICINE)

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DECLARATION

I HEREBY DECLARE THAT THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY

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DEDICATION

This thesis is dedicated to those who strive not to equal but to excel
and to my wife Zipporah Wambui, sons Steven Ndeereh and Charles Gichere and my loving mother Ruth Kimoi Ndeereh

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ATBF	African Tick Bite fever
BLAST	Basic Local Alignment Search Tool
CBPP	Contagious Bovine Pleuro-Pneumonia
CCPP	Contagious Caprine Pleuro-Pneuminia
CDC	Centers for Disease Control and Prevention
CFT	Complement Fixation Test
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded Deoxyribonucleic Acid
ECF	East Coast Fever
EDTA	Ethylene-Diamine Tetraacetic Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
FMD	Foot and Mouth Disease
GIS	Geographical Information System
GPS	Global Positioning System
HIV	Human Immunodeficiency Virus
HRM	High Resolution Melting
icipe	International Centre for Insect Physiology and Ecology
IFA	Indirect Fluorescence Antibody
KAP	Knowledge Attitudes and Practices
KWS	Kenya Wildlife Service
MCF	Malignant Catarrhal Fever
MS	Microsoft
MSF	Mediterranean Spotted Fever
NACOSTI	National Council for Science Technology and Innovation
OIE	World Organization for Animal Health
ompA	Outer Membrane Protein A
ompB	Outer Membrane Protein B
PBS	Phosphate Buffered Saline

PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RMSF	Rocky Mountain Spotted Fever
rpm	Revolutions Per Minute
rRNA	Ribosomal Ribonucleic Acid
RTqPCR	Real-Time Quantitative Polymerase Chain Reaction
SAS	Statistical Analysis Software
SFG	Spotted Fever Group
STG	Scrub Typhus Group
Taq	*Thermus aquaticus*
TG	Typhus Group
UK	United Kingdom
USA	United States of America
α	Alpha
µl	Microlitre

ABSTRACT

Spotted fever group (SFG) rickettsioses and Q fever are zoonotic diseases caused by the intracellular bacteria in the genus Rickettsia and *Coxiella burnetii* respectively. The pathogens continue to be described in livestock and their ticks in Kenya yet no information is available about them in wildlife and their ticks. This is despite wildlife having been identified as important sources of zoonotic pathogens. This study investigated the presence, prevalence and species of SFG *Rickettsia* species and strains of *C. burnetii* in wildlife and their ticks in Laikipia and Maasai Mara ecosystems. It also evaluated the knowledge, attitudes and practices (KAP) of the local residents towards the diseases.

A total of 152 animals (79 in Laikipia and 73 in Maasai Mara) comprising 8 species were sampled and 851 ixodid ticks comprising 10 species collected from the animals. The ticks were pooled into 166 pools (137 in Laikipia and 29 in Maasai Mara) of 1-8 ticks according to species, sampling site and the animal host. To detect SFG *Rickettsia* species and *C. burnetii*, DNA extracted from EDTA blood and ticks was tested using real-time PCR targeting the intergenic spacer *rpmE*-tRNA^{fMet} and the repetitive insertion element IS*1111a* of the transposase gene, respectively. To identify the SFG *Rickettsia* species, genes *ompA*, *ompB* and *gltA* were amplified and the PCR positive products sequenced. To identify the strains of *C. burnetii*, the insertion element *IS1111a* was sequenced in PCR positive products.

The prevalence of SFG rickettsioses in wildlife was 2/79 (2.5%) in Laikipia and 4/73 (5.5%) in Maasai Mara. The prevalence in ticks was 30/137 (21.9%) in Laikipia and 5/29 (17.2%) in Maasai Mara. The detection was in 30/135 (22.2%) and 2/24 (8.3%) of *Rhipicephalus* ticks sampled in Laikipia and Maasai Mara respectively, and 2/2 (100%) of *Amblyomma* and 1/3 (33.3%) of *Hyalomma* ticks sampled in Maasai Mara. In regard to individual tick species, the detection was in 4/11 (36.4%) of *Rh. evertsi*, 16/53 (30.2%) of *Rh. appendiculatus*, 6/33 (18.2%) of *Rh. pulchellus* and 4/38 (10.5%) of *Rh. evertsi evertsi* ticks sampled in Laikipia. In Maasai Mara, 2/10 (20%) *Rh. appendiculatus* and the only sample each of *H. dromedari*, *A. variegatum* and *A. truncatum* tested positive. Sequence analyses of amplified genes of SFG rickettsiae revealed *R. sibirica* in a Topi (*Damaliscus korrigum*), *Rh. evertsi evertsi*, *Rh. appendiculatus*, *A.*

variegatum and *A. truncatum* as well as *R. sibirica* subspecies *mongolotimonae* in *Rh. evertsi*, *Rh. pulchellus, A. variegatum* and *A. truncatum* ticks.

C. burnetii DNA was not detected in any of the animals. The prevalence in ticks was 4/137 (2.9%) in Laikipia. The pathogen was detected in 4/135 (3.0%) of *Rhipicephalus* ticks at prevalence of 2/53 (3.8%) in *Rh. appendiculatus*, 1/33 (3.0%) in *Rh. pulchellus* and 1/38 (2.6%) in *Rh. evertsi evertsi*. No ticks tested positive in Maasai Mara. Sequence analyses revealed the isolates to be identical and had 100% similarities to strains from other parts of the world.

To evaluate the KAP, a semi-structured questionnaire was administered to 101 respondents comprising 51 pastoralists, 17 human health providers, 28 wildlife sector personnel and 5 veterinarians. Thirteen out of 22 (59.1%) pastoralists in Laikipia and 18/29 (62.1%) in Maasai Mara were aware that some tick-borne diseases can infect humans but expressed low level of knowledge on SFG rickettsioses. Eleven out of 22 (50%) pastoralists in Laikipia and 16/29 (55.2%) in Maasai Mara expressed knowledge on non tick-borne zoonotic diseases but expressed no knowledge about Q fever. Six out of 15 (40%) wildlife sector personnel in Laikipia and 2/13 (15.4%) in Maasai Mara expressed knowledge on tick-borne zoonoses and 4/15 (26.7%) in Laikipia expressed some knowledge about African tick-bite fever and none expressed any knowledge on Q fever in both study areas. Five out of 11 (45.5%) of the health providers in Laikipia and 2/6 (33.3%) in Maasai Mara expressed knowledge on tick-borne zoonoses including African tick-bite fever and 1/11 (9.1%) in Laikipia expressed good knowledge on Q fever and none in Maasai Mara. Only one medical facility in Laikipia finds it necessary to confirm SFG rickettsioses and Q fever in febrile patients and none in Maasai Mara. The veterinarians expressed some level of knowledge on both diseases.

The study identified *R. sibirica* and *R. sibirica* subspecies *mongolotimonae*, two SFG rickettsial species not previously reported in Kenya in a Topi (*Damaliscus korrigum*) and *Rh. evertsi evertsi, Rh. evertsi*, *Rh. pulchellus, A. variegatum* and *A. truncatum* ticks. It also identified *C. burnetii* in *Rh. appendiculatus*, *Rh. pulchellus* and *Rh. evertsi evertsi* ticks. These findings demonstrate that wildlife and their ticks play a potential role in the epidemiology of these pathogens in Laikipia and Maasai Mara ecosystems. The study recommends that the role of these pathogens as causes of febrile illness in local residents in both areas be evaluated.

CHAPTER ONE: INTRODUCTION

To effectively mitigate disease threats in humans, livestock and wildlife, it is increasingly becoming important to understand the epidemiology of pathogens that can infect multiple hosts (Cleaveland *et al*., 2001). According to Jones *et al*. (2008), over 60% of emerging infectious diseases are zoonoses and over 70% of them are of wildlife origin. Many factors favour emergence and re-emergence of diseases including increased interaction between humans, domestic animals and wildlife (Jones *et al*., 2008) highlighting the need for a holistic approach to surveillance and detection of diseases so as to effectively manage them. This is particularly so at human-livestock-wildlife interfaces where disease transmission can occur across different species.

The spotted fever group (SFG) rickettsioses are zoonotic diseases caused by over 20 species of the intracellular bacteria in the genus Rickettsia and transmitted by ticks (Todar, 2012; Parola *et al*., 2013). They are diagnosed in a large number of international travellers visiting Africa (Jensenius *et al*., 2006; Freedman *et al*., 2006) including Kenya (Rutherford *et al*., 2004; Yoshikawa *et al*., 2005). The clinical manifestations in humans are non specific and mimic those of other diseases such as malaria and flu-like illnesses. These include fever, headache, malaise, myalgias, nausea and vomiting (Krauss *et al*., 2003; Roch *et al*., 2008). They are transmitted by different species of ixodidae ticks and reservoirs include various species of domestic and wild animals (Cowan, 2003; Todar, 2012). The diseases therefore can be of particular concern where wildlife shares habitats and other resources with humans and domestic animals (Grootenhuis and Olubayo, 1993).

Q fever is also a zoonotic disease now recognised as an important emerging or re-emerging vector-borne disease (Arricau and Rodolakis 2005; Porter *et al*., 2011) caused by the intracellular bacterium *Coxiella burnetii*, a potential agent for bioterrorism (Jones *et al.*, 2006; Porter *et al*., 2011). The disease has multiple transmission modes which include inhalation, contact with infected body fluids (Jones *et al*., 2006; Marrie 2009) and consumption of untreated milk (Marrie 2009). Ticks are known to be reservoirs of *C. burnetii* as well as being responsible for spreading the disease in wildlife and transmission to livestock (Jones *et al*., 2006; Marrie, 2009; Porter *et*

al., 2011) and over 40 species are naturally infected (Porter *et al*., 2011). Although human infection through tick bites is rare (Porter *et al*., 2011), it is possible (McQuiston *et al.,* 2002). In animals, Q fever is mostly associated with various reproductive problems (Marrie 2009; Porter *et al.*, 2011) while in humans, it presents either as an acute disease which is self limiting and characterised by non-specific symptoms which include fever, headache, pneumonia and hepatitis (Marrie 2009; Porter *et al.,* 2011) or a chronic form characterised by an often fatal endocarditis, hepatitis, osteomyelitis or endovascular infection (McQuiston *et al.*, 2002; Marrie 2009; Porter *et al.*, 2011) as well as spontaneous abortions in pregnant women (Porter *et al*., 2011).

The importance of SFG rickettsioses and Q fever as causes of illnesses in Kenya is underreported and underappreciated despite being reported among foreign travellers who visit game reserves (Potasman *et al.*, 2000; Rutherford *et al*., 2004; Yoshikawa *et al*., 2005; Richards *et al*., 2010). This may be attributed to low level of knowledge and the challenges of diagnosing diseases in Africa (Ari *et al.*, 2011; Brah *et al.*, 2015). These diseases therefore may be amongst the 'fevers of unknown origin' whose aetiologies are often not the focus of health providers or are impossible to diagnose because of lack of resources (Brah *et al.*, 2015). Nevertheless, there has been increasing reports in Kenya of SFG rickettsioses (Macaluso *et al*., 2003; Rutherford *et al*., 2004; Yoshikawa *et al*., 2005; Richards *et al*., 2010; Maina, 2012; Mutai *et al*., 2013) and Q fever (Potasman *et al*., 2000; Knobel *et al*., 2013; DePuy *et al*., 2014) in humans, domestic animals and ticks from domestic animals. However, there is no information about these pathogens in wildlife and their ticks locally. Given the increasing incidences and geographical ranges of emerging zoonotic diseases with over 70% originating from wildlife (Jones *et al*., 2008), there is need to understand the dynamics of infectious zoonotic pathogens in multiple hosts for their effective management. Wildlife plays an important role of disease epidemiology but are often neglected in surveillance and detection of diseases. They are often infested with high numbers of ticks and can be important reservoirs of many tick-borne pathogens including SFG rickettsiae and *C. burnetii* which can be transmitted to domestic animals and humans in areas where wildlife and domestic animals share habitats and other resources. This study investigated SFG rickettsioses and Q fever at the wildlife-livestock interfaces in Laikipia County and Maasai Mara national reserve. It evaluated the presence, prevalence and species of SFG

rickettsiae and *C. burnetii* strains circulating in wildlife and their ticks as well as the knowledge, attitudes and practices of the local residents towards the diseases.

1.1 OVERALL OBJECTIVE

To determine the epidemiology of SFG rickettsioses and Q fever at the wildlife-livestock interfaces in Laikipia and Maasai Mara ecosystems.

1.2 SPECIFIC OBJECTIVES

(i) Determine the presence and prevalence of SFG rickettsioses and Q fever in wildlife and ticks in Laikipia and Maasai Mara

(ii) Identify the SFG *Rickettsia* species and *C. burnetii* strains present in wildlife and ticks in Laikipia and Maasai Mara

(iii) Evaluate the knowledge, attitudes and practices (KAP) on SFG rickettsioses and Q fever among the local residents in Laikipia and Maasai Mara

(iv) Evaluate potential risk factors that could predispose the local residents in Laikipia and Maasai Mara to SFG rickettsioses and Q fever

CHAPTER TWO: LITERATURE REVIEW

2.1 RICKETTSIOSES

2.1.1 Description of rickettsioses

Rickettsioses are zoonotic diseases caused by various species of the intracellular bacteria of the genus Rickettsia (Eremeeva and Dasch, 2011; Todar, 2012; Parola *et al*., 2013). The diseases have a worldwide distribution and are usually transmitted by arthropods such as ticks, lice, fleas and mites (Socolovschi *et al*., 2008).

The taxonomical classification of the bacteria is Tribe Rickettsiae, Family Rickettsiaceae, Order Rickettsiales and Class α-proteobacteria (Todar, 2012; Parola *et al*., 2013). Rickettsiae are small (0.8-2.0µm long and 0.3-0.5µm in diameter), pleomorphic (coccal, oval, or rod-shaped), aerobic, non-motile and Gram-negative bacteria that are obligate intracellular parasites of vertebrate and arthropod host cells in which they replicate freely in the cytoplasm and sometimes in the nucleus (Krauss *et al*., 2003; Petri, 2007; Todar, 2012). They reproduce by binary fission and lack flagella while their genomes are very small measuring about 1.0 to 1.5 million base pairs (Todar, 2012). Rickettsiae were once thought to be viruses because of their small size and obligate intracellular life cycle but are now classified as bacteria (Petri, 2007).

2.1.2 Historical perspectives of rickettsioses

Rickettsioses are amongst the oldest known arthropod-transmitted diseases (Parola *et al*., 2005). The louse-borne epidemic typhus for example has plagued humanity throughout history (Todar, 2012) such as the Athens plague in the 5th Century and was certainly recognised in the 16th Century due to skin eruptions or rashes occurring as symptoms of an acute disease allowing its distinction among diseases with high fever (Raoult and Roux, 1997). Between 1917 and 1923, epidemic typhus is suspected of having caused deaths of about three million people in Russia (Perlman *et al*., 2006).

Tick transmitted rickettsioses have been described since the 19th Century. The first clinical description of the prototype tick-borne rickettsioses, the Rocky Mountain spotted fever (RMSF),

was in 1899 by Edward E. Maxey (Parola *et al*., 2005). Latter in 1906, Howard Ricketts documented tick transmission of RMSF in mammals and the presence of the causative agent in infected humans and the trans-ovarian transmission of the agent in ticks (Thorner *et al*., 1998; Dantas-Torres, 2007). Ricketts contracted epidemic typhus and died in 1910 as he was investigating its outbreak in Mexico City having already described the causing agent, *Rickettsia prowazekii*, isolated from lice and blood from infected humans (Thorner *et al*., 1998; Dantas-Torres, 2007). *Rickettsia prowazekii* is named after Stanislaus Von Prowazek who had earlier in 1909 discovered the organism as the cause of epidemic typhus (Hechemy *et al*., 2006). Later in 1919, Wolbach S. Burt described *R. rickettsii* which causes RMSF as an intracellular pathogen and confirmed ticks to be vectors. *Rickettsia rickettsii* is named after Howard Ricketts (Dantas-Torres, 2007).

Other tick-transmitted rickettsioses have been described after these early discoveries including Mediterranean spotted fever (MSF) first described in Tunisia in 1910 by Conor and Bruch and the causative agent named *R. conorii* after Conor (Rovery *et al*., 2008). Thereafter in 1930, African tick-bite fever (ATBF) was discovered when its causative agent was isolated but lost (Althaus *et al*., 2010) until 1990 when isolation was done in humans (Kelly *et al*., 1992) and given the name *R. africae* in 1996 (Kelly *et al*., 1996).

Since these early discoveries, many other tick-transmitted rickettsiae have been described (Fournier *et al*., 2003; Perlman *et al*., 2006; Todar 2012; Parola *et al*., 2013). Vector-borne rickettsiae are currently considered amongst diseases important in understanding emergence of infectious diseases (Raoult and Roux, 1997; Perlman *et al*., 2006) with some getting particular attention because of their bioterrorism potential (Azad and Beard 1998; Azad and Radulovic, 2003; Perlman *et al*., 2006).

2.1.3 Classification of rickettsioses

To date, there are many species of pathogenic rickessiae that have been described. These are classified into three major groups according to the distinctive diseases which they cause namely spotted fever, typhus and scrub-typhus groups (Parola *et al*., 2005; Perlman *et al*., 2006; Socolovschi *et al*., 2008).

2.1.3.1 Spotted fever group rickettsioses

These are a group of diseases distributed worldwide apart from Antarctica usually transmitted by ticks and caused by over twenty species of rickettsiae (Todar, 2012; Parola *et al*., 2013). The species reported in Africa include *R. conorii*, *R. africae*, *R. aeschlimannii*, *R. sibirica*, *R. massiliae* and *R. mongolotimonae* (Todar, 2012; Mediannikov *et al*., 2012; Znazen *et al*., 2013; Kleinerman *et al*., 2013; Kamani *et al*., 2015) including Kenya (Macaluso *et al*., 2003; Maina, 2012; Mutai *et al*., 2013). Of these species, R. *conorii* and *R. africae* are the most widespread and causative agents of Kenyan tick typhus (commonly called MSF) and ATBF respectively (Rovery *et al*., 2008; Todar, 2012). The two species are now recognised as important causes of febrile illnesses in foreign travellers visiting Africa after malaria (Freedman *et al*., 2006).

Previously, MSF was thought to be the major cause of tick-transmitted rickettsioses in Africa (Jensenius *et al*., 2003) before *R. africae*, the aetiological agent of ATBF was isolated in 1990 (Kelly *et al*.,1992). It is now known that ATBF is the predominant SFG rickettsiosis in Africa (Jensenius *et al*., 2003; Jensenius *et al*., 2004). Whereas MSF can cause serious disease and mortality can be as high as 32% as was reported in Portugal in 1997 (Rovery *et al*., 2008), ATBF causes a less severe disease (Todar, 2012).

In the USA, RMSF caused by *R. rickettsii* is the predominant SFG rickettsiosis (Todar, 2012). It is amongst the most virulent infections in human beings and potentially fatal even in previously healthy people (Dantas-Torres, 2007; Todar, 2012). Its mortality rate is 20-25% if untreated and up to 5% in delayed or ineffective antimicrobial therapy (Thorner *et al*., 1998; Todar, 2012). The name RMSF is somewhat a misnomer because beginning 1930, the disease has been reported in other parts of the USA other than the Rocky Mountain region as well as parts of Canada, Central America, Mexico and South America (Todar, 2012).

Many other SFG rickettsioses have been described from other parts of the world including Boutonneuse fever caused by *R. mongolotimonae* and *R. slovaca*, Japanese spotted fever caused by *R. japonica*, North Asian tick typhus (Siberian tick typhus) caused by *R. sibirica*, lymphangitis-associated rickettsiosis caused by *R. sibirica mongolotimonae*, Queensland tick typhus caused by *R. australis*, rickettsialpox (vesicular rickettsioses) caused by *R. akari* and

Flinders Island spotted fever caused by *R. honei* (Jensenius *et al*., 2004; Fournier *et al*., 2005; Todar, 2012). Other pathogenic rickettsiae include R. *rhipicephali, R. helvetica, R. montana, R. amblyomii* and *R. cooleyi* (Azad and Beard, 1998; Fournier *et al*., 2003; Jensenius *et al*., 2004; Parola *et al*., 2005; Moncayo *et al*., 2010; Todar, 2012).

2.1.3.2 Typhus group rickettsioses

The typhus group (TG) rickettsiae include lice transmitted *R. prowazekii*, the causative agent of the epidemic typhus (Todar, 2012). The disease is most common in overpopulated poor settlements that provide suitable environments for the spread of the vector, *Pediculus humanus corporis* lice (Socolovschi *et al.*, 2008). The disease has a global distribution and is historically known to have caused severe outbreaks in humans (Todar, 2012) that include the death of up to three million people in Russia between 1917 and 1923 (Perlman *et al*., 2006). *Rickettsia prowazekii* and the tick-borne *R. rickettsii* are the most virulent rickettsiae with significant mortalities if early treatment is not instituted (Todar, 2012).

The other TG rickettsia is the flea-transmitted *R. typhi*, the aetiological agent of murine typhus which also has a global distribution (Todar, 2012). Fleas that infest rats and cats such as *Xenopsylla cheopis* and *Ctenocephalides felis* are responsible for transmission of the pathogen (Socolovschi *et al*., 2008). Also in this group is *R. felis* which is phylogenetically more related to SFG rickettsiae than the TG but has similar antigens with *R. typhi* and causes a murine typhus-like disease that is transmitted by the same fleas as *R. typhi* (Todar, 2012).

2.1.3.3 Scrub-typhus group rickettsiosis

The scrub-typhus group (STG) rickettsiae comprise the mite-transmitted *Orientia tsutsugamushi* which was previously called *R. tsutsugamushi*. The pathogen has lately been designated a genus as it has significant phylogenetic differences with other rickettsiae (Tamura *et al*., 1995; Todar, 2012). It is transmitted through bites of several species of trombiculid mites (chiggers) particularly *Leptotrombidium deliense* and is prevalent in most of Asia and Australia (Socolovschi *et al*., 2008; Todar, 2012).

2.1.4 Epidemiological aspects of SFG rickettsioses

2.1.4.1 Ticks as vectors and reservoirs of SFG rickettsiae

Ticks are blood-sucking arthropod parasites of all vertebrates and are widely distributed in most parts of the world and are known to be vectors of bacterial, viral and parasitic pathogens (Socolovschi *et al*., 2008, Olson and Patz, 2010). Over 850 species of ticks are known which are grouped into either Ixodidae (hard-bodied) or Argasidae (soft-bodied) ticks (Olson and Patz, 2010). The SFG rickettsiae are transmitted by Ixodidae ticks (Parola *et al*., 2005; Todar, 2012). Ticks are among the most efficient vectors of disease causing pathogens because they attach tightly on their hosts and suck blood slowly and may sometimes be unnoticed when feeding (Balashov, 1972).

Ticks inhabit wildlife and human settlement areas and also interfaces in which wildlife and domestic animals share pastures and water. Disease transmission between wildlife, humans and domestic animals therefore can be of particular concern where wildlife shares habitats and other resources with domestic animals and humans (Grootenhuis and Olubayo, 1993).

The diversity and distribution of tick-borne pathogens has been shown to be changing due to warmer temperatures as a result of climate change which influences tick development, dispersal and host diversity (Olson and Patz, 2010) emphasising the need for concerted efforts in understanding all aspects of disease epidemiology. Ticks serve as natural hosts (reservoirs, vectors and amplifiers) of many pathogens including SFG rickettsiae. They transmit disease causing pathogens mainly through bites and less commonly exposure to tissues, fluids or faeces (Todar, 2012).

Ticks infected with SFG rickettsiae carry the pathogens for life and generations of infected ticks are sustained through various methods. These include trans-stadial passage whereby pathogens are spread during the life cycle of an infected tick from one stage to the next, trans-ovarial passage by females to eggs and offsprings as well as during mating from males to females through body fluids or spermatozoa (Parola *et al*., 2005; Todar, 2012). Additionally, horizontal spread can also occur through coxal fluids when feeding (Parola *et al*., 2005) when ticks excrete excess fluids of the blood meal via the coxal organs (Kaufman *et al*., 1982). Ticks may also get

infected horizontally through saliva when infected and non-infected ticks feed together in the absence of an infection in the host (Parola *et al*., 2005).

Ticks have been extensively studied as reservoirs and vectors of SFG rickettsiae than any other species (Perlman *et al*., 2006). In Eastern Africa, Mutai *et al*. (2013) reported a prevalence of 23.3% in ticks collected from cattle, sheep and goats presented at slaughter houses in Mombasa and Nairobi. The highest detection rate was in *Amblyomma* species at 62.3%, followed by *Rhipicephalus* species at 45.5%, *Hyalomma* species at 35.9% and *Boophilus* species at 34.9%. They also characterised the SFG rickettsiae and found 93% of the ticks to be infected with *R. africae* while the infection with other *Rickettsia* species was much lower. *Rickettsia aeschlimannii* was present in 1.9% of the ticks and *R. mongolotimonae, R. conorii* subspecies *israelensis* and *Candidatus Rickettsia kulagini* in 0.96% of the ticks sampled.

Earlier, Macaluso *et al*. (2003) had reported SFG rickettsiae at a prevalence of 15.8% in *Amblyomma variegatum* and 1% in *Rhipicephalus* species of ticks collected from livestock and vegetation in private and public land surrounding the Maasai Mara national reserve. Maina (2012) has also documented an infection rate of 96.9% and 20.34% in ticks from cattle and dogs respectively in Western part of Kenya. In Uganda, *R. africae* was reported in *A. variegatum* species of ticks at a prevalence of 97.1% (Nakao *et al*., 2013).

In Western Africa, Mediannikov *et al*. (2012) reported presence of SFG rickettsiae in ticks from domestic animals and wildlife in Guinea and Liberia. They reported *R. africae* in 93 - 100% of *A. variegatum*, in 14 - 93% of *Rhipicephalus* (*B.*) *geigyi, Rh.* (*B.*) *annulatus* and *Rh.* (*B.*) *decoloratus* as well as *Hyalomma marginatum rufipes* and *Haemaphysalis paraleachi*. In addition, they also found *R. massiliae* in 16% of *Rh. senegalensis* ticks and in 2% of *H. paraleachi* ticks collected from dogs. The presence of *R. aeschlimannii* in *Hyalomma* ticks collected from camels has also been reported by Kamani *et al*. (2015) in Nigeria.

In Northern Africa, Znazen *et al*. (2013) characterised *Rickettsia* species detected in ticks in Tunisia and reported a prevalence of 23.7% in ticks allowing the identification of *R. conorii* subspecies *israelensis, R. massiliae* and *R. conorii* subspecies *conorii*. *Rickettsia africae* and *R.*

aeschlimannii were reported at a prevalence of 26.7-73.3% in different *Hyalomma* species of ticks collected from camels in Egypt (Abdel-Shafy *et al*., 2012).

Boretti *et al.* (2009) detected *R. helvetica* in ticks collected from wild foxes, humans and domestic dogs using PCR at a prevalence of 36% in Switzerland while ticks collected from vegetation had a prevalence of 12%. In Israel, Kleinerman *et al*. (2013) identified and genetically characterised SFG rickettsiae in ticks. *Rickettsia aeschlimannii* was detected in *H. dromedarii*, *H. turanicum* and *H. excavatum* species of ticks. In addition, they reported *R. africae* in *H. turanicum*, *H. impeltatum*, *H. dromedarii* and *H. excavatum* species of ticks while *R. sibirica mongolitimonae* was detected in one *H. turanicum* tick.

Other studies on the presence of SFG rickettsiae in ticks have been by Moncayo *et al*. (2010) who reported the presence of *R. Montana, R. amblyommii* and *R. cooleyi* in *Amblyomma* and *Dermacentor* species of ticks in the USA at a prevalence of 32% in ticks collected from various species of wildlife as well as humans and domestic canines and also from flannel drags. Frankie *et al*. (2010) reported the presence of SFG rickettsiae in ticks collected from various species of wild birds and small mammals (rodents) in Germany at a prevalence of 2.1% and 1.8% respectively.

Shpynov *et al*. (2004) reported SFG rickettsiae in ticks collected from vegetation in Russia at a prevalence of 15.6%, while Zhang *et al*. (1995) studied the same and reported a prevalence of 70% in ticks in China. In Greece, Psaroulaki *et al*. (2003) reported an infection rate of 1.6% in ticks. Other reports also show that *R. rickettsii* is predominantly transmitted by *Dermacentor* species of ticks (Socolovschi *et al*., 2008, Moncayo *et al*., 2010). Many other studies have been conducted in ticks including China (Zhang *et al*., 2006), Japan (Fournier *et al*., 2002), USA (Eremeeva *et al*., 2006) and Netherlands (Sprong *et al*., 2009).

2.1.4.2 Spotted fever group rickettsioses in animals

Studies conducted over the years have documented various mammalian species, both domestic and wild, as reservoirs of the SFG rickettsiae. In Kenya, Mutai *et al.* (2013) reported SFG rickettsiae DNA in cattle at 16.3%, sheep at 15.1% and a lower rate in goats at 7.1%. In their study that involved sampling livestock presented at slaughter houses in Mombasa and Nairobi

from all over Kenya, they found presence of SFG rickettsiae in 25 of the 32 counties (78.1%) from which the animals came. Laikipia County had a prevalence of 27.8%. There were no animals from Narok County where the Maasai Mara national reserve is located but Mau Narok in Nakuru County at the border with Narok County had a prevalence of 100%. Maina (2012) reported SFG rickettsiae DNA in 3.7% and 7.7% of dogs and cats respectively in western Kenya and no infection in cattle, sheep and goats. In the same study, 7.2% of febrile patients and 3.4% of asymptomatic humans tested positive for SFG rickettsiae DNA and a higher proportion of humans (56%) tested positive using serology.

In western Africa, Kamani *et al*. (2015) reported *Rickettsia* species in 18.8% of camels. In northern Africa, Znazen *et al*. (2013) characterised *Rickettsia* species detected in human patients in Tunisia and reported a prevalence of 60% and identified the SFG rickettsiae as *R. conorii* subspecies *israelensis*, *R. massiliae* and *R. conorii* subspecies *conorii*.

In Israel, Kleinerman *et al*. (2013) identified and genetically characterised *R. aeschlimannii* in camels but did not detect any SFG rickettsiae in horses while Zhang *et al.* (1995) studied SFG rickettsiae in wild mice in China and reported 7.4% as positive. Inokuma *et al*. (2008) reported SFG rickettsiae DNA in peripheral blood of a deer in Japan while Ortuno *et al*. (2007) reported the same in a wild boar in Spain.

2.1.4.3 Spotted fever group rickettsioses around the world

Ticks inhabit wildlife and human settlement areas and also interfaces in which wildlife and domestic animals share pastures and water. People at risk of being infected with SFG rickettsiae are those whose activities involve walking through bushes. The diseases have a worldwide distribution (Parola *et al*., 2005) and infections have been demonstrated in international travellers who have visited Africa (Kelly *et al*., 1996; Jensenius *et al*., 2004; Jensenius *et al*., 2006; Roch *et al*., 2008). Both MSF and ATBF have been described as the second most important causes of febrile illnesses in the travellers after malaria followed by dengue and typhoid fever (Freedman *et al*., 2006). In Kenya, a fatal infection by MSF was reported in a woman from USA (Rutherford *et al*., 2004). Yoshikawa *et al*. (2005) also reports of a Japanese tourist infected with

SFG rickettsioses in Kenya. Some species of SFG rickettsiae have been reported to have potential for use in bioterrorism (Azad and Radulovic, 2003).

2.1.5 Pathogenesis and clinical signs of SFG rickettsioses

Following infection of a vertebrate host through a tick bite, the primary target cells of SFG rickettsiae is the endothelium of small blood vessels where they cause a generalised vasculitis which results to hypoperfusion and hypoxia of neighbouring tissues as well as increased vascular permeability with oedema, hypovolemia, hypotention, hypalbuminemia, hyponatremia and increased platelet adherence (Krauss *et al*., 2003). This explains the broad range of clinical signs and serious complications that sometimes occur in some patients (Krauss *et al*., 2003; Todar 2012). These signs are non-specific and similar to those of other diseases such as malaria, typhoid fever and flu-like illnesses often leading to misdiagnoses. The signs include fever, headache, malaise, myalgias and loss of appetite, nausea and vomiting as well as cutanenous eschar/eruptions at the tick bite sites, regional lymphadenopathy and vesicular rush (Scola and Raoult, 1997; Jensenius *et al*., 2004; Krauss *et al*., 2003; Roch *et al*., 2008; Frean *et al*., 2008).

Sometimes, the diseases can be very severe and can lead to death (Jensenius *et al*., 2004; Rutherford *et al*., 2004) particularly if no treatment is given or if treatment is delayed or if wrong anti-microbial therapy is provided (Lee *et al*., 2008). Effective antibiotics include tetracyclines and chloramphenicol (Lee *et al*., 2008). In patients with concurrent diseases such as diabetes, alcoholism or heart diseases, severe complications can sometimes occur resulting to mortality of up to 50% (Todar, 2012). However, ATBF is often less severe with fewer complications (Frean *et al*., 2008).

2.1.6 Laboratory diagnosis of SFG rickettsioses

Various serological tests are used in the diagnosis of rickettsioses. These include the Immuno-Fluorescence Assay (IFA), Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Complement Fixation Test (CFT) which are highly sensitive and specific and useful for sero-epidemiology. In addition, IFA and ELISA are also useful for detection of acute cases (Scola and Raoult, 1997). Other conventional methods used for diagnoses of bacterial infections such as culture are not

usually used because of the obligate intracellular nature of the pathogens (Parola *et al*., 2005) which makes them very difficult to cultivate in the laboratory (Weiss *et al*., 1987).

Diagnoses can also be done using PCR to amplify different genes such as the citrate synthase encoding gene *gltA*, the outer membrane protein A encoding gene *ompA*, the outer membrane protein encoding gene *ompB* and gene D which encodes the outer membrane protein PS120 (Roux and Raoult, 2000; Fournier *et al.*, 2003; Fournier *et al*., 2004; Parola *et al*., 2005; Merhej and Raoult, 2011). The DNA for PCR amplification can be obtained from EDTA blood, Buffy coat, tissue biopsies and ticks (Raoult *et al*., 1997; Ndip *et al*., 2004; Whitman *et al*., 2007). To identify SFG rickettsial species, comparative analyses of gene sequences after amplification by PCR is done (Parola *et al*., 2005).

Besides PCR amplification of the coding genes, intergenic spacers that include *dksA-verC*, *mppA-purC*, *rmpE-tRNA*^{fMet} and *tRNA*^{Gly}*-tRNA*^{Tyr} are also amplified to detect and identify rickettsiae (Fournier *et al*., 2004; Zhu *et al*., 2005; Jado *et al*., 2006; Fournier and Raoult, 2007; Eldin *et al*., 2011; Abdel-Shafy *et al*., 2012). Intergenic spacers refer to non-transcribed lengths of DNA separating repeated lengths of DNA containing genes encoding ribosomal RNA (rRNA) (Fournier *et al*., 2004). They have been demonstrated to be more variable and therefore more discriminatory in the detection and identification of SFG rickettsiae than the coding genes (Fournier and Raoult, 2007).

The use of highly sensitive methods that can detect low DNA concentration in specimens is important because as demonstrated before, rickettsiae in rickettsiemic patients may be very few (Kaplowitz *et al*., 1983; Norment and Burgdorfer 1984; Kidd *et al*., 2008). Thus, intergenic spacers are very useful in detection of rickettsiae because they are easily amplified even from low quantities of DNA because of high copies of rRNA genes (Fournier *et al*., 2004; Zhu *et al*., 2005). They also highly vary between species because they are subjected to lower evolutionary pressure than the coding genes. This means that they are highly conserved among the genus Rickettsia which makes them ideal for real time PCR (Fournier *et al*., 2004; Zhu *et al*., 2005).

The use of intergenic spacers for the detection of pathogens including SFG rickettsiae is useful in both clinical and arthropod specimens for diagnosis and epidemiological surveillance of

rickettsial diseases (Fournier *et al*., 2002; Zhu *et al*., 2005). Fournier and Raoult (2007) have reported amplification of all strains of 23 species of *Rickettsia* using the intergenic spacers. They have also been used for detection and identification of other organisms (Mateos and Markow, 2005; Osorio *et al*., 2005).

2.1.7 Prevention and control of SFG rickettsioses

There is no vaccine to prevent SFG rickettsial diseases. To prevent infection, persons are encouraged to avoid tick bites by wearing protective clothing such as long sleeved shirts and trousers as well as tucking trousers into socks so that ticks cannot crawl under clothing and applying insect repellents. In addition, persons should inspect themselves regularly for ticks and remove them immediately as well as practising tick control in animals (Socolovschi *et al*., 2008).

2.2 Q FEVER

2.2.1 Description of Q fever

Q fever is a zoonotic disease caused by the obligate intracellular bacterium *Coxiella burnetii* (Dorko *et al*., 2009; Marrie, 2009). *Coxiella burnetii* is a pleomorphic Gram-negative coccobacillus that has high resistance to harsh environmental conditions (Marrie, 2009).

2.2.2 Historical perspectives of Q fever

Q fever was first reported in 1935 during an outbreak of an undiagnosed febrile disease among abattoir workers in Brisbane, Queensland, Australia (Marrie, 2009; Porter *et al*., 2011). According to Marrie (2009), the infection was characterised by nonspecific clinical signs and samples collected were negative to other known febrile diseases.

Marrie (2009) further reports that in 1936, Frank MacFarlane Burnet described rickettsial like bodies in tissue smears of the spleen from experimentally infected mouse. Thereafter in 1938, Herald Rea Cox managed to culture the organism in the laboratory. Since these early discoveries, the organism has been given several names. Firstly, it was named *Rickettsia diaporica*, then *R. burnetii* before it was elevated to a subgenus, *Coxiella* and is currently called *Coxiella burnetii* in

honour of Cox and Burnet (Marrie, 2009). Q fever was first reported in Kenya in 1955 (Craddock and Gear, 1955).

2.2.3 Epidemiological aspects of Q fever

2.2.3.1 Transmission of Q fever

Q fever is transmitted through direct or indirect contact with infected animals or their body fluids (Jones *et al*., 2006; Marrie, 2009). Its main mode of transmission is inhalation of contaminated dust but it can also be transmitted through contact with materials contaminated with body fluids and secretions from infected animals (Jones *et al*., 2006; Marrie, 2009). Humans can also get infected orally by consuming raw milk from infected animals (Marrie, 2009; OIE, 2012).

Ticks are reservoirs of *C. burnetii* and transmit Q fever between domestic and wild animals (Masala *et al*., 2004; Porter *et al*., 2011). The possibility of infected ticks transmitting the disease to humans through their bites is rare (Porter *et al*., 2011) but is possible (McQuiston *et al*., 2002; Jones *et al*., 2006; Medeannikov *et al*., 2010) as well as per-cutaneous for instance by crushing infected ticks between the fingers (Marrie, 2009).

The main sources of Q fever transmission to humans are domestic animals such as cattle, sheep and goats (Kersh *et al*., 2012) and rarely does transmission occur from wild animals (Porter *et al*., 2011). The bacteria is shed through secretions and body fluids of infected animals such as urine, faeces, milk and placental and amniotic fluids (Porter *et al*., 2011; Kersh *et al*., 2012) sometimes for a prolonged period of time (Marrie, 2009). The bacterium is highly resistant to harsh environmental conditions and can survive in the environment for a long period of time (Jones *et al*., 2006).

2.2.3.2 Q fever in animals

Q fever infection has been reported in a wide range of domestic and wild animals as well as marine mammals in different parts of the world (Binninger *et al*., 1980; Zarnke 1983; Marrie *et al*., 1986; Sawyer *et al*., 1987; Marrie *et al*., 1993; Serbezov *et al*., 1999; Gardon *et al*., 2001; McQuiston *et al*., 2002; Barandika *et al*. 2007; Hernandez *et al*., 2007; Dorko *et al*., 2009; Kersh *et al*., 2012).

In Kenya, DePuy *et al.* (2014) reported sero-prevalence of Q fever in livestock in Laikipia with the lowest prevalence in cattle (3-4%), followed by sheep (13-20%), then goats (31-40%) and highest in camels (5-46%). And in Turkey, Kirkan *et al*. (2008) reported a prevalence of 4.3% in cattle. Knobel *et al*. (2013) reported Q fever infection through serology in 30.9% of humans sampled in western Kenya and 3% in patients with acute lower respiratory infections. Further, they reported prevalence of 28.3% in cattle, 32.0% in goats and 18.2% in sheep.

Wild animals are known to infect domestic animals particularly where the source of the infection in domestic animals is not found (Marrie, 2009). Birds have also been reported to play a significant role in Q fever epidemiology by transporting infected ticks across distances. They have also been incriminated in direct transmission of the disease to humans due to aerosols from faeces (Stein and Raoult, 1999).

2.2.3.3 Ticks as vectors and reservoirs of Q fever

Infected ticks play a significant role in maintaining *C. burnetii* infection in the environment (Loukaides *et al*., 2006; Marrie, 2009) and the pathogen has been detected in many species of ticks (Porter *et al*., 2011). Ticks transmit *C. burnetii* between wild and domestic animals through their bites (McQuiston *et al*., 2002; Medeannikov *et al*., 2012). Human infection through tick bites is rarely reported (Porter *et al*., 2011) but is possible (McQuiston *et al*., 2002; Jones *et al*., 2006; Medeannikov *et al*., 2010).

There are several reports of detection of *C. burnetii* by molecular analysis in Kenya. In western Kenya, Knobel *et al.* (2013) detected *C. burnetii* by PCR in 2.5% of *Amblyomma variegatum* pool samples collected from cattle while in ticks collected from domestic dogs, it was detected in 20% *Rhipicephalus Sanguineus*, 11.1% in *Rh. appendiculatus* and 20% in an un-speciated *Rhipicephalus* as well as 20% of *A. variegatum*, 50% of *Hyalomma leachi* and 20% of *Rh.* (*Boophilus*) *decoloratus* ticks.

2.2.3.4 Q fever around the world

Q fever is now recognised as an important emerging vector-borne pathogen (Porter *et al*., 2011) of great public health concern (Angelakis and Raoult, 2010). Despite being a notifiable disease

under the World Organisation for Animal Health (OIE), it is poorly understand in most parts of the world (Porter *et al*., 2011). *Coxiella burnetii* is considered a potential bioterrorism agent because of the aerosol route of transmission, ability to infect large groups of people, its low infectious dose and high resistance to harsh environment conditions (Jones *et al*., 2006).

Marrie (2009) has described various outbreaks of Q fever at community level. For example in three provinces in Canada and one area in Japan, she described small outbreaks involving family units exposed to infected cats. Other outbreaks she reported were from exposure to contaminated materials in Britain and Switzerland as well as exposure to various species of animals. Roest *et al*. (2011) has also reported Q fever outbreak in the Netherlands associated with domestic ruminants.

In Kenya, an outbreak of Q fever in international travellers who visited a Maasai Manyata in Maasai Mara has been reported by Potasman *et al*. (2000). The travellers reported having entered a Manyatta made of cattle hides and straw and covered with mud and/or manure and had two goats inside. It was postulated that either the Manyatta or the goats were the source of the infection.

2.2.4 Pathogenesis and clinical manifestation of Q fever

Once an animal is infected, *C. burnetii* targets the uterus and mammary glands (Marrie, 2009). The clinical signs in infected animals include abortions, stillbirths, weak offsprings and infertility which can severely impact on livestock production (Masala *et al*., 2004; Porter *et al*., 2011). Also reported in cattle is metritis (Marrie, 2009).

In humans, the disease occurs in either acute or chronic forms (Marrie, 2009; Mediannikov *et al*., 2010; Porter *et al*., 2011). The acute form is often a mild infection while the chronic can be severe with significant morbidity and mortality (Marrie, 2009).

The predominant clinical manifestation for the acute form is a self limiting febrile illness which manifests with non specific signs (Marrie, 2009). These include chills, night sweats, headache and fatigue (Mediannikov *et al*., 2010; OIE, 2012). Other signs include atypical pneumonia

(Marrie, 2009; Mediannikov *et al*., 2010; OIE, 2012) which manifests with fever, a non-productive cough and shortness of breath in some severe cases (Marrie, 2009).

Acute Q fever may also manifest as granulomatous hepatitis particularly in severe infections (Marrie, 2009; Mediannikov *et al*., 2010; OIE, 2012) manifesting with fever and elevated liver enzymes and sometimes jaundice (Marrie, 2009). Other miscellaneous manifestations of acute Q fever are premature deliveries and abortions in pregnant women (Mediannikov *et al*., 2010; OIE, 2012). Q fever has also been reported in immuno-compromised patients such as those with Human Immuno-deficiency Virus (HIV) infection (Marrie, 2009).

Chronic Q fever on the other hand usually manifests with an endocarditis, hepatitis, osteomyelitis, or endovascular infection (infected aortic aneurysms) (Marrie, 2009; Mediannikov *et al*., 2010; OIE, 2012).

2.2.5 Laboratory diagnosis of Q fever

Because of the highly infectious and resistance nature of *Coxiella burnetii*, it is recommended that only bio-safety level IV laboratories can isolate the organism (Marrie, 2009; Porter *et al*., 2011). For clinical diagnosis, serology is the commonly used method using either the indirect immuno-fluorescence assay (IFA), the enzyme-linked immuno-sorbent assay (ELISA) or the complement fixation test (CFT) (Marrie, 2009; OIE, 2012, Porter *et al*., 2011) with ELISA being the most preferred because of its higher sensitivity and specificity (OIE, 2012).

Molecular diagnosis is now being used more frequently by employing PCR amplifications of various genes. The DNA for PCR amplification is obtained from EDTA blood, serum, tissue biopsies, ticks or bacterial cultures (Porter *et al*., 2011). These methods are not only highly sensitive and specific, but they are also rapid and reliable in the identification of *C. burnetii* particularly while screening large numbers and different types of samples (OIE, 2012; Porter *et al*., 2011). To characterise isolated *C. burnetii* in order to understand Q fever epidemiology in a geographical area including circulating strains and potential sources of infection, several genotyping methods are used including comparative analysis of sequences following DNA amplification (OIE, 2012).

The repetitive insertion element IS*1111* of the transposase gene is the most commonly used for PCR detection and identification of *C. burnetii* (OIE, 2012; Porter *et al*., 2011). It is frequently repeated in the *Coxiella* genome with 7 to 120 copies per genome therefore making PCR detection very sensitive (Fournier *et al*., 2003; OIE, 2012; Porter *et al*., 2011). Several other genes are also used that include *sodB* which encodes superoxide dismutase, *com1* which encodes an outer membrane protein, *htpA* and *htpB* which encode two heat shock proteins, *icd* which encodes Isocitrate dehydrogenase, and *cbmip* which encodes the macrophage infectivity potentiator protein (OIE, 2012).

2.2.6 Prevention and control of Q fever

Q fever can be prevented by limiting spread of infection for instance by isolating aborting animals and raising feeding troughs to avoid contamination of feeds by faecal material and urine (Marrie, 2009). Spread of infection can also be achieved through prompt treatment of infected animals with effective antibiotics such tetracyclines (Bossi *et al*., 2004). Other preventive measures include consumption of pasteurised dairy products, tick control in animals and proper handling and disposal of placenta, foetal membranes and aborted foetuses (Marrie, 2009; Siqueira-Batista *et al*., 2016). There is no sufficient information on the effectiveness of animal vaccines in preventing Q fever (Marrie, 2009; Porter *et al*., 2011). Effective vaccines however exist for humans although they are not available in most countries (Maurin and Raoult, 1999).

CHAPTER THREE: MATERIALS AND METHODS

3.1 STUDY AREAS

The study areas were Laikipia and Maasai Mara ecosystems. The main population composition in both areas are pastoralists whose livelihoods are dependent on livestock keeping although other forms of land uses are emerging particularly crop farming. Both areas have large wildlife populations which share habitats and other resources with humans and domestic animals.

Laikipia ecosystem is about 9,500 km^2 and is located within Laikipia County in the central region of Kenya to the North-west of Mt. Kenya between 0.88N 36.18E and 0.2667S 37.38E (Figure 1). It forms one of the most important areas for biodiversity in Kenya with much of it covered by large privately or community owned ranches populated by livestock sharing the land with free ranging wildlife. Sampling was done in areas of high wildlife-livestock interactions which included Ol Pejeta Conservancy, Mpala ranch, ADC Mutara ranch and Kiamariga sub-location.

The Maasai Mara ecosystem is approximately 1,510 km^2 between 1.22S 34.75E and 1.75S 35.42E within Narok County in South-western Kenya along the border with Tanzania (Figure 1). It is a contiguous ecosystem with the Serengeti national park in Tanzania. It is one of the most important protected wildlife areas in Kenya accounting for about 25% of Kenya's wildlife (Reid *et al*., 2003). It forms part of the greater Mara ecosystem which also includes the Mara Triangle and numerous group ranches. This is an open ecosystem without fences and free ranging wildlife share resources with livestock. Sampling was done within and around the Maasai Mara national reserves.

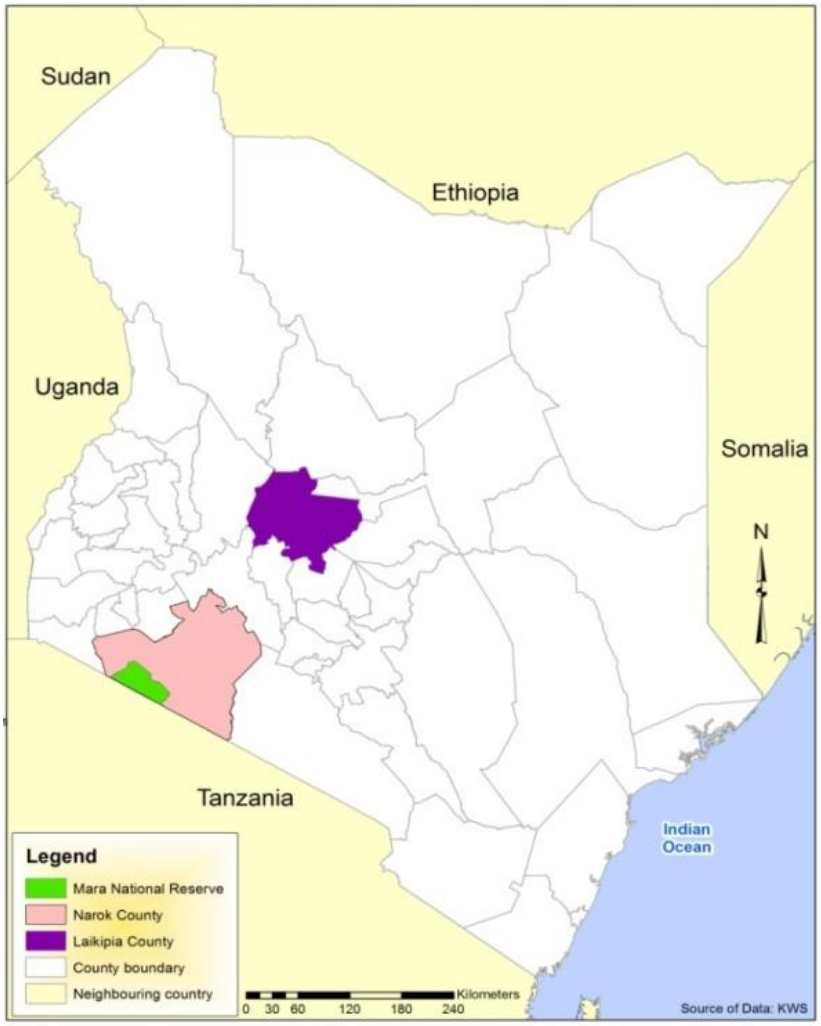

Figure 1: The geographical locations of Laikipia County and Maasai Mara national reserve

3.2 DETERMINATION OF PRESENCE AND PREVALENCE OF SFG RICKETTSIAE AND *C. BURNETII* IN WILDLIFE AND TICKS

3.2.1 Sampling techniques

The sampling sites were selected purposively such that sites sampled were those where wildlife have highest interaction with livestock and are accessible with vehicles to allow for darting of the animals. The geo-referenced position of each sampling site was recorded using a Global Positioning System (GPS) (Garmin GPS 12 XL, Garmin Olathe, KS, USA) and entered into a Geographical Information System (GIS) database to permit a detailed epidemiological study.

Convenience sampling of the animals was employed because of the difficulties of constructing a sampling frame in wildlife to allow random sampling. This method allowed for readily available animals of the target species to be sampled. The target species were those most common in the study areas and have high tendency to interact with livestock. These included buffaloes, zebras, impalas, Topis, Coke's hartebeests, Grant's gazelles, waterbucks and wildebeests.

3.2.2 Sample size determination

In determining the sample size, the following formula described by Naing *et al*. (2006) was used to calculate the minimum number of animals for the study:

$$n = \frac{Z^2P(1-P)}{d^2}$$, where:

n = Sample size

Z = Statistic for the confidence level. A 95% confidence level was used for the study. Thus, the Z value was 1.96

P = Expected prevalence. No information was available on prevalence of SFG rickettsioses and Q fever in wildlife in Kenya. Available reports in Kenya are in different species of domestic animals. Mutai *et al*. (2013) have reported 7-16% prevalence for SFG rickettsioses while Knobel *et al*. (2013) and DePuy *et al*. (2014) have reported Q fever prevalence of 18-32% and 3-46%, respectively. Information available on Q fever was used to determine the *P* value as it had higher prevalence than SFG rickettsioses. An expected prevalence of 25% was used for this study. Thus, the *P* value was 0.25.

d = The precision. A precision of 10% (0.1) was used in view of the immense resources required to sample wild animals namely the costs of immobilisation drugs and reversal agents, darting accessories and transport. The two study areas are far and most of the sampling areas have poor road infrastructure.

The minimum sample size was therefore determined to be 72 animals for each study area.

3.2.3 Collection of blood samples from wildlife

Animals were immobilised with etorphine hydrochloride (M99®, Verico, UK) combined with azaperone tartarate (Kyron Laboratories, S. Africa) at dosages recommended by McKenzie (1993) depending on the animal species, age, degree of excitation as well as the sex and terrain. After the sampling procedure, etorphine hydrochloride was reversed with diprenorphine hydrochloride (M5050®, Verico, UK) calculated at three times the amount in milligrams of etorphine hydrochloride used for each individual animal. Azaperone tartarate was the sedative drug that was used to calm the animal in the initial central nervous system (CNS) excitatory phase of etorphine hydrochloride before its CNS depressive properties took effect. It does not have an antidote and was left to be metabolised.

The drugs were delivered remotely from a vehicle by use of projectile darts using a carbon dioxide (CO_2) operated darting rifle (Dan-Inject®, Dan-Inject APS, Denmark) into parts of the body with well covered muscles such as the hindquarters . This darting system is gentle and causes minimal pain and trauma on the animal.

Immediately the animals went down, they were put on sternal recumbency to decrease the incidence of bloat and regurgitation as well as protect the airways by decreasing the pressure of the abdominal viscera on the diaphragm. They were also blindfolded to minimise stress from visual stimulation. The head was placed low to allow saliva or regurgitated ruminal contents to drain out.

Breathing was monitored throughout the sampling procedure to ensure respiratory sufficiency and avoid hypoxia. The body temperature was also monitored with a thermometer for signs of hyperthermia, a common problem during immobilisation. The immobilisation of the animals was undertaken by experienced personnel to ensure as humane process as much as possible. Sampled animals were marked with a coloured spray to avoid re-sampling (Figure 2).

Figure 2: A Coke's hartebeest (*Alcelaphus buselaphus*) reversed from anaesthesia after sampling

At least 30 ml of blood was collected from each animal by jugular venipuncture into plain and EDTA coated tubes. Blood in EDTA tubes was then split into at least four aliquots and stored frozen in liquid nitrogen (-196°C) until processed. Serum was separated by centrifugation from blood in plain tubes after at least 6 hours of standing to allow for clot formation. Serum was split into at least 4 aliquots and also preserved frozen for future studies.

3.2.4 Collection and identification of ticks

Ticks were collected from immobilised animals using tweezers from all over the body including ears, underneath the legs and at the base of the tail. After collection, ticks were immersed in 1.5ml vials which were then labelled with information identifying the date, location and host and stored in liquid nitrogen (-196°C) until processed.

Ticks were transported to the laboratory where they were identified and separated into pools of 1 to 8 non-engorged ticks according to tick species, sampling site and the individual animal host. Identification of the ticks was done using a dissecting microscope (Leica Microsystems, Wetzlar, Germany) to the species level as described by Walker *et al*. (2003). Morphological characteristics as well as host and predilection attachment sites and the geographical location were used as aids in the identification.

3.2.5 Extraction of DNA from blood and ticks

The extraction of genomic DNA (gDNA) from EDTA blood samples was done using the DNeasy® Blood and Tissue Kits (QIAGEN GmbH, Hilden, Germany) following some modification of the manufacturer's instructions as summarised in Appendix 1. The MagNA 96 Pure DNA® and Viral NA Small Volume Kit® (Roche Diagnostics Ltd, Sussex, UK) was used to extract DNA from ticks as summarised in Appendix 2.

3.2.6 Polymerase chain reaction-high resolution melting analyses

3.2.6.1 Polymerase chain reaction analyses

To detect SFG rickettsiae and *C. burnetii* in gDNA extracted from blood and ticks, real-time fluorescence touch-down PCR followed by High Resolution Melting (HRM) using the Rotor-Gene® Q thermocycler (QIAGEN GmbH, Hilden, Germany) was used. The PCR conditions were an initial DNA denaturation at 95°C for 15 minutes, then 43 cycles of denaturation for 20 seconds at 94°C, annealing for 25-50 seconds at temperatures ranging from 65°C decreasing by 1°C to 50°C and extension for 5-30 seconds at 72°C. The reaction mixture was then held for three minutes at 72°C for final elongation followed by a final hold for one minute at 45°C for complete annealing. The detailed PCR conditions for the detection of each pathogen are presented in appendices 3 and 4.

3.2.6.2 High resolution melting analyses

Immediately after the amplification, the high resolution melting (HRM) analysis was done. This involved gradual melting (denaturing or dissociating) the PCR product's dsDNA by gradually increasing the temperature from 75°C to 90°C in 0.1°C temperature increments, at intervals of two seconds. The decrease in fluorescence as the dye was released when dsDNA melted into single strands was measured on a specialised optical system to generate characteristic sequence-dependent melting curve profiles. The curve showed fluorescence at its highest when the dye was in its bound state before the dsDNA was melted and lowest in its solution form after being released once the melting was complete. The melt curves raw data was plotted as fluorescence verses temperature. Upon completion of the run, the melting curve profiles were analysed with

the Rotor-Gene Q Software V2.1.0. (SABioSciences, Frederick, USA) to show the melting temperatures peaks (points in the melting curves when 50% of the DNA is double stranded and 50% is single stranded). These were plotted for each PCR product. Samples were determined positive if they displayed melting curves and melting temperature peaks profiles that were similar in shape to the positive control sample and negative if the shapes were distinct from the positive control.

3.2.6.3 Polymerase chain reaction-high resolution melting reaction mix

The PCR-HRM detection was done in 10 µl reaction mix consisting of 5 µl of 5X HOT FIREPol® EvaGreen® HRM mix (no ROX) (Solis BioDyne, Tartu, Estonia), 0.5 µL of 10 nanomoles of primers and 1 µl of DNA template in UlraPure® DNase/RNase-Free PCR-grade water (Thermo Fisher Scientific Inc, Waltham, Massachusets, USA). The positive controls used were DNA extracted from *R. africae* isolated from a tick in Kenya and the IS*1111* plasmid pBluescript® SH6E (Stratagene, La Jolla, CA, USA) in Elution Buffer for SFG rickettsiae and *C. burnetii* assays respectively. UlraPure® DNase/RNase-Free PCR-grade water (Thermo Fisher Scientific Inc, Waltham, Massachusets, USA) was used as the negative control for both assays. The thermocycler operated on the Rotor-Gene Q series Software V2.1.0 (Build 9) (SABioSciences, Frederick, USA).

3.2.6.4 Primers for PCR analyses

The primers and target genes for PCR detection of the pathogens are shown in Table 1. The primers were obtained from Eurogentec S.A (Seraing, Belgium).

Table 1: Primers and target genes used for PCR analyses of SFG rickettsiae and *C. burnetii*

Pathogen	**Primer**	**Gene**	**5'-Primers Sequences-3'**	**Reference**
SFG rickettsiae	*rpmE*F	*rpmE*-tRNA^{fMet}	TTCCGGAAATGTAGTAAATCAATC	Fournier *et al*. (2004)
	*rpmE*R		TCAGGTTATGAGCCTGACGA	
C. burnetii	IS*1111*F	IS*1111a*	GCTCCTCCACACGCTTCCAT	Tokarz *et al*. (2009)
	IS*1111*R		GGTTCAACTTGTGTGGAATTGATGAGT	

3.3 IDENTIFICATION OF SFG RICKKETTSIAE AND *C. BURNETII* PRESENT IN WILDLIFE AND TICKS

3.3.1 Identification of SFG rickettsiae

The PCR-HRM analysis targeting the intergenic spacer *rpmE*-tRNA^{fMet} was initially used to screen the 152 mammalian EDTA blood and 166 tick pool samples for the presence of SFG rickettsiae. For species identification in the positive samples, other genes were amplified by PCR and subsequently sequenced.

3.3.1.1 Re-amplification of PCR-HRM positive samples

Single step PCR was used to amplify gene *ompB* which encodes the outer membrane protein B while nested PCR was used to amplify genes *ompA* and *gltA* which encode the outer membrane protein A and citrate synthase respectively using previously described primer sets shown in Table 2.

Table 2: Target genes and primers used to amplify and sequence SFG rickettsiae in wildlife and ticks

Gene for amplification	Primer	5'-Primers Sequences-3'	Annealing Temperature	Expected product size	References
ompA	RR 190-70	ATGGCGAATATTTCTCCAAA	50°C	590 bp	Blair *et al*. (2004)
	RR190-701	GTTCCGTTAATGGCAGCATCT	50°C		
	190-FN1	AAGCAATACAACAAGGTC	45°C	540 bp	
	190-RN1	TGACAGTTATTATACCTC	45°C		
gltA	CS1dF	ATGACTAATGGCAATAATAA	47°C	1254 bp	Jiang *et al*. (2005)
	CS1273R	CATAACCAGTGTAAAGCTG	47°C		
	CS1234R	TCTAGGTCTGCTGATTTTTTGTTCA	50°C		
	Rp CS877F	GGGGGCCTGCTCACGGCGG	50°C	382 bp	
	Rp CS1258R	ATTGCAAAAAGTACAGTGAACA	50°C		
ompB	120-2788F	AAACAATAATCAAGGTACTGT	55°C	790 bp	Roux and Raoult 2000; Jiang *et al*. (2005)
	120-3599R	TACTTCCGGTTACAGCAAAGT	55°C		

The PCR amplifications were carried out in a total volume of 25 µl reaction mix containing 1 µl deoxynucleotides solution (dNTPs), 2.5 µl Standard *Taq* Buffer (Biolabs®, New England, UK), 1 µl each of reverse and forward primers, 17.25 µl of DNase/RNase-Free® PCR grade water (QIAGEN, Hilden, Germany), 0.25 µl of *Taq* DNA polymerase (Biolabs®, New England, UK) and 2µl of template DNA.

The amplifications were performed in Applied Biosystems Veriti® 96 well thermocycler (Applied Biosystems, California, USA). The PCR conditions were as follows: 3 minutes initial denaturation at 95°C, 35 cycles of 30 seconds denaturation at 95°C, 30 seconds primer annealing at temperatures specific for each of the primers (Table 3), one minute extension at 72°C and a final 10 minute extension at 72°C. The mixture was then maintained at 4°C. A negative control using DNase/RNase-Free® PCR grade water (QIAGEN, Hilden, Germany) was added for quality control.

The PCR products were run on agarose gel electrophoresis to check for presence of DNA band and size. The gel was prepared by dissolving 1 gram of Agarose LE Molecular Biology Grade® (Benchmark Scientific Inc, New Jersey, USA) in 100 ml of TAE Buffer. This was heated to dissolve the agarose and allowed to cool on an electrophoresis tray with combs inserted to form wells. Then 4 µl of the PCR product was mixed with 1 µl of GelPilot® DNA loading dye (QIAGEN, Hilden, Germany) and added into the wells. The size of the amplified product was determined by comparison with a GelPilot® 100 bp Plus Ladder (QIAGEN, Hilden, Germany) molecular marker which was added to one of the wells to give indication of the size of amplified product. The gel was subjected to electrophoresis at 80 volts for 40 minutes then imaged under UV light to show the bands.

3.3.1.2 Sequencing of PCR positive samples

Positive PCR products of, or close to, the expected product size for the three target genes (*ompA*, *ompB* and *gltA*) were subsequently purified using QIAquick® purification kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. This was aimed at recovering DNA free of left over primers, excess deoxynucleotides and buffer salts for sequencing.

The purification involved addition of 5 volumes Buffer PB to 1 volume of the PCR product in a QIAquick® column and mixing the two. The QIAquick® column was then placed in a 2 ml collection tube. To bind the DNA, the mixture was centrifuged for 30-60 seconds. The flow-through was discarded and the QIAquick® column placed back in the same collection tube. To wash, 750 µl Buffer PE was added into the QIAquick® column. The mixture was then centrifuged for 30-60 seconds. The flow-through was discarded and the QIAquick® column placed back in the same collection tube and then centrifuged once more for 1 minute to remove residual wash buffer. The QIAquick® column was then placed in a clean 1.5 ml micro-centrifuge tube. To elute the DNA, 50 µl Buffer EB was added to the centre of the QIAquick® column membrane and the column centrifuged for 1 minute. In order to increase the DNA concentration, 30µl elution buffer was added to the centre of the QIAquick® column membrane. The membrane was allowed to stand for 1 minute and then centrifuged.

The DNA sequencing was performed by Inqaba Biotechnical Industries (Pty) Ltd, South Africa (http://www.inqababiotec.co.za/). This was carried out by direct cycle sequencing on both strands of purified positive DNA products. Sequencing reactions were carried out with the ABI PRISM BigDye Terminator V3.1 cycle sequencing kit and analyzed on an ABI310 DNA sequencer (Applied Biosystems, California, USA). The same primers as those used for the PCR amplifications as listed in Table 2 were used for both forward and reverse sequencing reactions.

3.3.1.3 Phylogenetic analysis of SFG rickettsiae

Phylogenetic and molecular evolutionary analyses for the sequences obtained from the isolated SFG rickettsiae were conducted using MEGA version 6 sequence analyses software (Tamura *et al*., 2013) in order to compare their relationship with other reference strains from different geographical locations sourced from the Genbank.

To construct the phylogenetic trees for each of the three genes used, the traces (i.e. the raw sequences obtained before editing) were firstly assembled using Chromas Lite Version 2.1 to generate consensus sequences. BLAST searches were managed on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.hih.gov/) using Blastn tool. The sequences obtained in the study were aligned with similar sequences sourced from the

GenBank using Clustal X version 2.0 analyses software (Larkin *et al.*, 2007). This was based on the sequences that blasted with a high expectation value to the detected SFG rickettsiae sequences using Blastn tool in NCBI. The phylogenetic trees were constructed using the Maximum Likelihood method and branch support was evaluated using bootstrap analyses with 1,000 replications using MEGA V6 sequence analyses software (Tamura *et al*., 2013).

3.3.2 Identification of *C. burnetii* isolates

3.3.2.1 Sequencing of PCR-HRM positive amplicons

To identify the *C. burnetii* isolates detected in this study, the PCR-HRM positive amplicons were purified for direct sequencing by enzymatic treatment using exonuclease I and shrimp alkaline phosphatase (PCR Product Pre-sequencing Kit, Amersham). This was aimed at recovering DNA free of excess nucleotides, excess dideoxynucleotides and buffer salts.

The DNA sequencing was carried out at Macrogen Inc, Seoul, South Korea (www.macrogen.com/) by direct cycle sequencing on both strands of purified PCR DNA products from PCR amplification. Sequencing reactions were carried out with the ABI PRISM BigDye Terminator V3.1 cycle sequencing kit and analyzed on an ABI310 DNA sequencer (Applied Biosystems, CA). BLAST searches were managed on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.hih.gov).

3.3.2.2 Phylogenetic analysis of *C. burnetii*

To compare the *C. burnetii* isolates obtained in this study with other reference strains downloaded from the GenBank, a phylogenetic tree using *IS1111* gene was constructed to illustrate the evolutionary relationship of isolates obtained to strains in the GenBank.

Reverse and forward trace files were assembled using Geneious version 8.1.6 sequence analyses software (Kearse *et al*., 2012; http://www.geneious.com). To assess phylogenetic relationship of the study sequences with others from different geographical locations, blast searches were performed on NCBI website (Altschul *et al*., 1990; http://blast.ncbi.nlm.hih.gov). Matches with a query cover of over 98% and identity of 95% and above were used for phylogenetic analyses.

Sequences were aligned using MUSCLE (Edgar, 2004; http://www.drive5.com/muscle). The phylogenetic tree was constructed using MEGA version 6 sequence analyses software (Tamura *et al*., 2013). The maximum likelihood tree was drawn using the most appropriate model namely Jukes-Cantor (Jukes and Cantor, 1969). Gamma shape parameter and proportion of invariant sites were estimated from the data. Polymorphism and divergence of the study sequences and references from Genbank was checked using DNASP and a graph generated as described by Librado *et al*. (2009).

3.4 EVALUATION OF KNOWLEDGE, ATTITUDES AND PRACTICES ON SFG RICKETTSIOSES AND Q FEVER

A semi-structured questionnaire (Appendix 5) was used to assess the knowledge, attitudes and practices (KAP) of local residents towards the diseases. The respondents comprised pastoralists, medical health providers as well as veterinary and wildlife sector personnel.

Given that the predominant local populations in both study areas are pastoralists who are widely dispersed and hold deep cultural practices, the respondents were selected through key informants who included village elders, local administration (chiefs and assistant chiefs) and local opinion leaders such as human health providers and veterinarians. The key informants also provided other relevant information about the areas such as accessibility and availability of respondents.

The questionnaire covered a range of topics relevant for each category of respondents. For the pastoralists and wildlife sector personnel, these included but not limited to types of livestock kept, interactions between livestock and wildlife and the types of problems encountered, diseases of importance shared between livestock and wildlife, zoonotic diseases including tick-borne, clinical manifestations of tick-borne zoonotic diseases and measures undertaken to prevent tick bites. For the medical health and veterinary personnel, the topics included zoonotic diseases found in patients including tick-borne, clinical signs of SFG rickettsioses and Q fever, number of patients diagnosed with SFG rickettsioses and Q fever in the past one year, differential diagnosis of SFG rickettsioses and Q fever, laboratory diagnostic methods for SFG rickettsioses and Q fever and whether confirmatory diagnosis is routinely done in non-specific febrile illnesses.

To ensure accuracy of the information, the data collection tool was translated from English to Kiswahili or the local language and then back-translated for respondents not conversant with the English language.

3.5 EVALUATION OF POTENTIAL RISK FACTORS TO SFG RICKETTSIOSES AND Q FEVER INFECTION IN HUMANS

The evaluation of potential risk factors that could predispose the local residents in Laikipia and Maasai Mara to SFG rickettsioses and Q fever were derived from the findings of the study. These included the finding of the presence or absence of the diseases, the responses to the semi-structured questionnaire, discussions with the respondents and any other observations made during the study.

3.6 DATA ANALYSES

Statistical analysis was carried out using relevant statistical packages that included MS Access data base, MS Excel packages, STATA/SE 11.2 and the SAS statistical package. The prevalence of SFG rickettsioses and Q fever were analysed using a descriptive approach according to independent variables such as host species (wildlife or tick) and the study area using the SAS statistical package. The questionnaire data was entered into MS Access data base and analysed using STATA/SE 11.2 and MS Excel packages. The Z-test and Chi square test were used to test hypotheses.

CHAPTER FOUR: RESULTS

4.1 PRESENCE AND PREVALENCE OF SFG RICKETSIAE AND *C. BURNETII* IN WILDLIFE AND TICKS

4.1.1 Sampling locations

The sampling sites in Laikipia included Ol Pejeta conservancy, ADC Mutara ranch, Mpala ranch and Kiamariga sub-location (Figure 3). The sampling sites in Maasai Mara included different locations within and outside the reserve (Figure 4).

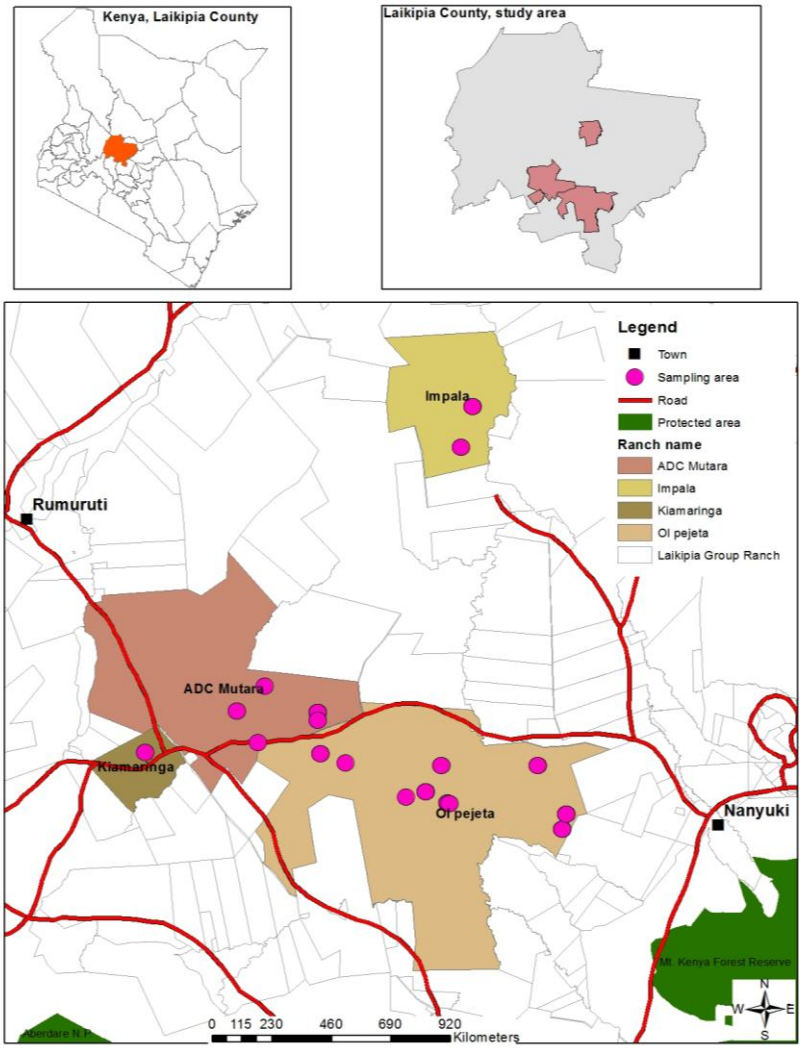

Figure 3: Sampling locations of wildlife and ticks in Laikipia County

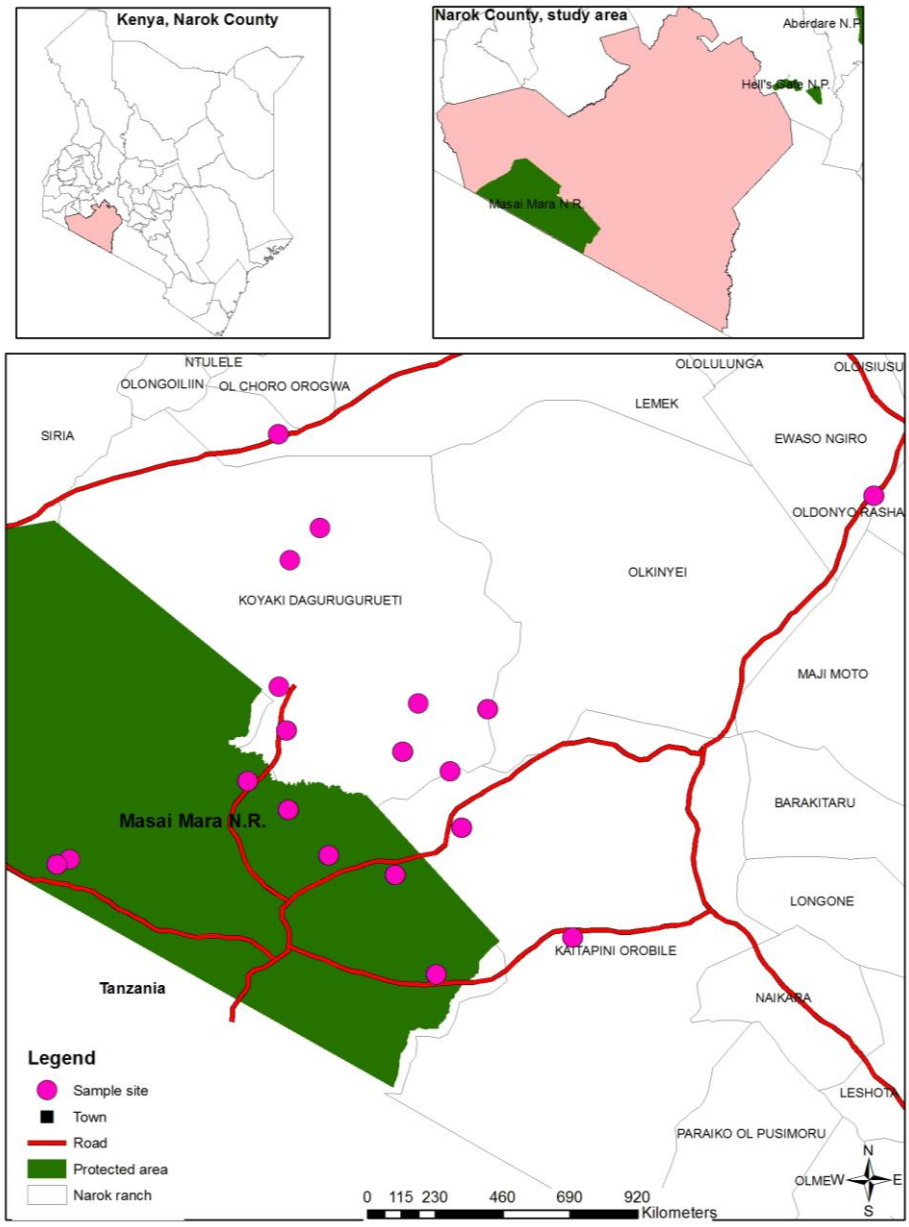

Figure 4: Sampling locations of wildlife and ticks in Maasai Mara

4.1.2 Wildlife species sampled

One hundred and fifty two (152) animals comprising 8 species were sampled in both Laikipia (79/152) and Maasai Mara (73/152) as summarised in Table 3.

Table 3: Species of wildlife sampled in Laikipia and Maasai Mara

Study Area	Animal species								
	Zebra (*Equus burchellii*)	Buffalo (*Syncerus caffer*)	Grant's Gazelle (*Nanger granti*)	Common Waterbuck (*Kobus ellipsiprymnus ellipsiprymnus*)	Impala (*Aepyceros melampus*)	Wildebeest (*Connochaetes taurinus*)	Topi (*Damaliscus korrigum*)	Coke's Hartebeest (*Alcelaphus buselaphus*)	**Total**
Laikipia	39	31	7	2	-	-	-	-	**79**
Maasai Mara	21	-	-	-	2	35	10	5	**73**
Total	**60**	**31**	**7**	**2**	**2**	**35**	**10**	**5**	**152**

All the animals responded well to the immobilisation drugs and induction times ranged between 8-12 minutes. No complications were encountered during immobilisation and handling except for a few animals which had slightly elevated body temperatures that were attributed to physical exertion during darting as well as psychological stress and fear. These animals were cooled by applying copious amounts of water on the whole body.

4.1.3 Tick species sampled

A total of 851 ixodid ticks were collected from immobilised animals in Laikipia and Maasai Mara. Most of the ticks were from Laikipia (756/851) and the rest (95/851) from Maasai Mara. The ticks were pooled according to species, sampling site and the individual animal host into 166 pools, majority of which were from Laikipia (137/166) and the rest (29/166) from Maasai Mara.

Rhipicephalus ticks comprised the majority of the tick samples at 135/137 and 24/29 in Laikipia and Maasai Mara respectively. The rest of the samples in Laikipia were one each of

Dermacentor and *Amblyomma* ticks. The rest of the samples in Maasai Mara were 3/29 *Hyalomma* and 2/29 *Amblyomma* ticks. Table 4 summarises the tick samples by species for each study area.

Table 4: Tick pool samples collected from wildlife in Laikipia and Maasai Mara

Study area	Tick Pool Samples										
	Rh. appendiculatus	*Rh. evertsi evertsi*	*Rh. pulchellus*	*Rh. evertsi*	*H. dromedari*	*D. rhinocerinus*	*H. albiparmatum*	*A. variegatum*	*A. truncatum*	*A. gemma*	**Total**
Laikipia	53	38	33	11	-	1	-	-	-	1	**137**
Maasai Mara	10	12	2	-	1	-	2	1	1	-	**29**
Total	**63**	**50**	**35**	**11**	**1**	**1**	**2**	**1**	**1**	**1**	**166**

Key: *Rh-Rhipicephalus*; *H- Hyalomma*; *D- Dermacentor*; *A- Amblyomma*

Rhipicephalus pulchellus, *Rh. evertsi evertsi* and *Rh. appendiculatus* were collected in both Laikipia and Maasai Mara, while *Rh. evertsi*, *D. rhinocerinus* and *A. gemma* were collected in Laikipia only and *H. Dromedari*, *H. Albiparmatum*, *A. variegatum* and *A. truncatum* were collected in Maasai Mara only. *Rh. appendiculatus* comprised the most tick pools in Laikipia (53/137), followed by *Rh. evertsi evertsi* (38/137), *Rh. pulchellus* (33/137) and *Rh. evertsi* (11/137). Both *D. rhinocerinus* and *A. gemma* comprised 1/137 of the tick pools in Laikipia.

In Maasai Mara, *Rh. evertsi evertsi* comprised the most tick pools (12/29), followed by *Rh. appendiculatus* (10/29). Both *Rh. pulchellus* and *H. albiparmatum* comprised 2/29 pools each while *H. dromedari*, *A. variegatum* and *A. truncatum* comprised 1/29 pool each.

4.1.4 Quality of DNA extracted from EDTA blood and ticks

The gDNA extracted from EDTA blood and tick samples was determined to be of high quality by agarose gel electrophoresis protocol as shown by representative gel image of 24 mammalian EDTA blood samples in Figure 5.

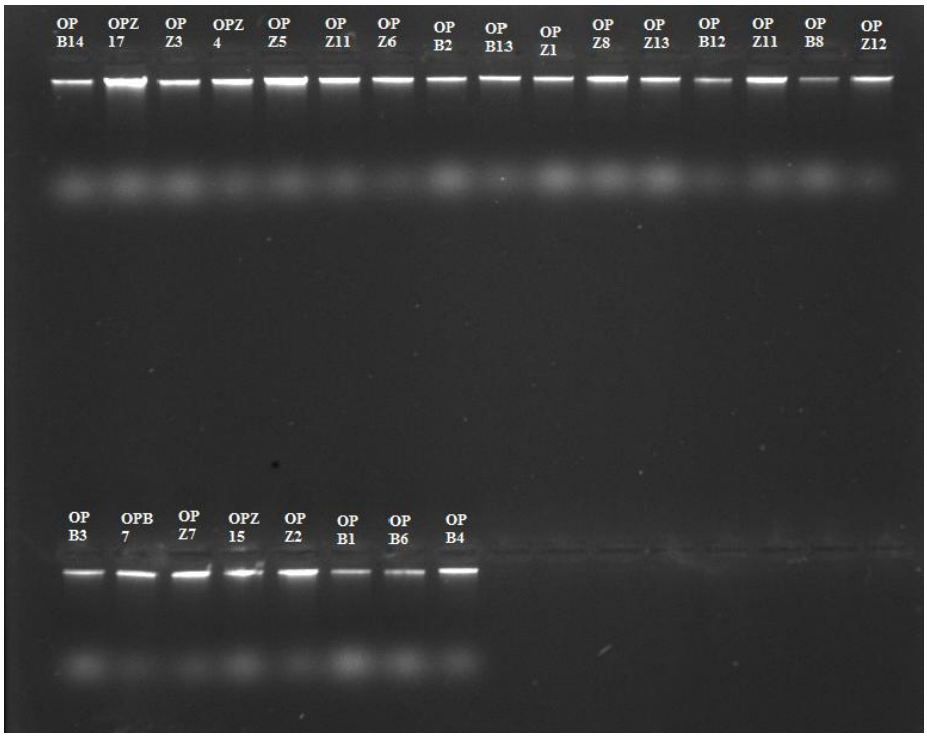

Figure 5: Gel-image of representative gDNA extracted from EDTA blood samples

4.1.5 Prevalence of SFG rickettsioses in wildlife

Two animals in Laikipia and 4 in Maasai Mara tested positive for SFG rickettsioses representing a prevalence of 2 out 79 (2.5%) in Laikipia and 4 out 73 (5.5%) in Maasai Mara.

The animals that tested positive in Laikipia were a zebra (*Equus burchellii*) sampled at ADC Mutara ranch and a buffalo (*Syncerus caffer*) sampled in Ol Pejeta conservancy (Appendix 6), representing a prevalence of 1 out of 39 (2.6%) and 1 out of 31 (3.23%) in these species respectively. Spotted fever group rickettsiae DNA was not detected in the other animal species sampled in Laikipia namely Grant's gazelle (*Nanger granti*) and common waterbuck (*Kobus ellipsiprymnus ellipsiprymnus*).

Three Topi (*Damaliscus korrigum)* and one wildebeest (*Connochaetes taurinus*) tested positive in Maasai Mara (Appendix 6) representing a prevalence of 3 out of 10 (30%) and 1 out of 35 (2.9%) respectively in these species. Spotted fever group rickettsiae DNA was not detected in the other animal species sampled in Maasai Mara namely common zebra (*Equus burchellii*), impala (*Aepyceros melampus*) and Coke's hartebeest (*Alcelaphus buselaphus*).

4.1.6 Prevalence of Q fever in wildlife

Coxiella burnetii DNA was not detected in any of the animal species sampled in both Laikipia and Maasai Mara (Appendix 6).

4.1.7 Prevalence of SFG rickettsiae in ticks

Thirty tick samples in Laikipia and 5 in Maasai Mara tested positive for SFG rickettsial DNA representing a prevalence of 30 out of 137 (21.9%) in Laikipia and 5 out of 29 (17.2%) in Maasai Mara. Table 5 summarises the prevalence of SFG rickettsiae according to tick species for each study area while the detailed results are presented in Appendix 7.

Table 5: Prevalence of SFG rickettsiae in ticks collected from Laikipia and Maasai Mara

Study area	Tick species and prevalence of SFG rickettsiae										
	Rh. pulchellus	*Rh. evertsi evertsi*	*Rh. appendiculatus*	*Rh. evertsi*	*H. dromedari*	*H. albiparmatum*	*D. rhinocerinus*	*A. variegatum*	*A. truncatum*	*A. gemma*	**Total**
Laikipia	6/33 (18.2%)	4/38 (10.5%)	16/53 (30.2%)	4/11 (36.4%	-	-	0/1 (0%)	-	-	0/1 (0%)	**30/137 (21.9%)**
Maasai Mara	0/2 (0%)	0/12 (0%)	2/10 (20.0%)	-	1/1 (100%)	0/2 (0%)	-	1/1 (100%)	1/1 (100%)	-	**5/29 (17.2%)**
Total	**6/35 (17.1%)**	**4/50 (8.0%)**	**18/63 (28.6%)**	**4/11 (36.4%)**	**1/1 (100%)**	**0/2 (0%)**	**0/1 (0%)**	**1/1 (100%)**	**1/1 (100%)**	**0/1 (0%)**	**35/166 (21.1%)**

Thirty samples of *Rhipicephalus* ticks in Laikipia and two in Maasai Mara tested positive representing a prevalence of 30 out of 135 (22.2%) in Laikipia and 2 out of 24 (8.3%) in Maasai Mara in these ticks.

In terms of individual *Rhipicephalus* species in Laikipia, *Rh. evertsi* had the highest prevalence of 4 out of 11 (36.4%), followed by *Rh. appendiculatus* in 16 out of 53 (30.2%), *Rh. pulchellus* in 6 out of 33 (18.2%) and *Rh. evertsi evertsi* in 4 out of 38 (10.5%) samples. In Maasai Mara, 2 out of 10 (20%) samples of *Rh. appendiculatus* tested positive.

Two samples of *Amblyomma* ticks in Maasai Mara tested positive representing a prevalence of 2 out of 2 (100%) in these ticks. These samples were one each of *A. variegatum* and *A. truncatum* representing a prevalence of 1 out of 1 (100%) in each of these species.

One tick sample of *Hyalomma* ticks in Maasai Mara tested positive representing a prevalence of 1 out of 3 (33.3%) in these ticks. This sample was *H. dromedari* representing a prevalence of 1 out of 1 (100%) in the species.

The other tick species sampled did not test positive for SFG rickettsiae. These were *D. rhinocerinus* and *A. gemma* in Laikipia and *Rh. pulchellus*, *Rh. evertsi evertsi* and *H. albiparmatum* in Maasai Mara.

The combined detection was 35 out of 166 (21.1%) tick samples in both Laikipia and Maasai Mara with the highest detection in *Rh. appendiculatus* at 18 out of 53 (28.6%), followed by *Rh. pulchellus* in 6 out of 35 (17.1%) and *Rh. evertsi evertsi* at 4 out of 50 (8.0%).

In Laikipia, SFG rickettsiae were detected in several locations in two of the areas sampled namely Ol Pejeta conservancy and ADC Mutara ranch (Figure 6). There was no detection in the other areas sampled in Laikipia namely Kiamariga sub-location and Mpala ranch. One tick pool of *Rh. appendiculatus* collected from a buffalo in Ol Pejeta conservancy in Laikipia tested positive for both SFG rickettsioses and Q fever. However, the buffalo tested negative for both diseases.

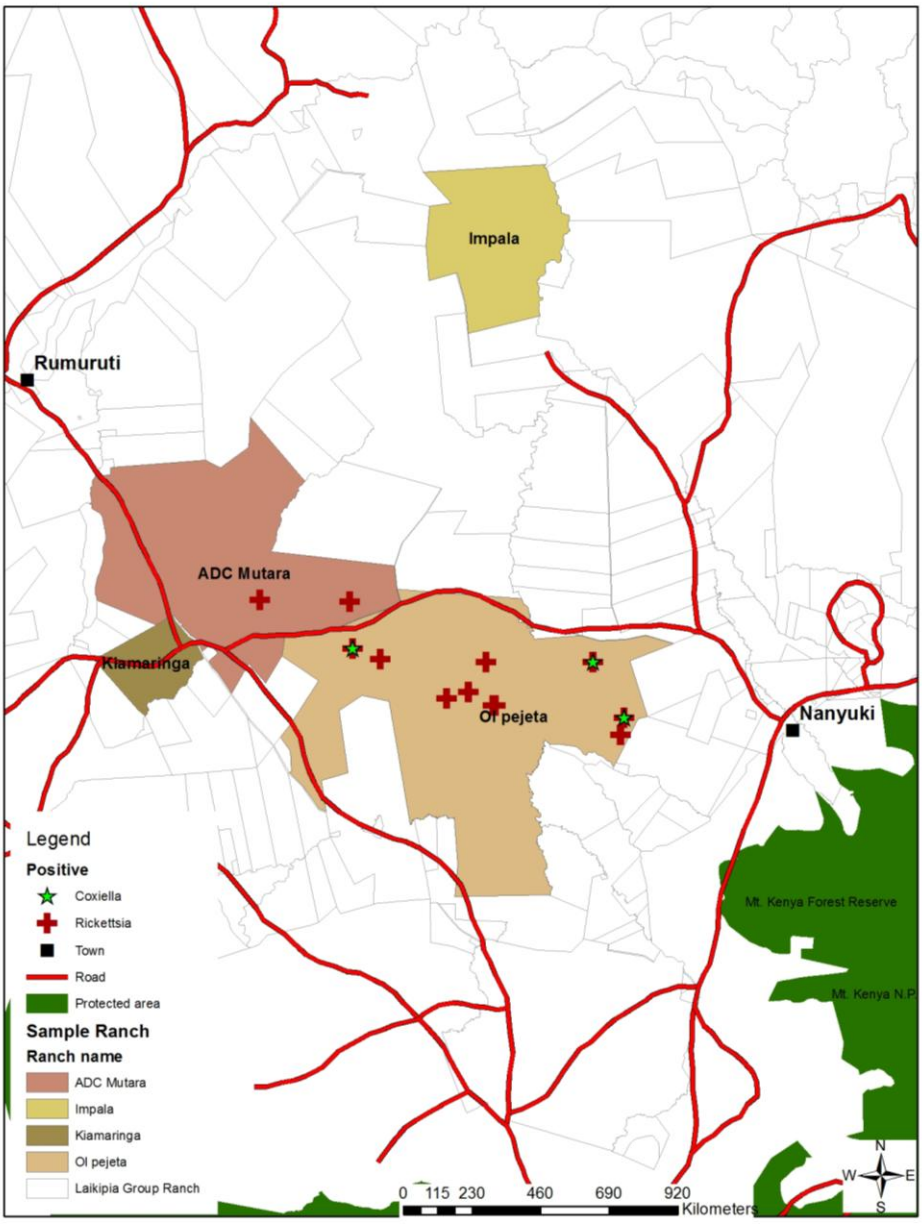

Figure 6: Distribution of SFG rickettsiae and *C. burnetii* positive wildlife and tick pool samples in Laikipia

In Maasai Mara, SFG rickettsiae were detected in two locations inside the reserve and one location outside the reserve (Figure 7).

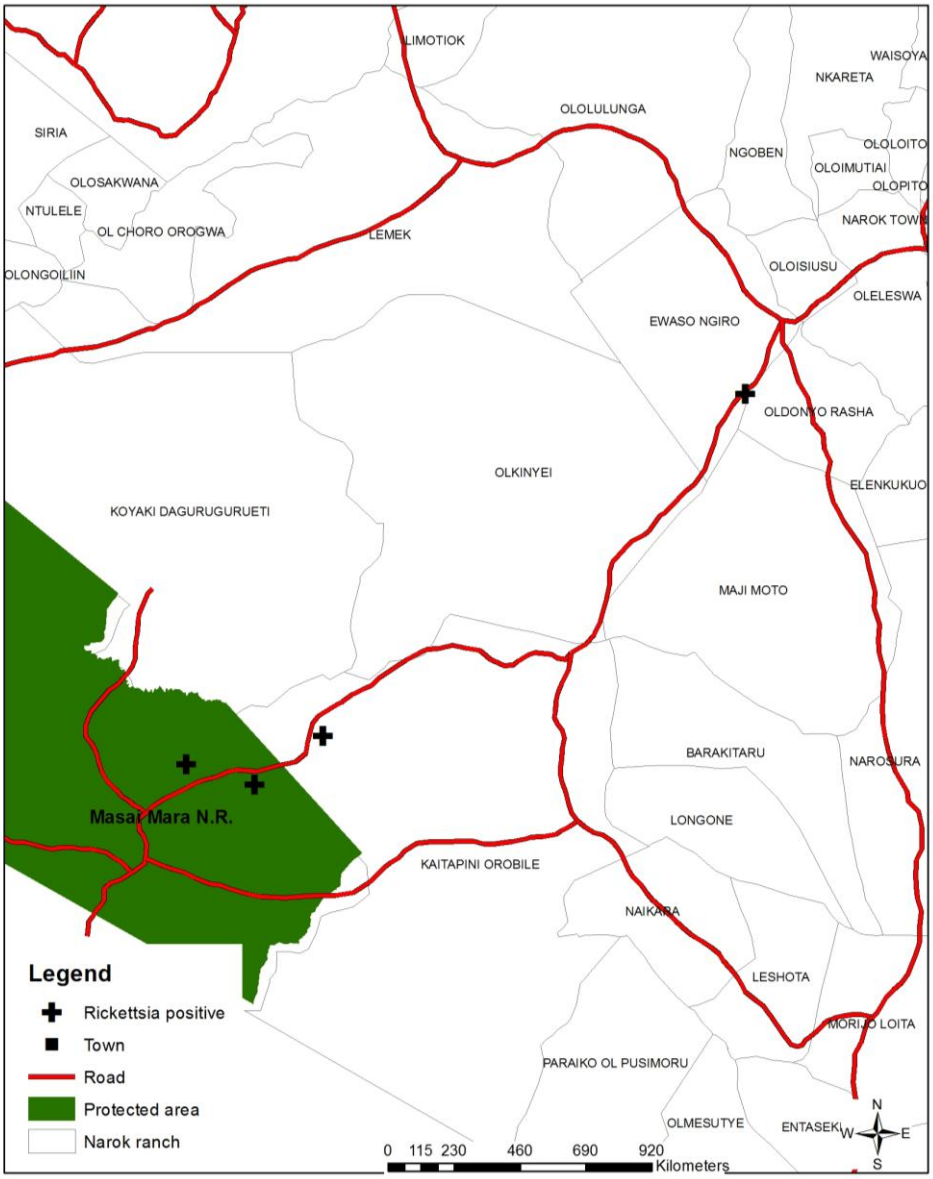

Figure 7: Distribution of SFG rickettsiae positive wildlife and tick samples in Maasai Mara

4.1.8 Prevalence of *C. burnetii* in ticks

Coxiella burnetii DNA was detected in 4 out of 137 (2.9%) tick samples in Laikipia in locations shown in Figure 6. The detection was in 4 out 135 (3.0%) of *Rhipicephalus* ticks. The species that tested positive in Laikipia were *Rh. appendiculatus* in 2 out of 53 (3.8%), *Rh. pulchellus* in 1 out of 33 (3.0%) and *Rh. evertsi evertsi* in 1 out of 38 (2.6%) samples. No sample tested positive in Maasai Mara. The combined prevalence for both Laikipia and Maasai Mara was 4 out of 166 (2.4%). Table 6 summarises the prevalence of *C. burnetii* according to tick species.

Table 6: Prevalence of *C. burnetii* in ticks collected from Laikipia and Maasai Mara

Study area	Tick species and prevalence of *C. burnetii*										
	Rh. pulchellus	*Rh. evertsi evertsi*	*Rh. appendiculatus*	*Rh. evertsi*	*H. dromedari*	*D. rhinocerinus*	*H. albiparmatum*	*A. variegatum*	*A. truncatum*	*A. gemma*	**Total**
Laikipia	1/33 (3.0%)	1/38 (2.6%)	2/53 (3.8%)	0/11 (0%)	-	0/1 (0%)	-	-	-	0/1 (0%)	**4/137 (2.9%)**
Maasai Mara	0/2 (0%)	0/12 (0%)	0/10 (0%)	-	0/1 (0%)	-	0/2 (0%)	0/1 (0%)	0/1 (0%)	-	**0/29 (0%)**
Total	**1/35 (2.9%)**	**1/50 (2.0%)**	**1/63 (1.6%)**	**0/11 (0%)**	**0/1 (0%)**	**0/1 (0%)**	**0/2 (0%)**	**0/1 (0%)**	**0/1 (0%)**	**0/1 (0%)**	**4/166 (2.4%)**

4.2 IDENTIFICATION OF SFG RICKETTSIAE AND *C. BURNETII* DETECTED IN WILDLIFE AND TICKS

4.2.1 Identification of SFG rickettsiae

4.2.1.1 Amplification of PCR-HRM positive samples

To identify the SFG rickettsiae detected in wildlife and ticks, the 41 PCR-HRM positive samples comprising 6 EDTA blood and 35 tick samples were further subjected to standard and nested PCR amplifications using primers targeting *ompA*, *ompB* and *gltA* genes. Of these samples, 35 amplified with either one or a combination of these genes as shown in the gel images in Figures 8, 9 and 10. The amplified samples are summarised in Table 7.

Primers targeting the *ompA* and *ompB* genes amplified six and three tick pool samples respectively while those targeting the *gltA* gene amplified a total of 31 samples comprising five blood and 26 tick-pool samples. Three samples were amplified by a combination of the three genes.

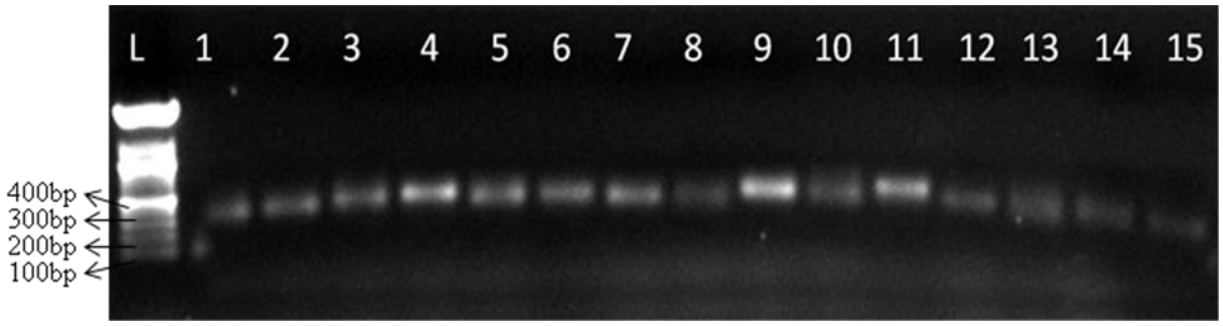

Figure 8: Gel-image of representative nested PCR amplifications of the *gltA* gene for detection of SFG rickettsiae (L is the GelPilot® 100bp molecular marker (QIAGEN, Germany); samples are loaded on lanes 1-15 samples)

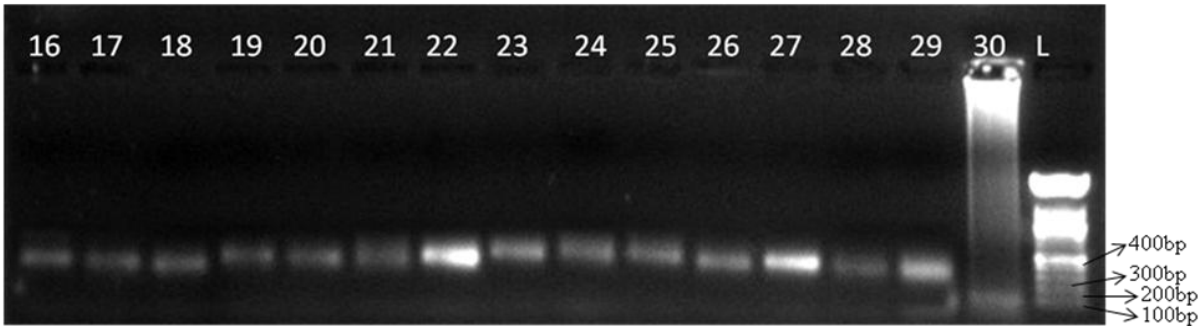

Figure 9: Gel-image of representative nested PCR amplifications of the *gltA* gene for detection of SFG rickettsiae (L is the GelPilot® 100bp molecular marker (QIAGEN, Germany); samples are loaded on lanes 16-30)

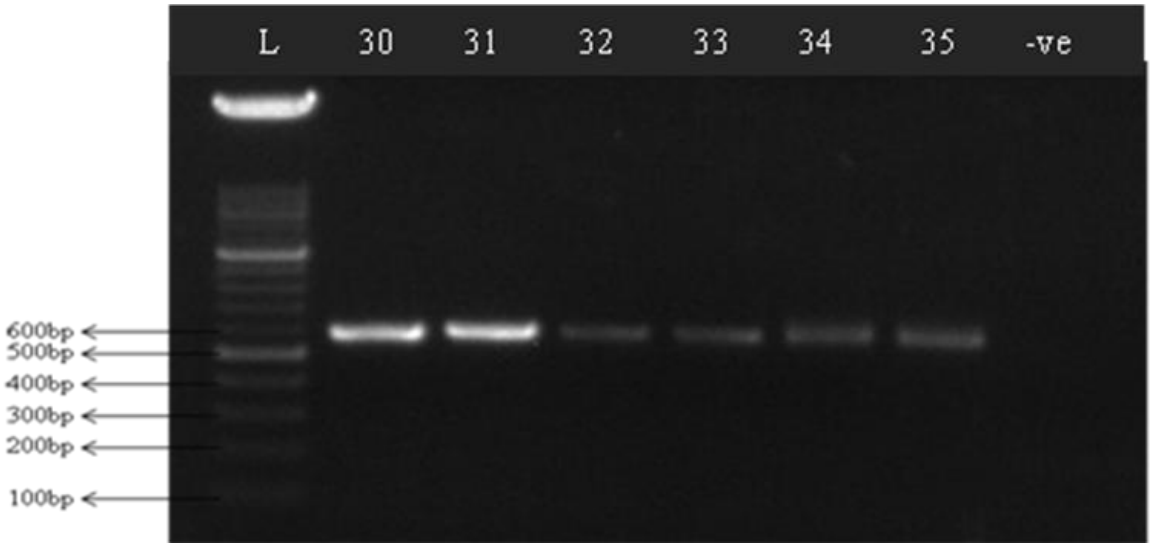

Figure 10: Gel-image of representative nested PCR amplification of *ompA* gene for detection of SFG rickettsiae (L is the GelPilot® 100bp molecular marker (QIAGEN, Germany); samples are loaded on lanes 30-35)

Table 7: PCR amplification of *ompA*, *ompB* and *gltA* genes for SFG rickettsiae detection in wildlife and ticks

No.	Sample ID	Host Species	Target Gene		
			ompA	***ompB***	***gltA***
1.	WB37	Wildebeest (*Connochaetes taurinus*)	-	-	+
2.	T4	Topi (*Damaliscus korrigum*)	-	-	+
3.	T2	Topi (*Damaliscus korrigum*)	-	-	+
4.	T8	Topi (*Damaliscus korrigum*)	-	-	+
5.	OPB23	Buffalo (*Syncerus caffer*)	-	-	+
6.	OPB8	*Rh. pulchellus*	-	-	+
7.	OPB8	*Rh. evertsi evertsi*	-	-	+
8.	OPB8	*Rh. appendiculatus*	-	-	+
9.	OPB8	*Rh. appendiculatus*	-	-	+
10.	OPZ16	*Rh. evertsi evertsi*	-	+	+
11.	OPZ17	*Rh. evertsi*	-	-	+
12.	OPZ17	*Rh. evertsi*	-	-	+
13.	OPB21	*Rh. appendiculatus*	-	-	+
14.	OPB21	*Rh. appendiculatus*	-	-	+
15.	OPB21	*Rh. appendiculatus*	-	-	+
16.	OPB21	*Rh. appendiculatus*	-	-	+
17.	OPB19	*Rh. appendiculatus*	-	-	+
18.	OPB19	*Rh. appendiculatus*	-	-	+
19.	OPB19	*Rh. pulchellus*	-	-	+
20.	MMK8/10	*Rh. appendiculatus*	-	-	+
21.	MM/TP/11	*H. dromedari*	-	-	+
22.	OPZ18	*Rh. evertsi evertsi*	-	-	+
23.	OPB6	*Rh. appendiculatus*	-	-	+
24.	OPB6	*Rh. appendiculatus*	-	-	+
25.	OPB20	*Rh. appendiculatus*	-	-	+
26.	OPB20	*Rh. evertsi evertsi*	-	-	+
27.	OPZ26	*Rh. pulchellus*	-	-	+
28.	OPB4	*Rh. appendiculatus*	-	-	+
29.	OPZ3	*Rh. pulchellus*	-	-	+
30.	OPZ13	*Rh. appendiculatus*	+	-	-
31.	MZ11	*Rh. evertsi*	+	-	-
32.	OPZ27	*Rh. evertsi*	+	-	-
33.	OPB28	*Rh. pulchellus*	+	-	-
34.	MM/WB/50	*A. variegatum*	+	+	+
35.	MM/WB/50	*A. truncatum*	+	+	+

4.2.1.2 Sequence analyses of detected SFG rickettsiae

The PCR positive products from the amplifications of the three genes namely *ompA*, *ompB* and *gltA* were sequenced to enable identification of the detected SFG *Rickettsia* species. Of the 35 samples amplified with these genes, 14 were sequenced as shown in Table 8. One of these samples was EDTA blood collected from a Topi in Maasai Mara and the remaining 13 were tick samples collected from both study areas. The nucleotide sequences of the three amplified genes were submitted to the Genbank and were allocated accession numbers as shown in Appendix 8. The rest of the samples could not be sequenced because they did not yield enough DNA for sequence analyses.

The EDTA blood from the Topi yielded a sequence of 779 bp with *gltA* gene that had 99% identity to *R. sibirica* Accession number KM288711 in the GenBank.

Two *Rh. evertsi evertsi* tick samples collected from buffaloes in Laikipia yielded sequences of 779 bp with *gltA* gene. Blast searches conducted showed the sequences to have 99% identity to *R. sibirica* Accession number KM288711 in the GenBank. Sequences of the same size (779 bp) with *gltA* gene were also obtained from two *Rh. appendiculatus* tick samples collected from buffaloes in Laikipia. These sequences also had 99% identity to *R. sibirica* Accession number KM288711.

Two tick samples one each of *A. variegatum* and *A. truncatum* both collected from a wildebeest in Maasai Mara yielded sequences of 790 bp with *gltA* gene and had 99% identity to *R. sibirica* Accession number KM28711.

In addition, two other tick samples one each of *Rh. appendiculatus* and *Rh. evertsi* collected from a buffalo and a zebra respectively in Laikipia yielded sequences of 526 bp and 591 bp respectively with *gltA* gene which had 87% and 94% identity respectively with an uncultured *Rickettsia* species Accession number KT257872.

Three tick samples were sequenced with primers targeting *ompB* gene. These were one each of *Rh. evertsi evertsi* from a buffalo in Laikipia and *A. variegatum* and *A. truncutum* from a wildebeest in Maasai Mara. The *Rh. evertsi evertsi* tick sample yielded sequences of 780 bp

while the other two yielded sequences of 768 bp. The sequences had 99% identity to *R. sibirica* Accession number HM050273.

Six tick samples sequenced with primers targeting the *ompA* gene revealed *R. sibirica* subspecies *mongolotimonae* with identities of 99-100% to the same species Accession number KT345980. Two of these tick samples were *Rh. evertsi evertsi* collected from two zebras in Laikipia which yielded sequences of 523 bp and 529 bp. Two other samples were *Rh .pulchellus* collected from a buffalo also in Laikipia which generated sequences of 523 bp and 553 bp. The other two were *A. variegatum* and *A. trucatum* tick samples collected from a wildebeest in Maasai Mara that yielded sequences of 541 bp and 542 bp respectively.

Table 8: GenBank BLAST hit results for SFG rickettsiae detected in wildlife and ticks

No.	Sample ID	Tick Pool	Host species	Size (bp)	Target gene	Closest Genbank hit	Identity	Accession number	E value
1.	T8	-	Topi (*Damaliscus korrigum*)	779	*gltA*	*R. sibirica*	99%	KM288711	0.0
2.	OPB8	10	*Rh. evertsi evertsi*	779	*gltA*	*R. sibirica*	99%	KM288711	0.0
3.	OPB8	15	*Rh. appendiculatus*	526	*gltA*	Uncultured *Rickettsia*	87%	KT257872	8e-158
4.	OPB8	17	*Rh. evertsi evertsi*	780	*ompB*	*R. sibirica*	99%	HM050273	0.0
5.	OPZ17	24	*Rh. evertsi*	591	*gltA*	Uncultured *Rickettsia*	94%	KT257872	0.0
6.	OPB21	29	*Rh. appendiculatus*	779	*gltA*	*R. sibirica*	99%	KM288711	0.0
7.	OPB18	52	*Rh. evertsi evertsi*	779	*gltA*	*R. sibirica*	99%	KM288711	0.0
8.	OPB6	53	*Rh. appendiculatus*	779	*gltA*	*R. sibirica*	99%	KM288711	0.0
9.	MZ11	79	*Rh. evertsi*	523	*ompA*	*R. sibirica subsp. mongolotimonae*	100%	KT345980	0.0
10.	OPZ27	82	*Rh. evertsi*	529	*ompA*	*R. sibirica subsp. mongolotimonae*	100%	KT345980	0.0
11.	OPB18	89	*Rh. pulchellus*	523	*ompA*	*R. sibirica subsp. mongolotimonae*	100%	KT345980	0.0
12.	OPB18	91	*Rh. pulchellus*	553	*ompA*	*R. sibirica subsp. mongolotimonae*	99%	KT345980	0.0
13.	MM/ WB/50	145	*A. variegatum*	541	*ompA*	*R. sibirica subsp. mongolotimonae*	99%	KT345980	0.0
				780	*ompB*	*R. sibirica*	99%	HM050273	0.0
				790	*gltA*	*R. sibirica*	99%	KM288711	0.0
14.	MM/ WB/50	146	*A. truncatum*	542	*ompA*	*R. sibirica subsp. mongolotimonae*	99%	KT345980	0.0
				768	*ompB*	*R. sibirica*	99%	HM050273	0.0
				790	*gltA*	*R. sibirica*	99%	KM288711	0.0

4.2.1.3 Phylogenetic analyses of detected SFG rickettsiae

The phylogenetic analyses were conducted for the detected SFG *Rickettsia* species to determine the relationship with other reference strains in the GenBank. The phylogenetic tree constructed using the *ompA* gene (Figure 11) and *ompB* gene (Figure 12) revealed that the detected rickettsial isolates in ticks clustered together (i.e. were identical) and formed a sister cluster with *R. sibirica* but were not identical to it. They formed distinct clades from the other reference strains in the GenBank.

Likewise, the phylogenetic tree constructed using the *gltA* gene (Figure 13) revealed the same findings that the detected rickettsial isolates in a topi (*Damaliscus korrigum*) and ticks were identical and they clustered with *R. conorii* but were not identical to it.

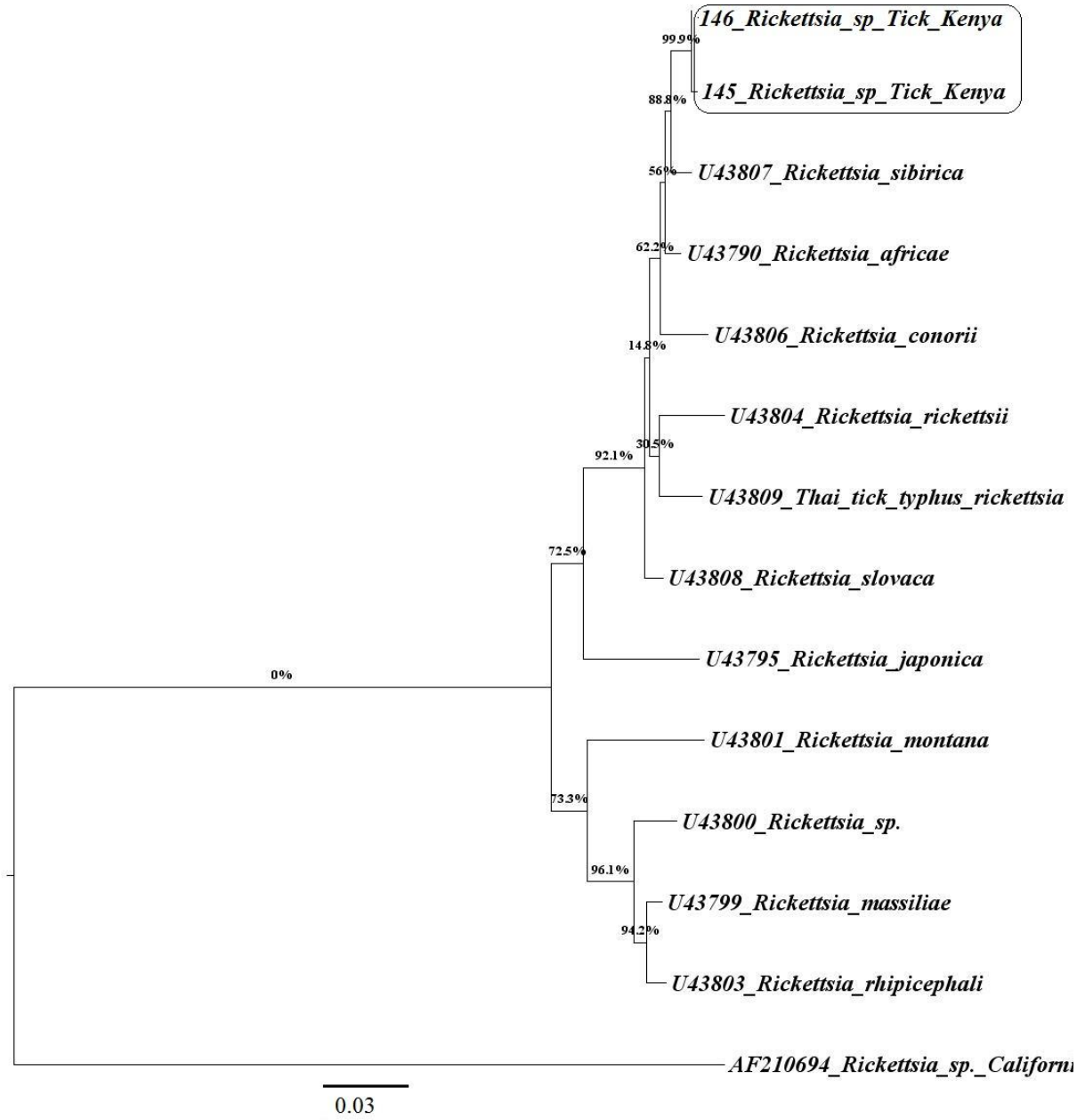

Figure 11: A phylogenetic tree derived from *ompA* gene of SFG rickettsiae isolated from ticks in Laikipia and Maasai Mara

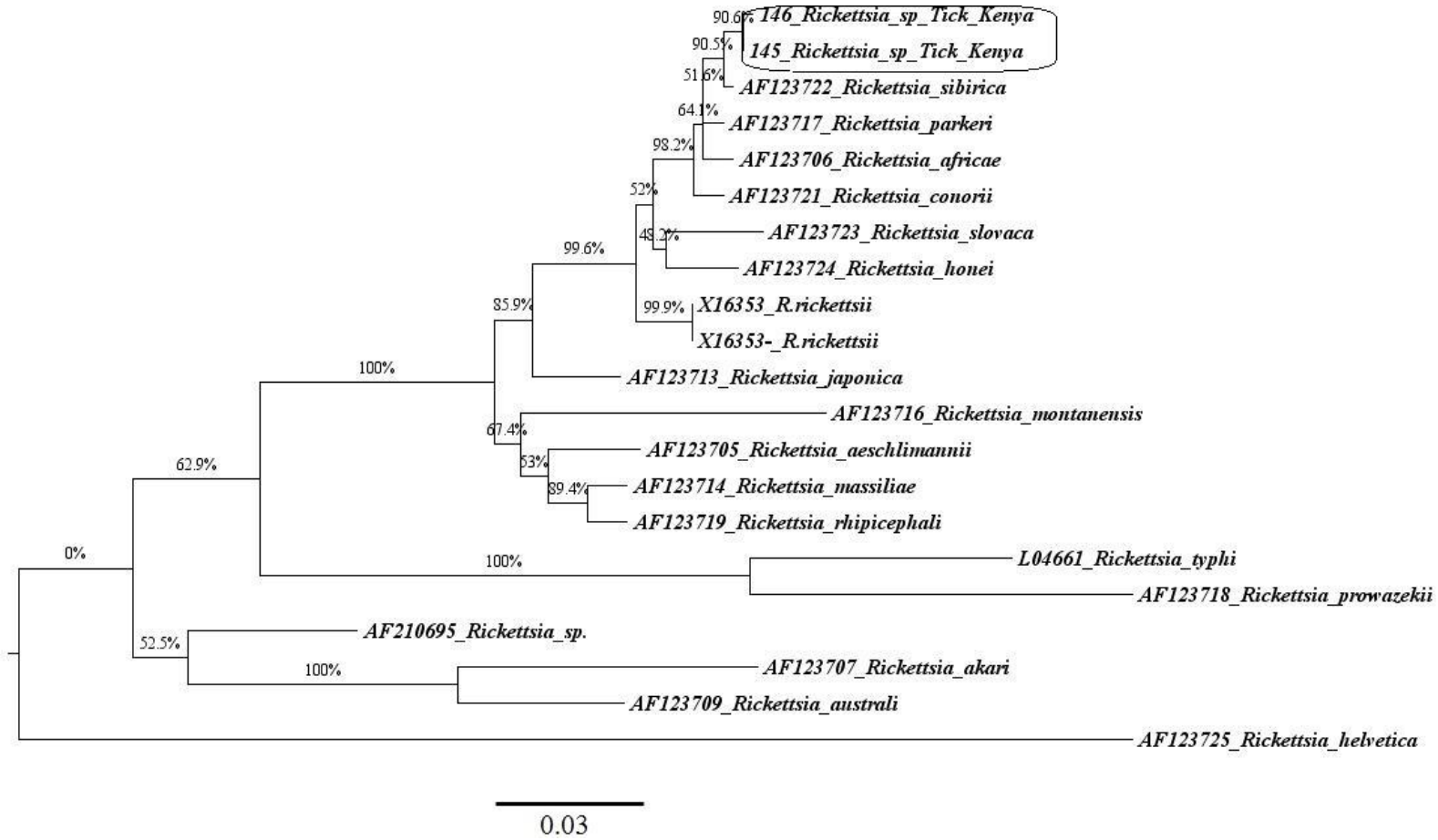

Figure 12: A phylogenetic tree derived from *ompB* gene of SFG rickettsiae isolated from ticks in Laikipia and Maasai Mara

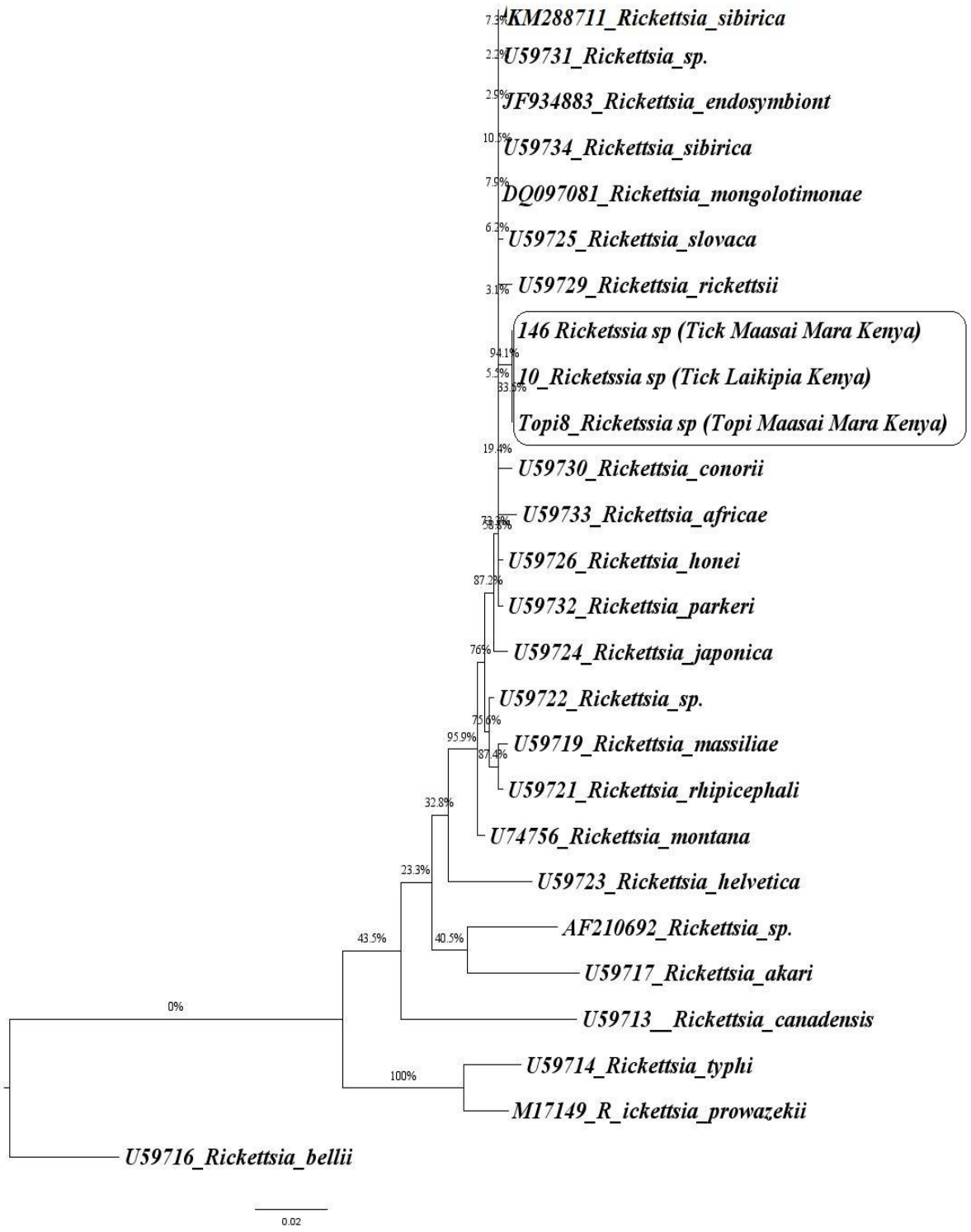

Figure 13: A phylogenetic tree derived from *gltA* gene of SFG rickettsiae isolated from wildlife and ticks in Laikipia and Maasai Mara

4.2.2 Identification of detected *C. burnetii* isolates

4.2.2.1 Sequence analyses of *C. burnetii*

The alignment of the nucleotide sequences of the *C. burnetii* isolates obtained from the study resulted in a 129 base pair consensus sequence. The BLAST analysis revealed that the sequences of three samples were indeed *C. burnetii* with 100% identity to different strains in the Genbank isolated from different hosts and countries as summarised in Appendix 9. Some of these strains included strain Namibia accession number CP007555.1 isolated from a goat in Namibia, strain Cb175 accession number HG825990.3 isolated from humans in French Guyana, strain RSA 493 close 7 IS1111A accession number M80806.1 and strain RSA493 accession number AE016828.2 both isolated from *Dermacentor andersoni* ticks in USA, strain WAV IS1111A accession number DQ882629.1 isolated from human in USA and strain Z3055 accession number LK937696.1 isolated from sheep in Germany amongst other strains. The sequences obtained were submitted to the GenBank and were allocated accession number KU994893 (Appendix 10).

The isolates were detected in ticks collected in Laikipia namely *Rh. appendiculatus* collected from a buffalo as well as *Rh. evertsi evertsi* and *Rh. pulchellus* both collected from a zebra. It was not possible to sequence a fourth PCR positive product because it yielded low concentration of DNA that was not enough for sequence analyses.

4.2.2.2 Phylogenetic analyses of *C. burnetii*

Only one haplotype was recovered from the three sequences. There was no heterogeneity between them since the gamma shape parameter was 2.00, a value > 1 thus indicating homozygosity. There was no polymorphism and nucleotide diversity Pi, Jukes-Cantor Pi and Theta totals were all 0. In addition, there were no substitutions as well as nucleotide divergence between the sequences of the three samples and those from other geographical locations and the divergence K and Jukes-Cantor totals were both 0. This is represented in Figures 14 and 15 while the sequences obtained are presented in Appendix 10.

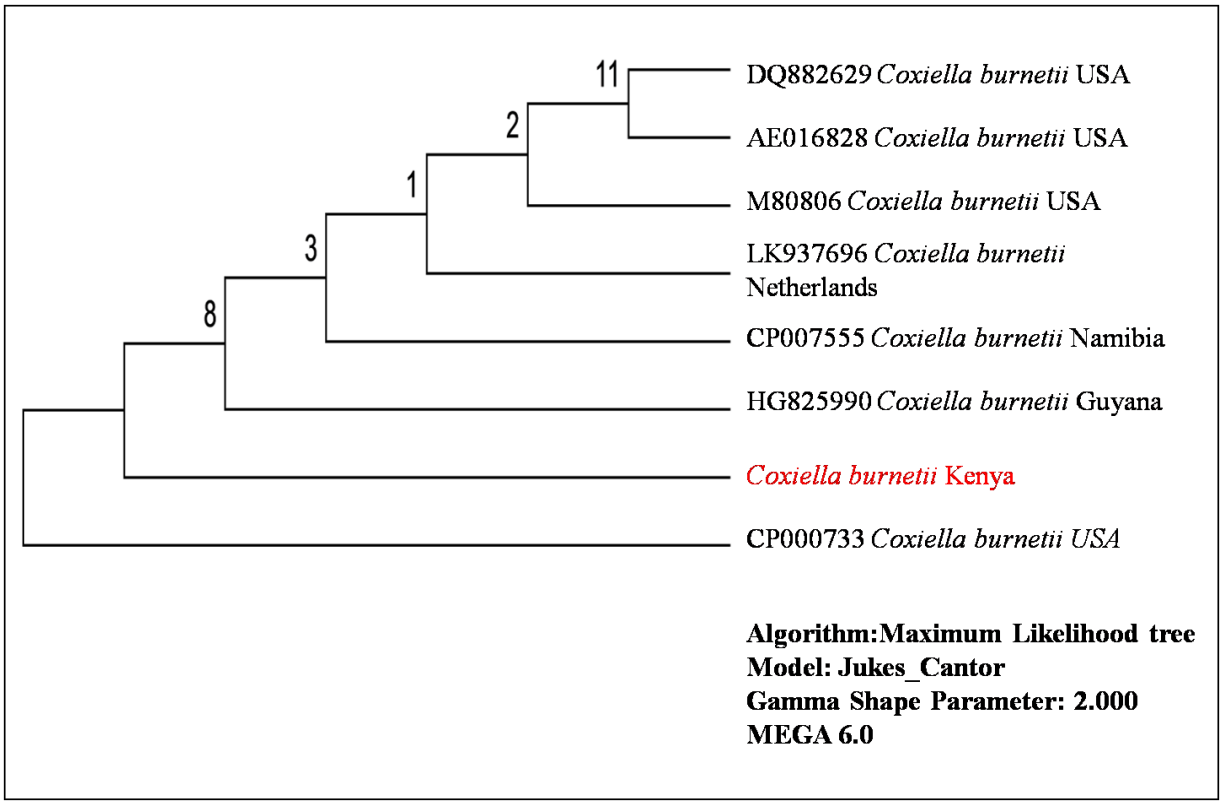

Figure 14: A phylogenetic tree derived from the target sequence IS*1111a* of C. *burnetii* isolated from ticks in Laikipia

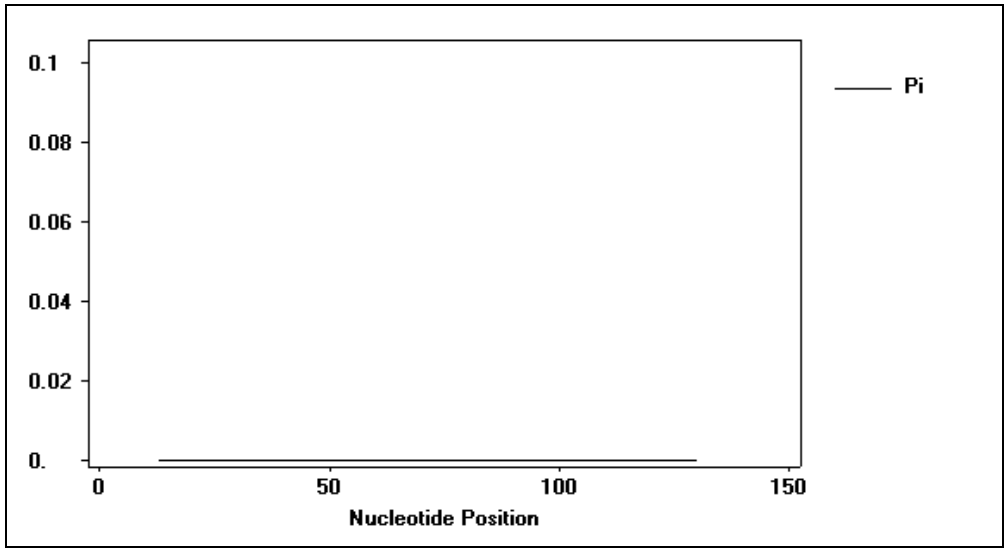

Figure 15: Graph showing 0 Pi of the *C. burnetii* isolates indicating no polymorphism

4.3 KNOWLEDGE, ATTITUDES AND PRACTICIES ON SFG RICKETTSIOSES AND Q FEVER

The semi-structured questionnaire was administered to a total of 101 respondents comprising of pastoralists (51), human health providers (17), wildlife sector personnel (28) and veterinarians (5) in different locations in both Laikipia (Figure 16) and Maasai Mara (Figure 17).

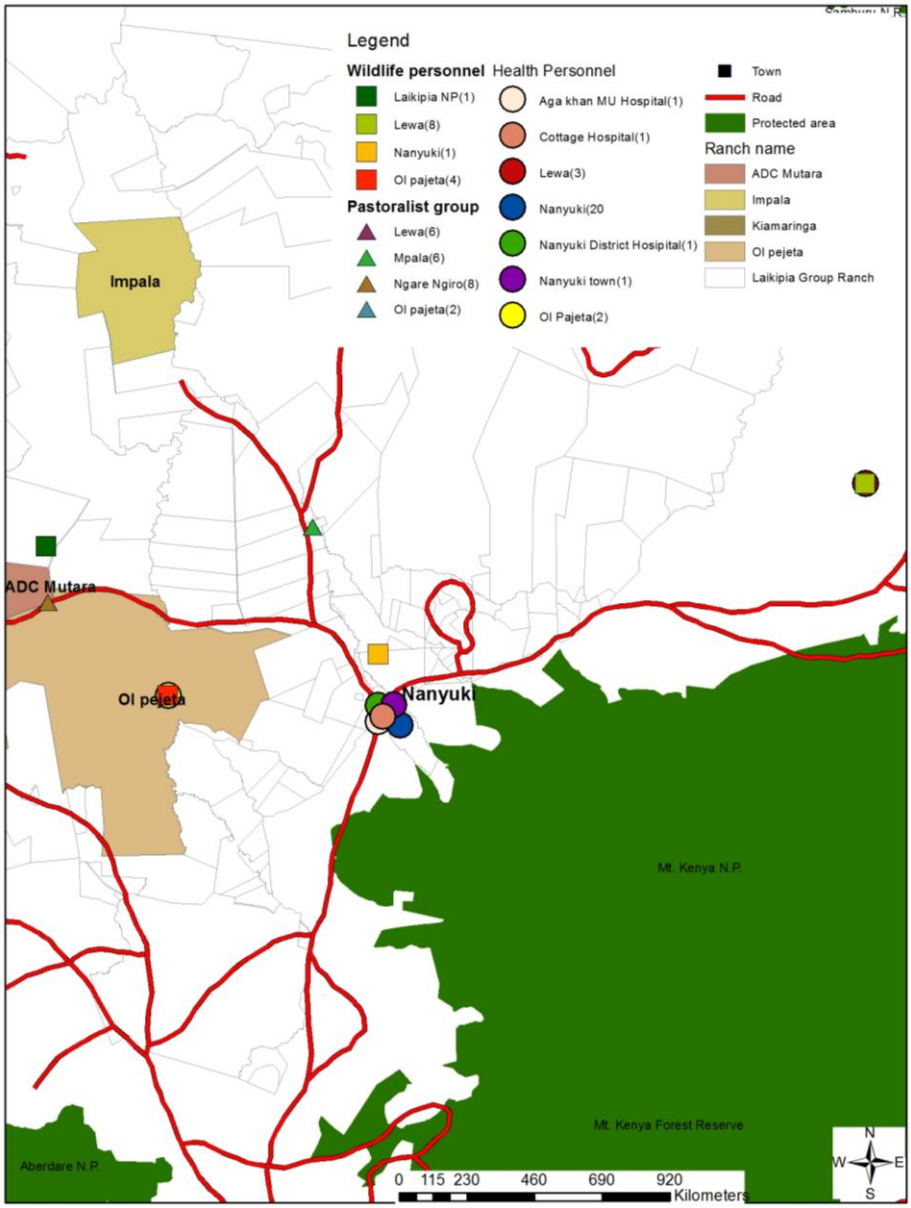

Figure 16: Distribution of respondents for the KAP questionnaire in Laikipia

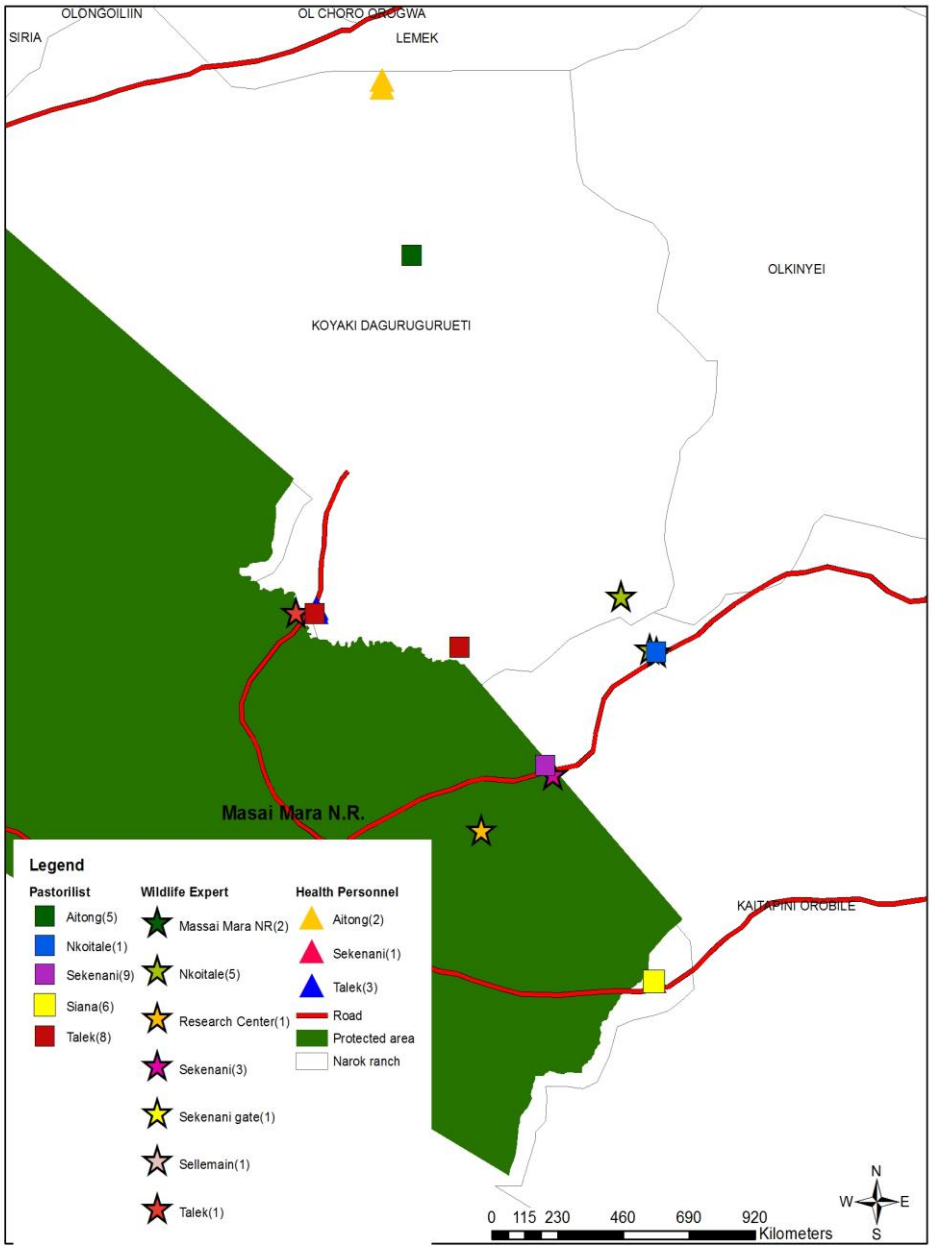

Figure 17: Distribution of respondents for the KAP questionnaire in Maasai Mara

4.3.1 Pastoralists

Of the 51 pastoralists interviewed, 22 (43.1%) were from Laikipia and 29 (56.9%) from the Maasai Mara. In Laikipia, the sites were Mpala sub-location (6/22), Ngare Ngiro sub-location (8/22), Ol Pejeta conservancy (2/22) and Lewa wildlife conservancy (6/22) while in Maasai Mara they were Aitong (5/29), Nkoilale (1/29), Sekenani (9/29), Siana (6/29) and Talek (8/29). Majority of the pastoralists interviewed in both study areas were household heads with 17/22

(77.3%) in Laikipia and 26/29 (89.7%) in Maasai Mara. The remainder of the respondents were spouses, children or employees of the household heads. The mean age of the respondents was 50 years in Laikipia and 40 years in Maasai Mara.

Figure 18: The study team interviewing some pastoralists in Maasai Mara

The main livestock kept by the respondents were cattle, sheep and goats. Cattle were kept by all households in both study areas. Seventeen out of 22 (77.3%) of the households in Laikipia kept goats and sheep. In Maasai Mara, goats were kept by all the households and sheep by 28/29 (96.6%) of the households. Other livestock kept included donkeys, camels, chicken and rabbits by much smaller proportions of the households.

All respondents in both study areas expressed that wildlife was present in their localities where it interacted with their livestock in grazing fields and watering points. Transmission of diseases and predation were listed as the main problems arising from this interaction by 21/22 (95.5%) and 27/29 (93.1%) of the respondents in Laikipia and Maasai Mara respectively. This was followed by competition for pastures and water at 18/22 (81.8%) in Laikipia and 20/29 (69.0%) in Maasai Mara. Other problems such as destruction of properties and injuries to humans and livestock were listed by less than 20% of the respondents.

All respondents in both study areas had knowledge about tick-borne diseases and gave examples that included anaplasmosis, East Coast Fever (ECF) and babesiosis. A significant proportion of

the respondents had knowledge that tick-borne diseases can infect humans. These were 13/22 (59.1%) in Laikipia and 18/29 (62.1%) in Maasai Mara. A smaller proportion of 6/22 (27.3%) in Laikipia and 8/29 (27.6%) in Maasai Mara said there are no tick-borne diseases that can infect humans while 3/22 (13.6%) in Laikipia and 3/29 (10.3%) in Maasai Mara said they did not know.

The respondents who were aware about tick-borne zoonotic diseases however listed diseases such as ECF, babesiosis and anaplasmosis which are not zoonotic except one in Laikipia who listed African tick-bite fever. They described the clinical signs in humans to include fever, vomiting, wounds, abortion, coughing, headache, itching, joint pains, swelling and watery eyes. This was interpreted to mean that despite not knowing the diseases by specific names, they were aware tick-borne diseases could infect humans. The respondents in both study areas did not undertake any deliberate efforts to minimise tick bites.

The respondents were asked to give examples of other zoonotic diseases not necessarily tick transmitted. At least half of the respondents, 11/22 (50%) in Laikipia and 16/29 (55.2%) in Maasai Mara, listed either of the following diseases: anthrax, brucellosis, helminthoses, leptospirosis, trypanosomiasis and diarrhoeal diseases. They listed the modes of transmission to include consumption of meat and untreated milk, contact with sick animals, handling materials from sick animals, inhalation, ecto-parasites (lice, fleas and ticks) and sharing sleeping quarters with animals. Sharing of sleeping quarters with sheep and goats was a common practice identified during the study particularly for young boys and respiratory problems attributed to allergy were said to be common by 8/22 (36.4%) and 4/29 (13.8%) of the respondents in Laikipia and Maasai Mara respectively. The respondents did not name any diseases that resembled SFG rickettsioses or Q fever in livestock and humans.

Certain practices by the pastoralist communities in Laikipia and Maasai Mara were identified as potential risk factors that can predispose them to SFG rickettsioses and Q fever. These included consumption of raw milk, attending to parturition and sharing living accommodations with animals.

4.3.2 Wildlife sector personnel

The respondents included wardens, managers of private conservancies, rangers and researchers. Of the 28 respondents, 15 (53.6%) were from Laikipia and 13 (46.4%) from Maasai Mara. The respondents in Laikipia were from the Kenya Wildlife Service (KWS) (2/15), Lewa wildlife conservancy (8/15) and Ol Pejeta conservancy (5/15). The respondents in Maasai Mara were from the Maasai Mara national reserve (10/13), KWS (1/13) and Naboisho community conservancy (2/13).

Thirteen out of the 15 (86.7%) and 12/13 (92.3%) of the respondents in Laikipia and Maasai Mara respectively said there is interaction between livestock and wildlife in their localities. Transmission of diseases was placed third in both areas after predation and competition for water and pastures as the most common problem arising from this interaction. The proportions were 9/15 (60%) in Laikipia and 5/13 (38.5%) in Maasai Mara. Predation and competition had proportions of 13/15 (86.7%) and 12/15 (80%) of the respondents in Laikipia, and equal proportions of 6/13 (46.2%) in Maasai Mara. Habitat destruction was given by a small proportions of the respondents in both areas.

Significant proportions, 10/15 (66.7%) in Laikipia and 8/13 (61.5%) in Maasai Mara, had knowledge that diseases can be transmitted at the livestock-wildlife interfaces. These diseases were listed as Foot and Mouth disease (FMD), anthrax, East Coast Fever (ECF), Malignant Catarrhal Fever (MCF), anaplasmosis and babesiosis.

Some respondents in both study areas had knowledge that tick-borne diseases can affect humans. These were 6/15 (40%) in Laikipia and 2/13 (15.4%) in Maasai Mara. Six out of 15 (40%) of the respondents in Laikipia said there are no human tick-borne diseases and a slightly higher proportion of 7/13 (53.9%) in Maasai Mara responded the same. Smaller proportions of 1/15 (6.7%) in Laikipia and 1/13 (7.7%) in Maasai Mara said they did not know if this is possible. When the respondents were asked to give examples of human tick-borne diseases, only 4/15 (26.7%) of the respondents in Laikipia listed African tick-bite fever. There were no respondents in Maasai Mara who could name an example of a human tick-borne disease.

The respondents with knowledge of human tick-borne diseases listed the clinical signs as fever, headache, nausea, pneumonia and itching and skin rash on tick-bite areas. One out 15 (6.7%) respondent in Laikipia listed orchitis as a clinical sign.

Eleven out of 15 (73.3%) and 6/13 (46.2%) in Laikipia and Maasai Mara respectively, said people with tick associated problems sought medical attention but they were not aware of the diagnoses made.

Some of the measures undertaken to prevent tick bites were listed as use of insect repellents, tucking trousers inside socks, avoiding foot patrols in areas with thick vegetation as well as burning or trimming of grass and bushes.

4.3.3 Human health providers

Eleven out of 17 (64.7%) health providers interviewed were from Laikipia and the remaining 6/17 (35.3%) were from Maasai Mara from 7 medical facilities in each study area that were either public (government), private, community or church funded.

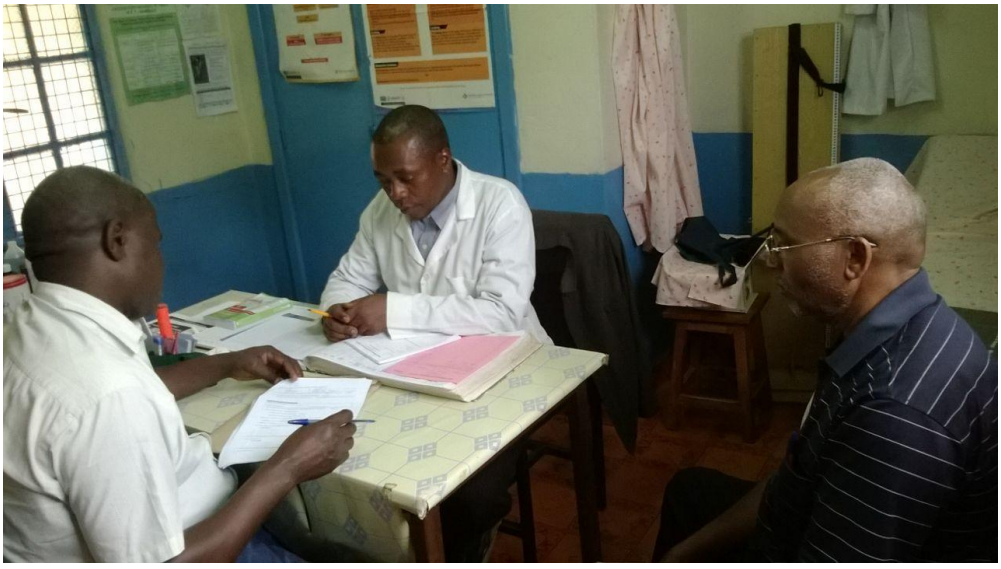

Figure 19: The study team interviewing a health provider in Laikipia

In Laikipia, one medical facility was government owned (Nanyuki district hospital), four were private (Aga Khan Hospital, Nanyuki cottage hospital, Lewa dispensary and Kamok dispensary) and two were church funded (Huruma Pope John Paul dispensary and Mary Immaculate

dispensary). In Maasai Mara, one was a government facility (Sekenani health centre), three were community owned (Aitong health centre, Koyoin community clinic and Talek community health centre) and the rest three were private (Manyatta medical clinic, Mara medical clinic, Naibor medical clinic).

The respondents consisted personnel who normally examine, diagnose and treat patients such as doctors, nurses, clinical officers or those who analyse samples such as laboratory technicians. In Talek community health centre in Maasai Mara however, a pharmacology technician was found to be examining, making diagnosis and treating patients and was subsequently interviewed. On average, the respondents had 3.7 and 2.7 years in the facilities they operated in Laikipia and Maasai Mara respectively.

Nine out of 11 (81.8%) and 5/6 (83.3%) of the respondents in Laikipia and Maasai Mara respectively indicated that they receive patients with zoonotic diseases. They gave examples as brucellosis, rabies, bovine tuberculosis, Rift Valley Fever, Echinococcosis (hydatid disease), trypanosomiasis, anthrax, helminthoses and sarcoptic mange (scabies).

Less than 50% of the respondents in both study areas had knowledge of human tick-borne diseases with a proportion of 5/11 (45.5%) in Laikipia and 2/6 (33.3%) in Maasai Mara expressing some good knowledge. These respondents listed African tick-bite fever as an example of a tick-borne zoonotic disease. The other disease listed was Lymes disease by 2/11 (18.2%) in Laikipia and no respondent in Maasai Mara. Four out of 11 (36.4%) human health providers in Laikipia and 4/6 (66.7%) in Maasai Mara expressed no knowledge on human tick-borne diseases while 2/11 (18.2%) in Laikipia and none in Maasai Mara said they did not know.

One out of 11 (9.1%) respondents in Laikipia expressed very good knowledge on Q fever while none expressed any knowledge on the disease in Maasai Mara. The respondent further indicated SFG rickettsioses and Q fever as illnesses diagnosed in foreign tourists in the medical facility. This was the only facility in both Laikipia and Maasai Mara that finds it necessary to confirm rickettsial infections and Q fever in patients with fever particularly foreign tourists.

4.3.4 Veterinary personnel

Four out of the five veterinary personnel interviewed were from Laikipia, two each from Ol Pejeta and Lewa wildlife conservancies and 1/5 was from Sekenani in Maasai Mara. On average, they had 3.6 and 2 years in their current stations in Laikipia and Maasai Mara respectively.

All the respondents had knowledge of tick-borne diseases in animals that included anaplasmosis, babesiosis, ECF and ehrlichiosis. They also had knowledge of potential zoonotic nature of tick-borne rickettsioses and Q fever amongst other diseases such as anthrax, bovine tuberculosis, rabies and brucellosis. None of the respondents had come across either disease in animals and humans and neither did they find it necessary to confirm these diseases in animals.

4.3 POTENTIAL RISK FACTORS TO SFG RICKETTSIOSES AND Q FEVER INFECTION IN HUMANS

The following were identified as potential risk factors that can predispose the local residents to SFG rickettsioses and Q fever in Laikipia and Maasai Mara:

(i) The sharing of habitats and other resources such as water between humans, livestock and wildlife which can potentially facilitate transmission of diseases across different species

(ii) Sharing of human living accommodations with livestock by most households

(iii) Consumption of raw milk which was reported common by most households

(iv) Own treatment of livestock by most pastoralists including attending to parturition due to inadequate veterinary presence in both Laikipia and Maasai Mara

(v) The presence of the diseases in some species of wildlife and ticks accompanied by low level of knowledge amongst most residents including health providers

(vi) The lack by most medical facilities to investigate the possibility of presence of the diseases in febrile patients even when the aetiology is not established.

CHAPTER FIVE: DISCUSSION

Both SFG rickettsioses and Q fever should be of public health concern in Laikipia and Maasai Mara which have unique human-livestock-wildlife interfaces that can potentially facilitate transmission of infectious pathogens across different species. With wildlife and arthropod vectors being important in disease transmission, it is important to understand the role of wildlife and ticks in the epidemiology of infectious pathogens including these zoonoses.

The two areas were selected for the study following recent reports of the diseases in both areas (Macaluso *et al*., 2003; Rutherford *et al*., 2004; Yoshikawa *et al*., 2005; Potasman *et al*., 2000; DePuy *et al*., 2014) and other parts of Kenya (Richards *et al*, 2010; Mutai *et al*., 2013; Knobel *et al*., 2013). However, the diseases remain unreported in wildlife and their ticks.

5.1 SPOTTED FEVER GROUP RICKETTSIOSES

Spotted fever group rickettsioses were detected in 2.5% and 5.5% of wildlife sampled in Laikipia and Maasai Mara respectively. This is the first report of the presence of the diseases in wildlife in Kenya which demonstrates that wildlife play a role in their epidemiology.

The finding of presence of SFG rickettsioses in wildlife is consistent with a study by Zhang *et al*. (1995) in China who reported a comparable prevalence of 7.4% in wild mice. Inokuma *et al*. (2008) and Ortuno *et al*. (2007) have also reported the presence of SFG rickettsioses in a deer in Japan and a wild boar in Spain respectively. In other studies by Boretti *et al*. (2009) and Barandika *et al*. (2007) however, 0% prevalence in wild foxes in Switzerland and wild small mammals in Spain respectively, was reported.

The finding of low prevalence in wildlife is also comparable to a study in domestic animals by Maina (2012) who reported a prevalence of 3.7% in dogs and 7.7% in cats in Western Kenya and no detection in cattle, sheep and goats. It is also comparable to a study by Kleinerman *et al*. (2013) who reported a prevalence of 2.0% in camels but no detection in horses in Israel.

The finding however contrasts several other studies which have reported higher prevalence in domestic animals. Mutai *et al*. (2013) reported a higher prevalence of 16.3% in cattle and 15.1%

in sheep but a lower prevalence of 7.1% in goats from various parts of Kenya. Likewise, Kamani *et al*. (2015) reported a higher prevalence of 18.8% in camels in Nigeria.

Despite the low detection of SFG rickettsial DNA in wildlife in this study, the pathogens were frequently detectable in different species of ticks at prevalence of 21.9% and 17.2% in Laikipia and Maasai Mara respectively. In Laikipia, they were detected in 22.2% of *Rhipicephalus* species and in Maasai Mara, they were detected in 100% of *Amblyomma*, 33.3% of *Hyalomma* and 8.3% of *Rhipicephalus* species. In Laikipia, the prevalence was highest in *Rh. evertsi* (36.4%) followed by *Rh. appendiculatus* (30.2%), *Rh. pulchellus* (18.2%) and *Rh. evertsi evertsi* (10.5%). The one tick sample each of *H. dromedari*, *A. variegatum* and *A. truncatum* tested positive in Maasai Mara as well as 20% of *Rh. appendiculatus* ticks. This is the first time these pathogens are being reported in ticks collected from wildlife in Kenya.

This finding is consistent with other studies which have reported the presence of the pathogens in various tick species but at different infection rates. Mutai *et al*. (2013) reported a comparable prevalence of 23.3% in ticks from various locations in Kenya with high detection in *Amblyomma* species (62.3%), *Rhipicephalus* species (45.5%), *Hyalomma* species (35.9%) and *Boophilus* species (34.9%).

In another study by Macaluso *et al*. (2003), prevalence of 15.8% in *A. variegatum* and 1% in *Rhipicephalus* species in private and public land surrounding the Maasai Mara national reserve was reported. Boretti *et al*. (2009) reported a prevalence of 36% in ticks collected from wild foxes, humans and domestic dogs in Switzerland and a prevalence of 12% in unfed ticks collected from vegetation. However, it contrasts studies by Znazen *et al*. (2013) who reported a low prevalence of 2.4% in ticks collected in Tunisia and Frankie *et al*. (2010) who reported prevalence of 2.1% and 1.8% in ticks collected from wild birds and small mammals respectively in Germany.

Almost comparable high infection rates in this study have been reported by Mediannikov *et al*. (2012) in *Amblyomma* and *Rhipicephalus* species of ticks. They reported 93-100% prevalence in *A. variegatum,* 14-93% prevalence in *Rhipicephalus* (*B.*) *geigyi*, *Rh.* (*B.*) *annulatus* and *Rh.* (*B.*) *decoloratus* but slightly lower prevalence of 16% in *Rh. senegalensis* ticks from domestic and wild animals in Guinea and Liberia.

Spotted fever group rickettsiae were detected in 100% of *H. dromedari*, *A. variegatum* and *A. truncatum* species of ticks collected in Maasai Mara. The finding of high prevalence in *Hyalomma* and *Amblyomma* ticks contrasts a study by Mutai *et al*. (2013) who reported an infection rate of 35.9% in *Hyalomma* species of ticks collected from livestock in Kenya. Kamani *et al*. (2015) also reported *Rickettsia aeschlimannii* at lower prevalence of 23.8%-64.3% depending on the gene used for PCR amplification in *Hyalomma* ticks from camels in Nigeria as well as Abdel-Shafy *et al*. (2012) who reported prevalence of 26.7-73.3% in different *Hyalomma* species ticks from camels in Egypt. An even lower prevalence of 0.2-2.3% depending on *Rickettsia* species has been reported in Tunisia by Kleinerman *et al*. (2013) in *Hyalomma* species from camels and horses.

However, the finding of high infection rate in *Amblyomma* ticks is consistent with previous reports. Socolovschi *et al*. (2009) has described *Amblyomma* ticks as the main vectors for *R. africae* with infection rates of up to 100% in *A. variegatum*. Maina (2012) also reported a high infection rate of 96.9% in *A. variegatum* ticks collected from cattle in Western Kenya but a lower prevalence of 20.34% in the same tick species collected from dogs in the same area. High infection rates of 93-100% in *A. variegatum* ticks collected from domestic and wild animals in Guinea and Liberia have also been reported by Mediannikov *et al*. (2012). Nakao *et al*. (2013) also reported a high prevalence of 97.1% in *A. variegatum* ticks in Uganda. Thus, the finding of high infection of these species was not unexpected. Nevertheless, a study by Mutai *et al*. (2013) reported a lower prevalence of 62.3% in *Amblyomma* species from livestock in Kenya while Macaluso *et al*. (2003) reported an even lower prevalence of 15.5% in the same species of ticks collected from livestock and vegetation in private and public land around the Maasai Mara.

The detection of *R. sibirica* and *R. sibirica* subspecies *mongolotimonae* has not been reported before in Kenya. *Rickettsia sibirica* is widely distributed in North Asia (Jensenius *et al*., 2004) with no reports available about its detection in Africa. The geographical distribution of *R. sibirica* subspecies *mongolotimonae* includes sub-Sahara Africa as well as Mongolia and parts of Asia and Europe (Jensenius *et al*., 2004; Psaroulaki *et al*., 2005; Fournier *et al*., 2005; Kleinerman *et al*., 2013).

The main vectors of *R. sibirica* are *Dermacentor* and *Haemaphysalis* species of ticks (Jensenius *et al*., 2004) but it has also been reported in *Rhipicephalus pusillus* and *Rh. bursa* (Toledo *et al*.,

2009) and *Amblyomma cooperi* (Labruna *et al*., 2004). The detection of the pathogen in *Rh. evertsi evertsi, Rh. appendiculatus, A. variegatum* and *A. truncatum* in this study has not been reported before making these species potential vectors of *R. sibirica.*

The main vectors of *R. sibirica* subspecies *mongolotimonae* are various *Hyalomma* species of ticks (Jensenius *et al*., 2004; Fournier *et al*., 2005; Psaroulaki *et al*., 2005; Kleinerman *et al*., 2013). The pathogen has also been detected in *Rh. pusillus* (Sousa *et al*., 2006; Edouard *et al*., 2013) but the detection in *Rh. evertsi* and *Rh. pulchellus* as well as *A. variegatum* and *A. truncatum* has not been reported previously making these species potential vectors of this pathogen.

Rickettsia sibirica is the causative agent of North Asian tick typhus also called Siberian tick typhus (Jensenius *et al*., 2004) and *R. sibirica* subspecies *mongolotimonae* causes a lymphangitis associated rickettsiosis (Fournier *et al*., 2005). These illnesses are characterised by fever, malaise, headache, myalgias and regional lymphadenopathy (Jensenius *et al*., 2004; Fournier *et al*., 2005; Sousa *et al*., 2008; Ramos *et al*., 2013) which may be confused with those of other febrile infections leading to misdiagnosis. In addition, *R. sibirica* subspecies *mongolotimonae* may manifest with lymphangitis (Fournier *et al*., 2005; Jensenius *et al*., 2004; Sousa *et al*., 2008; Ramos *et al*., 2013). It is therefore of interest to understand how local populations in Laikipia and Maasai Mara cope with infections by *R. sibirica* and *R. sibirica* subspecies *mongolotimonae*.

The detection of an uncultured *Rickettsia* species in this study continues to add to the growing list of unknown SFG rickettsiae identified in recent years. It is important to do further molecular characterisation of this isolate to establish its identity and potential to cause infection in humans and animals.

Despite few wild animals testing positive for SFG rickettsioses in Laikipia and Maasai Mara, the finding demonstrates that wildlife plays a potential role in the epidemiology of the diseases. On the other hand, the broad range of tick species that tested positive and the relatively high prevalence of SFG rickettsiae in some species demonstrate that ticks play a potential role in the epidemiology of the diseases. These findings underscore the risks for zoonotic transmission of SFG rickettsioses to humans and domestic animals at the wildlife-livestock interfaces in Laikipia and Maasai Mara.

5.2 Q FEVER

Coxiella burnetii was not detected in blood collected from wild animals in Laikipia and Maasai Mara. This was the first study to investigate the epidemiology of the disease in wildlife in Kenya. The finding contrasts several studies in domestic animals in Kenya which have reported varied sero-prevalence of Q fever. DePuy *et al*. (2014) reported sero-prevalence of up to 3-4% in cattle, 13-20% in sheep, 31-40% in goats and 5-46% in camels across five ranches in Laikipia County. In western Kenya, Knobel *et al*. (2013) reported sero-prevalence of 28.3% in cattle, 32.0% in goats and 18.2% in sheep. .

In wildlife, several reports outside Kenya exist on sero-epidemiology of Q fever in different species that include various mammals, birds, reptiles and fish (Binninger *et al*., 1980; Zarnke 1983; Marrie *et al*., 1986; Sawyer *et al*., 1987; Marrie *et al*., 1993; Serbezov *et al*., 1999; Gardon *et al*., 2001; McQuiston *et al*., 2002; Barandika *et al*. 2007; Hernandez *et al*., 2007; Dorko *et al*., 2009; Kersh *et al*., 2012). Unlike these studies however, the current study used highly sensitive and specific molecular methods for the detection of the disease.

Molecular methods for the detection of Q fever in animals have been used successfully before. Barandika *et al*. (2007) for instance reported the prevalence of *C. burnetii* in wild and domestic small mammals in Spain at a low prevalence of 0.8%. Additionally, Kirkan *et al*. (2008) reported detection of *C. burnetii* at a higher prevalence of 4.3% in cattle in Turkey. Locally, Knobel *et al*. (2013) used PCR to detect the disease in small ruminants in Western Kenya following parturition and reported a prevalence of 50%.

Despite no detection in wildlife, *C. burnetii* was detected at an infection rate of 2.9% in ticks collected in Laikipia but no detection in ticks collected in Maasai Mara. In regard to individual tick species, the detection was in 3.0% of *Rhipicephalus* ticks with *Rh. appendiculatus* having the highest infection rate of 3.8% followed by *Rh. pulchellus* at 3.0% and *Rh. evertsi evertsi* at 2.6%. This is the first report of *C. burnetii* naturally infecting ticks collected from wildlife in Kenya.

The detection of *C. burnetii* in ticks is consistent with a study locally by Knobel *et al*. (2013) who documented infection of ticks collected from domestic animals in Western Kenya at a

relatively similar infection rate of 2.5% in *A. variegatum* ticks collected from cattle. In the same study, ticks collected from domestic dogs had a higher prevalence of 20% in *Rh. Sanguineus, Rh. (Boophilus) decoloratus, an unspeciated Rhipicephalus* and *A. variegatum* while *Rh. appendiculatus* and *Haemaphysalis leachi* had infection rates of 11.1% and 50% respectively.

The detection of *C. burnetii* in wildlife ticks demonstrates the likelihood of its transmission to domestic animals and humans. Since infected ticks are the most important reservoirs of *C. burnetii* and responsible for maintaining the disease in the environment (Loukaides *et al*., 2006; Marrie, 2009), this finding is important in better understanding the epidemiology of the disease in Laikipia. Ticks are responsible for transmission of the disease from wild to domestic animals (Masala *et al*., 2004; Porter *et al*., 2011) and sometimes to humans (McQuiston *et al*., 2002; Medeannikov *et al*., 2012).

5.3 KNOWLEDGE, ATTITUDES AND PRACTICES ON SFG RICKETTSIOSES AND Q FEVER

The pastoralists interviewed expressed knowledge of tick-borne diseases infecting livestock and a significant proportion (59.1% in Laikipia and 62.1% in Maasai Mara) was aware some of these diseases can infect humans. However, they expressed low knowledge of SFG rickettsioses. Despite >50% being aware of non tick-borne zoonotic diseases including methods of transmission, they did not describe any disease that resembled Q fever. Disease transmission at the wildlife-livestock interface was identified as the major problem encountered by the pastoralists.

Some of the wildlife sector personnel (40% in Laikipia and 15.5% in Maasai Mara) expressed knowledge that tick-borne diseases can infect humans with a few (26.7%) expressing knowledge about African tick-bite fever, a SFG rickettsial disease caused by *Rickettsia africae* (Todar, 2012). None of these respondents expressed knowledge about Q fever.

Less than 50% of the health personnel expressed knowledge that tick-borne diseases can also infect humans. In addition, very few expressed knowledge about Q fever. Likewise, very few medical facilities investigate the possibility of presence of these diseases in patients presenting with fever where the aetiology is not established. The veterinary personnel expressed good

knowledge about SFG rickettsioses and Q fever but they did not find it necessary to undertake laboratory confirmation of the diseases in animals.

This is the first study to investigate the level of awareness about SFG rickettsioses in both Laikipia and Maasai Mara and Q fever in Maasai Mara. A study by DePuy *et al*. (2014) in Laikipia reported similar findings about Q fever. They reported that the local pastoralists had no knowledge about Q fever but most of the other respondents that included conservation professionals, human healthcare providers, veterinary practitioners and rangeland management experts expressed both awareness and concern about Q fever. They attributed the pastoralists' lack of familiarity with this potential pathogen to the absence of a specific word for ''Q fever'' in local dialects. The same could be said for the findings in this study for despite demonstrating good familiarity with and concern about livestock and zoonotic diseases such as brucellosis, trypanosomiasis, helminthoses, anthrax, diarrhoeal diseases, leptospirosis, FMD, MCF, ECF, anaplasmosis and babesiosis amongst others, the diseases described in their local dialect did not resemble either SFG rickettsioses or Q fever.

Generally within the East African region, there seems to be a low level of knowledge towards many zoonotic diseases amongst communities and medical practitioners (Kunda *et al*., 2008; Omena *et al*., 2012; Chipwaza *et al*., 2014) which is consistent with the findings of this study raising concerns about the potential risks of zoonoses amongst local populations.

5.4 POTENTIAL RISK FACTORS FOR SFG RICKETTSIOSES AND Q FEVER INFECTION

The low level of knowledge on SFG rickettsioses and Q fever amongst most respondents in Laikipia and Maasai Mara and the detection of the diseases in some species of wildlife and ticks known to feed on humans raise concerns about the potential risks posed by the diseases in local residents. These findings also suggest that the diseases could be circulating unnoticed in the two areas especially because most medical facilities do not investigate the possibility of presence of the diseases in febrile patients even when the aetiology is not established. Thus, the diseases could be amongst the 'fevers of unknown origin' recorded in most medical facilities.

The study further identified certain practices which could also predispose the local residents to zoonotic transmission of the diseases. These included consumption of raw milk and treatment of own livestock including attending to parturition which can predispose humans to Q fever should the animals be infected (Marrie, 2009). Sharing living accommodations with livestock was also identified to be very common in most households particularly for young boys. This can promote transmission of Q fever through inhalation of dust contaminated with fluids and secretions from infected animals as well as direct contact with these materials (Jones *et al*., 2006; Marrie, 2009). Further, such close contact with the animals can expose the owners to tick bites, the main mode of transmission of SFG rickettsioses (Parola *et al*., 2005) and sometimes Q fever (McQuiston *et al*., 2002; Medeannikov *et al*., 2010).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

(i) The detection of SFG rickettsioses in some wildlife species suggests that wild animals are important in their epidemiology.

(ii) The detection of SFG rickettsiae and *C. burnetii* in a broad range of tick species suggests that ticks from wildlife areas are important in the epidemiology of the pathogens and demonstrates the likelihood of their transmission to tick exposed humans and domestic animals.

(iii) The study documented two pathogenic SFG rickettsiae previously unreported in Kenya, *R. sibirica* and *R. sibirica* subspecies *mongolotimonae*, suggesting these pathogens may be causes of 'fevers of unknown origin' in Laikipia and Maasai Mara.

(iv) The low level of knowledge on SFG rickettsioses and Q fever amongst most respondents raises concerns about the potential risks of these zoonoses amongst local populations.

6.1 RECOMMENDATIONS

(i) Initiate serological and molecular studies of SFG rickettsioses and Q fever in local residents in Laikipia and Maasai Mara to understand their potential role in causing febrile illnesses

(ii) Undertake full genome sequencing of the uncultured *Rickettsia* to establish its identity and pathogenicity

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

(iv) Institute immediate and long-term surveillance and dissemination of findings about SFG rickettsioses and Q fever

(v) Undertake sensitisation efforts related to SFG rickettsioses and Q fever awareness and prevention taking cognizance of the cultural practices of the main population composition in Laikipia and Maasai Mara. This will require multi-disciplinary and cross-cultural approaches in order to be fully effective.

REFERENCES

Abdel-Shafy S., Allam N.A.T., Mediannikov O., Parola P. and **Raoult D. (2012):** Molecular detection of spotted fever group rickettsiae associated with ixodid ticks in Egypt. Vector-Borne and Zoonotic Diseases **12** (5): 346-359

Althaus F., Greub G., Raoult D and **Genton B. (2010):** African tick-bite fever: A new entity in the differential diagnosis of multiple eschars in travellers: Description of five cases imported from South Africa to Switzerland. International Journal of Infectious Diseases **14**: 274-276

Altschul S., Glish W., Miller W., Myers E. and **Lipman D. (1990):** Basic local alignment search tool. Journal of Molecular Biology **215**: 403-410

Angelakis E. and **Raoult D. (2010):** Q fever. Veterinary Microbiology **140** (3-4): 297-309

Ari M.D., Guracha A., Fadeel M.A., Njuguna C., Njenga M.K., Kalani R., Abdi H., Warfu O., Omballa V., Tetteh C., Breiman R.F., Pimentel G. and **Feikin D.R. (2011):** Challenges of establishing the correct diagnosis of outbreaks of acute febrile illnesses in Africa: The case of a likely *Brucella* outbreak among nomadic pastoralists, northeast Kenya, March-July 2005. American Journal of Tropical Medicine and Hygiene **85** (5): 909-912

Arricau-Bouvery N. and **Rodolakis A. (2005):** Is Q fever an emerging or re-emerging zoonosis? Veterinary Research **36**: 327-349

Azad A.F. and **Beard C.B. (1998):** Rickettsial pathogens and their arthropod vectors. Emerging Infectious Diseases **4** (2): 179-186

Azad A.F. and **Radulovic S. (2003):** Pathogenic rickettsiae as bioterrorism agents. Annals of New York Academy of Sciences **990**: 734-738

Balashov Y.S. (1972): Blood sucking ticks (Ixodidae) - vectors of diseases of man and animals. Miscellaneous Publications of the Entomological Society of America **8**: 163-376

Barandika J.F., Hurtado A., Garcia-Esteban C., Gil H., Escudero R., Barral M., Jado I., Juste R.A., Anda P. and **Garcia-Perez A.L. (2007):** Tick-borne zoonotic bacteria in wild and domestic small mammals in northern Spain. Applied and Environmental Microbiology **73** (19): 6166-6171

Binninger C.E., Beecham J.J., Thomas L.A. and **Winward L.D. (1980):** A serological survey for selected infectious diseases of black bears in Idaho. Journal of Wildlife Diseases **16**: 423-430

Blair P.J., Jiang J., Schoeler G.B., Moron C., Anaya E., Cespedes M. and **Olson, J.G. (2004):** Characterisation of spotted fever group rickettsiae in flea and tick specimens from northern Peru. Journal of Clinical Microbiology **42** (11): 4961-4967

Boretti F.S., Perreten A., Meli M.L., Cattori V., Willi B., Wengi N., Hornok S., Honegger H., Hegglin D., Woelfel R., Reusch C.E., Lutz H. and **Hofmann-Lehmann R. (2009):** Molecular investigations of *Rickettsia helvetica* infection in dogs, foxes, humans and *Ixodes* ticks. Applied and Environmental Microbiology **75** (10): 3230-3237

Bossi P., Tegnell A., Baka A., van Loock F., Hendriks J., Werner A., Maidhof H. and **Gouvras G. (2004):** Bichat guidelines for the clinical management of Q fever and bioterrorism-related Q fever. Eurosurveillance **9**: 1-3

Brah S., Daou M., Salissou L.,Mahaman S.A., Alhousseini D., Amelie I.B., Moussa S., Malam-Abdou B., Adamou H. and **Adehossi E. (2015):** Fever of unknown origin in Africa: the causes are often determined!. Health Science and Diseases **16** (2):1-8. Available at www.hsd-fmsb.org

Chipwaza B., Mugasa J.P., Mayumana I., Amuri M., Makungu C. and **Gwakisa P.S. (2014):** Community knowledge and attitudes and health workers' practices regarding non-malaria febrile illnesses in eastern Tanzania. PLoS Neglected Tropical Diseases **8** (5): e2896. doi:10.1371/journal.pntd.0002896

Cleaveland S., Laurenson M.K. and **Taylor L.H. (2001):** Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. Philosophical Transaction of the Royal Society B **356**: 991-999

Cowan G.O. (2003): Rickettsial infections. In: Cook G.C. and Zumla A.I. (Editors). Manson's Tropical Diseases. 21st Edition. Edinburgh, Saunders: 891-906

Craddock A.L. and **Gear J. (1955):** Q fever in Nakuru, Kenya. The Lancet: 1167-1169

Dantas-Torres F (2007): Rocky Mountain spotted fever. The Lancet Infectious Diseases **7**: 724-732

DePuy W., Benka V., Massey A., Deem S.L., Kinnaird M., O'Brien T., Wanyoike S., Njoka J., Butt B., Foufopoulos J., Eisenberg J.N.S. and **Hardin R. (2014):** Short communication: Q fever risk across a dynamic, heterogeneous landscape in Laikipia County, Kenya. EcoHealth: Published online on 7th March 2014 by the International Association for Ecology and Health

Dorko E., Rimarova K., Pilipcinec E. and **Travnicek M. (2009):** Prevalence of *Coxiella burnetii* antibodies in wild ruminants in Kavecany zoo, Kosice, eastern Slovakia. Annals of Agriculture and Environmental Medicine **16**: 321-324

Edgar R.C. (2004): MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research **32** (5): 1792-1797

Edouard S., Parola P., Socolovschi C., Davoust B., Scola B.L. and **Raoult D (2013):** Clustered cases of *Rickettsia sibirica mongolotimonae* infection, France. Emerging Infectious Diseases **19** (2): 337-338. DOI: http://dx.doi.org/eid1902.120863

Eldin C., Mediannikov O., Davoust B., Cabre O., Barre N., Raoult D. and **Parola P. (2011):** Emergence of *Rickettsia africae*, Oceania. Emerging Infectious Diseases **17** (1): 100-102

Eremeeva M.E. and **Dasch G.A. (2011):** Infectious diseases related to travel: Rickettsial (spotted and typhus fevers) and related infections (Anaplasmosis and Ehrlichiosis). CDC Health Information for International Travel (Yellow Book). Centers for Disease Control and Prevention, Atlanta, USA. www.cdc.gov/ticks

Eremeeva M.E., Bosserman E.A., Demma L.J., Zambrano M.L., Blau D.M., and **Dasch G.A. (2006):** Isolation and identification of *Rickettsia massiliae* from *Rhipicephalus*

sanguineus ticks collected in Arizona. Applied and Environmental Microbiology **72**(8): 5569–5577

Fournier P.E. and **Raoult D. (2007):** Identification of rickettsial isolates at the species level using multi-spacer typing. BMC Microbiology **7**:72-78

Fournier P.E., Dumler J.S., Greub G., Zhang J., Wu Y. and **Raoult D. (2003):** Gene sequence-based criteria for identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. Journal of Clinical Microbiology **41** (12): 5456-5465

Fournier P.E., Fujita H., Takada N. and **Raoult D. (2002):** Genetic identification of rickettsiae isolated from ticks in Japan. Journal of Clinical Microbiology **40** (6): 2176-2181

Fournier P.E., Gouriet F., Brouqui P., Lucht F. and **Raoult D. (2005):** Lymphangitis-associated rickettsiosis- A new rickettsiosis caused by *Rickettsia sibirica mongolotimonae*: Seven new cases and review of the literature. Clinical Infectious Diseases **40***:* 1435-1444.

Fournier P.E., Zhu Y., Ogata H. and **Raoult D. (2004):** Use of highly variable intergenic spacer sequences for multispacer typing of *Rickettsia conorii* strains. Journal of Clinical Microbiology **42** (12): 5757-5766

Frankie J., Fritzsch J. and **Tomaso H. (2010):** Co-existence of pathogens in host-seeking and feeding ticks within a single natural habitat in central Germany. Applied and Environmental Microbiology **76** (20): 6829-6836

Frean J., Blumberg L. and **Ogunbanjo G.A. (2008):** Tick-bite fever in South Africa. South Africa Family Practice **50** (2): 33-35

Freedman D.O., Weld L.H., Kozarsky P.E., Fisk T., Robins R., Sonnenburg F.V., Keystone J.S., Pandey P. and **Cetron M.S. (2006):** Spectrum of disease and relation to place of exposure among ill returned travellers. New England Journal of Medicine **354** (2): 119-130

Gardon J., Heraud J.M., Laventure S., Ladam A., Capot P., Fouquet E., Favre J., Weber S., Hommel D., Hulin A., Couratte Y. and **Talarmin A. (2001):** Suburban transmission of Q fever in French Guyana: Evidence of a wild reservoir. Journal of Infectious Diseases **184**: 278-284

Grootenhuis J.G. and **Olubayo R.O. (1993):** Disease research in the wildlife-livestock interface in Kenya. Veterinary Quarterly **15** (2):55-59

Hechemy K., Oteo J.A., Raoult D., Silverman D.J. and **Blanco J.R. (2006):** A century of rickettsiology: Emerging, re-emerging rickettsioses, clinical epidemiologic and molecular diagnostic aspects and emerging veterinary rickettsioses. Annals of New York Academy of Sciences **1078**: 1-14

Hernandez S., Lyford-Pike V., Alvarez M.E. and **Tomasina F. (2007):** Q fever outbreak in an experimental wildlife breeding station in Uruguay. Revista de Patologia Tropical **36**: 129-140

Inokuma H., Seino N., Suzuki M., Kaji K., Takahashi H., Igota H. and **Inoue S. (2008):** Detection of *Rickettsia helvetica* DNA from peripheral blood of Sika deer (*Cervus nippon yesoensis*) in Japan. Journal of Wildlife Diseases **44** (1): 164–167

Jado I., Escudero R., Gil H., Jimenez-Alonso M.I., Sousa R., Garcia-Perez A.L., Rodriguez-Vargas M., Lobo B. and **Anda P. (2006):** Molecular method for identification of *Rickettsia* species in clinical and environmental samples. Journal of Clinical Microbiology **44** (12): 4572-4576

Jensenius M., Fournier P.E. and **Raoult D. (2004):** Tick-borne rickettsioses in international travellers. International Journal of Infectious Diseases **8** (3): 139-146. Doi:10.1016/j.ijid.200

Jensenius M., Fournier P.E., Kelly P., Myrvang B. and **Raoult D. (2003):** African tick-bite fever. Lancet Infectious Diseases **3**:557–64.

Jensenius M., Parola P. and **Raoult D. (2006):** Threats to international travellers posed by tick-borne diseases. Travel Medicine and Infectious Diseases **4**: 4-13

Jiang J., Blair P.J., Felices V., Moron C., Cespedes M., Anaya E. and **Richards A.L. (2005):** Phylogenetic analysis of a novel molecular isolate of spotted fever group rickettsiae from northern Peru: *Candidatus Rickettsia andeanae*. Annals of New York Academy of Sciences **1063 (1)**: 337-342

Jones K.E., Patel N.G., Levy M.A., Storeygard A., Balk D., Gittleman J.L. and **Daszak P. (2008):** Global trends in emerging infectious diseases. Nature **451**: 990-993

Jones R.M., Nicas M., Hubbard A.E. and **Reingold A.L. (2006):** The infectious dose of *Coxiella burnetii* (Q fever). Applied Biosafety: **11** (1): 32-41

Jukes T.H. and **Charles R.C. (1969):** Evolution of protein molecules. Mammalian Protein Metabolism **3**: 21-132

Kamani J., Baneth G., Apanaskevich D.A., Mumcuoglu K.Y. and **Harrus S. (2015):** Molecular detection of *Rickettsia aeschlimannii* in *Hyalomma* Spp. ticks from camels (*Camelus dromedarius*) in Nigeria, West Africa. Medical and Veterinary Entomology **29**: 205-209

Kaplowitz L.G., Lange J.V., Fischer J.J. and **Walker D.H. (1983):** Correlation of rickettsial titres, circulating endotoxin and clinical features in Rocky Mountain spotted fever. Archives of Internal Medicine **143:** 1149-1151

Kaufman S.E., Kaufman W.R. and **Phillips J.E. (1982):** Mechanism and characteristics of coxal fluid excretion in the Argasid tick *Ornithodorus moubata*. Journal of Experimental Biology **98**: 343-352

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., Mentjies P. and **Drummond A. (2012):** Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28** (12): 1647-1649

Kelly P., Matthewman L., Beati L., Raoult D., Mason P., Dreary M. and **Makombe R. (1992):** African tick-bite fever: A new spotted fever group rickettsiosis under an old name. Lancet **340**: 982-983.

Kelly P.J., Beati L., Mason P.R., Matthewman L.A. and **Roux D. (1996):** *Rickettsia africae* sp. nov., The aetiological agent of African tick-bite fever. International Journal of Systematic Bacteriology **46** (2): 611-614

Kersh G.J., Lambourn D.M., Raverty S.A., Fitzpatrick K.A., Self J.S., Akmajian A.M., Jeffries S.J., Huggins J., Drew C.P., Zaki S.R. and **Massung R.F. (2012):** *Coxiella burnetii* infection of marine mammals in the pacific northwest, 1997-2010. Journal of Wildlife Diseases 48 (1): 201-206

Kidd L., Maggi R., Diniz P.P., Hegarty B., Tucker M. and **Breitschwerdt E. (2008):** Evaluation of conventional and real-time PCR assays for detection and differentiation of spotted fever group Rickettsia in dog blood. Veterinary Microbiology Journal **129:**294-303

Kirkan F., Kaya O., Tekbiyik S. and **Parin U. (2008):** Detection of *Coxiella burnetii* in cattle by PCR. Turkish Journal of Veterinary and Animal Sciences **32** (3): 215-220

Kleinerman G., Baneth G., Mumcuoglu K.Y., van Straten M., Berlin D., Apanaskevich D.A., Abdeen Z., Nasereddin A. and **Harrus S. (2013):** Molecular detection of *Rickettsia africae*, *Rickettsia aeschlimannii*, and *Rickettsia sibirica mongolitimonae* in camels and *Hyalomma* spp. ticks from Israel. Vector-Borne Zoonotic Diseases **13** (12): 851-856

Knobel D.L., Maina A.N., Cutler S.J., Ogola E., Feikin D.R., Junghae M., Halliday J.E., Richards A.L., Breiman R.F., Cleaveland S. and **Njenga M.K. (2013):** *Coxiella burnetii* in humans, domestic ruminants and ticks in rural western Kenya. The American Journal of Tropical Medicine and Hygiene **88**: 513-518

Krauss H., Weber A., Appel M., Enders B., Isenberg H.D., Schiefer H.G., Slenczka W., von Graevenitz A. and **Zahner H. (2003):** Zoonoses: Infectious diseases transmissible from animals to humans. 3rd Edition. American Society for Microbiology (ASM) Press, Washington DC, USA: 221-234

Kunda J, Kazwala R and **Mfinanga GS (2008):** Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. BMC Infectious Diseases: **8**:162. doi:10.1186/1471-2334-8-162

Labruna M.B., Whitworth T., Horta M.C., Bouyer D.H., McBride J.W., Pinter A., Popov V., Gennari S.M. and **Walker D.H. (2004):** *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. Journal of Clinical Microbiology **42** (1): 90-98. Doi:10.1128/jcm.42.1.90-98.2004

Larkin M. A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. and **Higgins D.G. (2007):** Clustal W and Clustal X version 2.0. Bioinformatics **23** (21): 2947-2948

Lee N., Ip M., Wong B., Lui G., Tsang O.T.Y., Lai J.Y., Choi K.W., Lam R., Ng T.K., Ho J., Chan Y.Y., Cockram C.S. and **Lai S.T. (2008):** Risk factors associated with life-threatening rickettsial infections. American Journal of Tropical Medicine and Hygiene **78** (6): 973-978

Librado P. and **Julio R. (2009):** DNASP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25** (11): 1451-1452

Loukaides F., Hadjichristodoulou C., Soteriades E.S., Kolonia V., Ioannidou M.C., Psaroulaki A. and **Tselentis Y. (2006):** Active surveillance of Q fever in human and animal populations of Cyprus. BMC Infectious Diseases **6**: 48

Macaluso K.R., Davis J., Alam U., Korman A., Rutherford J.S., Rosenberg R. and **Azad A.F. (2003):** Spotted fever group rickettsiae in ticks from the Maasai Mara region of Kenya. American Journal of Tropical Medicine and Hygiene **68** (5): 551-553

Maina A.N. (2012): Sero-epidemiology and molecular characterisation of rickettsiae infecting humans, selected animals and arthropod vectors in Asembo, western Kenya, 2007-2010. PhD Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya

Marrie T.J. (2009): Q fever. In: Bacterial infections of humans: Epidemiology and control (Brachman P.S. and Elias A., editors), 4th Edition. Springer Science+Business Media, New York, USA: 643-660. DOI 10.1007/978-0-387-09843-2 30

Marrie T.J., Embil J. and **Yates L. (1993):** Sero-epidemiology of *Coxiella burnetii* among wildlife in Nova Scotia. American Journal of Tropical Medicine and Hygiene **49**: 613-615

Marrie T.J., Schlech W.F., Williams J.C. and **Yates L. (1986):** Q fever pneumonia associated with exposure to wild rabbits. Lancet **1**: 427-429

Masala G., Porcu R., Sanna G., Chessa G., Cillara G., Chisu V. and **Tola S. (2004):** Occurrence, distribution and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. Veterinary Microbiology **99**: 301-305

Mateos M. and **Markow T.A. (2005):** Ribosomal intergenic spacer (IGS) length variation across the Drosophilinae (Diptera: Drosophilidae). BMC Evolutionary Biology **5**: 46-50

Maurin M. and **Raoult D. (1999):** Q fever. Clinical Microbiology Reviews **12** (4):518–553

McKenzie A.A. (1993): The capture and care manual: Capture, care, accommodation and transportation of wild African animals. Lynnwood Ridge, South Africa, Wildlife Decision Support Services; Menlo Park, South Africa: South African Veterinary Foundation: 729

McQuiston J.H., Childs J.E. and **Thompson H.A. (2002):** Zoonosis update- Q fever. Journal of American Veterinary Medicine Association **221** (6): 796-799

Mediannikov O., Diatta G., Zolia Y., Balde M.C., Kohar H., Trape J.F. and **Raoult D. (2012):** Tick-borne rickettsiae in Guinea and Liberia. Ticks and Tick-borne Diseases **3** (1): 43-48

Mediannikov O., Fenolla F., Socoloschi C., Diatta G., Bassene H., Molez F., Sokhna C., Trape J.F. and **Raoult D. (2010):** *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS Neglected Tropical Diseases **4** (4): e654

Merhej V. and **Raoult D. (2011):** Rickettsial evolution in the light of comparative genomics. Biological Reviews **86**: 379-405

Moncayo A.C., Cohen S.B., Fritzen C.M., Huang E., Yabsley M.J., Freye J.D., Dunlap B.G., Huang J., Mead D.G., Jones T.F. and **Dunn J.R. (2010):** Absence of *Rickettsia rickettsii* and occurrence of other spotted fever group rickettsiae in ticks from Tennessee. American Journal of Tropical Medicine and Hygiene **83** (3): 653-657

Mutai B.K., Wainaina J.M., Magiri C.G., Nganga J.K., Ithondeka P.M., Njagi O.N., Jiang J., Richards A.L. and **Waitumbi J.N. (2013):** Zoonotic surveillance for rickettsiae in domestic animals in Kenya. Vector-Borne and Zoonotic Diseases **13** (12): 851-856

Naing L., Winn T., and **Rusli B.N. (2006):** Practical issues in calculating the sample size for prevalence studies. Archives of Orofacial Sciences **1**: 9-14

Nakao R., Qiu Y., Igarashi M., Magona J.W., Zhou L., Ito K. and **Sugimoto C. (2013):** High prevalence of spotted fever group rickettsiae in *Amblyomma variegatum* from Uganda and their identification using sizes of intergenic spacers. Ticks and Tick-borne Diseases **4** (6): 506–51

Ndip, L.M., Bouyer, D.H., Amelia, P.A., Travassos, D.A., Titanji, V.P.K., Tesh, R.B. and **Walker, D.H**. **(2004):** Acute spotted fever rickettsioses among febrile patients, Cameroon. Emerging Infectious Diseases **10** (3): 432-437

Norment B.R. and **Burgdorfer W. (1984):** Susceptibility and reservoir potential of the dog to spotted fever-group rickettsiae. American Journal of Veterinary Research **45:** 1706-1710

OIE (2012): Manual of diagnostic tests and vaccines for terrestrial animal (Mammals, birds and bees). 7th Edition Vol. 1. World Organisation for Animal Health, Paris, France: 250-262. http://www.oie.int

Olson S.H. and **Patz J.A. (2010):** Global environmental change and tick-borne disease incidence; draft background paper written to stimulate discussion for the institute of medicine committee on Lyme disease and other tick-borne diseases: the state of the science workshop entitled: "critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes" Washington, D. C., October 11-12, 2010

Omemo P., Ogola E, Omondi G., Wasonga J. and **Knobel D. (2012):** Knowledge, attitude and practice towards zoonoses among public health workers in Nyanza province, Kenya. Journal of Public Health in Africa **3**: 92-93

Ortuno A.**, Quesada M., Lopez-Claessens S., Castella J., Sanfeliu I., Anton E.** and **Segura F. (2007):** The role of wild boar (*Sus scrofa*) in the eco-epidemiology of *R. slovaca* in north-eastern Spain. Vector-Borne and Zoonotic Diseases **7**: 59-64.

Osorio C.R., Collins M.D., Romalde J.L. and **Toranzo1 A.E. (2005):** Variation in 16S-23S rRNA intergenic spacer regions in *Photobacterium damselae*: a mosaic-like structure. Applied and Environmental Microbiology **71** (2): 636-645

Parola P., Paddock C.D. and **Raoult D. (2005):** Tick-borne rickettsioses around the world: Emerging diseases challenging old concepts. Clinical Microbiology Reviews **18** (4): 719-756

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

Perlman S.J., Hunter M.S. and **Zchori-Fein E. (2006):** Review: The emerging diversity of *Rickettsia*. Proceedings of the Royal Society **273**: 2097-2106

Petri W.A. (2007): Overview of rickettsial infections. The Merck Manual, Downloaded from: www.merckmanuals.com

Porter S.R., Czaplicki G., Mainil J., Guatteo R. and **Saegerman C. (2011):** Q fever: current state of knowledge and perspectives of research of a neglected zoonosis. International Journal of Microbiology **11**: Article ID 248418, 22 pages doi:10.1155/2011/248418

Potasman I., Rzotkiewicz S., Pick N. and **Keysary A. (2000):** Outbreak of Q fever following a safari trip. Clinical Infectious Diseases **30**: 214-215

Psaroulaki A., Germanakis A., Gikas A., Scoulica E. and **Tselentis Y. (2005):** Simultaneous detection of *Rickettsia mongolotimonae* in a patient and in tick in Greece. Journal of Clinical Microbiology **43**: 3559-3559

Psaroulaki A., Spyridaki I., Ioannidis A., Babalis T., Gikas A. and **Tselentis Y. (2003):** First isolation and identification of *Rickettsia conorii* from ticks collected in the region of Fokida in central Greece. Journal of Clinical Microbiology **41** (7): 3317-3319

Ramos J.M., Jado I., Padilla S., Masia M., Anda P. and **Gutierrez F. (2013):** Human infection with *Rickettsia sibirica mongolotimonae*, Spain, 2007-2011. Emerging Infectious Diseases **19** (2):267-269

Raoult D. and **Roux V. (1997):** Rickettsioses as paradigms of new or emerging infectious diseases. Clinical Microbiology Reviews **10**: 694-719.

Raoult D., Roux V., Ndihokubwayo J.B., Bise G., Baudon D., Martet, G. and **Birtles R. (1997):** Jail fever (Epidemic Typhus) outbreak in Burundi. Emerging Infectious Diseases **3**: 357-359

Reid R.S., Rainy M., Ogutu J., Kruska R.L., McCartney M., Nyabenge M., Kimani K., Kshatriya M., Worden J., Nganga L., Owuor J., Kinoti J., Njuguna E., Wilson C.J. and **Lamprey R. (2003):** People, wildlife and livestock in the Mara ecosystem: The Mara count 2002 report, International Livestock Research Institute, Nairobi, Kenya

Richards A.L.,Jiang J., Omulo S., Dare R., Abdirahman K., Ali A., Sharif S.K., Feikin D.R., Breiman R.F. and **Njenga M.K. (2010):** Human infection with *Rickettsia felis*, Kenya. Emerging Infectious Diseases **16**: 1081-1086

Roch N., Epaulard O., Pelloux I., Pavese P., Brion J.P., Raoult D. and **Maurin M. (2008):** African tick bite fever in elderly patients: 8 cases in French tourists returning from South Africa. Clinical Infectious Diseases **47:** 28-35

Roest H.I.J., Ruuls R.C., Tilburg J.J.H.C., Nabuurs-Franssen M.H., Klaassen C.H.W., Vellema P., Brom R., Dercksen D., Wouda W., Spierenburg M.A.H., Spek A.N., Buijs R., Boer A.G., Willemsen P.T.J. and **Zijderveld F.G. (2011):** Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerging Infectious Diseases **17** (4): 669-675

Roux V. and **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**: 1449-1455

Rovery C., Brouqui and **Raoult D. (2008):** Questions on Mediterranean spotted fever a century after its discovery. Emerging Infectious Diseases **14** (9): 1360-1367

Rutherford J.S., Macaluso K.R., Smith N., Zaki S.R., Paddock C.D., Davis J., Peterson N., Azad A.F. and **Rosenberg R. (2004):** Fatal spotted fever rickettsioses, Kenya. Emerging Infectious Diseases **10** (5): 910-913

Sawyer L.A., Fishbein D.B. and **McDade J.E. (1987):** Q fever: Current concepts. Reviews of Infectious Diseases Journal **9**: 935-946.

Scola B.L. and **Raoult D. (1997).** Laboratory diagnosis of rickettsioses: Current approaches to diagnosis of old and new rickettsial diseases. Journal of Clinical Microbiology **35** (11): 2715-2727

Serbezov V., Kazar J., Novkirishki V., Gatcheva N., Kovacova E. and **Voyonova V. (1999):** Q fever in Bulgaria and Slovakia. Emerging Infectious Diseases **5**: 388-394

Shpynov S., Fournier P.E., Rudakov N., Tankibaev M., Tarasevich I. and **Raoult D. (2004):** Detection of a *Rickettsia* closely related to *Rickettsia aeschlimannii*, "*Rickettsia heilongjiangensis*", *Rickettsia* sp. strain RpA4 and *Ehrlichia muris* in ticks collected in Russia and Kazakhstan. Journal of Clinical Microbiology **42** (5): 2221-2223

Siqueira-Batista R., Gazineo J.L.D., Gomes A.P., Miguel P.S.B., Santana L.A. and **Geller M. (2016):** Human rickettsiosis: An epidemiological and clinical update. Journal of Tropical Diseases **4** (3): 205. http://dx.doi.org/10.4172/2329-891X.1000205

Socolovschi C., Doudier B., Pages F. and **Parola P. (2008):** Ticks and human tick-borne diseases in Africa. Medecine Tropicale (Marseilles) **68** (2): 119-33

Socolovschi C., Mediannikov O., Raoult. D. and **Parola P. (2009):** The relationship between spotted fever group *Rickettsiae* and ixodid ticks. Veterinary Research **40**:34-53

Sousa R., Barata C., Vitorino L., Santos-Silva M., Carrapato C., Torgal J., Walker D. and Bacellar F. (2006): Isolation of *Rickettsia sibirica* (strain mongolotimonae) from a patient and detection in a *Rhipicephalus pusillus* tick in Portugal. Emerging Infectious Diseases **12**:1103- 1108

Sousa R., **Duque L., Anes M., Pocas J., Torgal J.** and **Bacellar F. (2008):** Lymphangitis in a Portuguese patient infected with *Rickettsia sibirica*. Emerging Infectious Diseases **14** (3): 529-530. http://dx.doi.org/10.3201/eid1403.070680

Sprong H., Wielinga P.R., Fonville M., Reusken C., Brandenburg A.H., Borgsteede F., Gaasenbeek C. and **van der Giessen J.W.B. (2009):** *Ixodes ricinus* ticks are reservoir hosts for *Rickettsia helvetica* and potentially carry flea-borne *Rickettsia* species. Parasites and Vectors: 2: 41

Stein A. and **Raoult D. (1999):** Pigeon pneumonia in Provence: A bird borne Q fever outbreak. Clinical Infectious Diseases **29**: 617–620

Tamura A., Ohashi N., Urakami H. and **Miyamura S. (1995):** Classification of *Rickettsia tsutsugamushi i*n a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. International Journal of Systemic Bacteriology **45:**589-591

Tamura K., Stecher G., Peterson D., Filipski A. and **Kumar S. (2013):** MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. doi:10.1093/molbev/mst197. http://mbe.oxfordjournals.org/

Thorner A.R., Walker D.H. and **Petri Jr. W.A. (1998):** Rocky Mountain spotted fever. Clinical Infectious Diseases **27**: 1353-1360

Todar K. (2012): Rickettsial diseases including typhus and Rocky Mountain spotted fever. Todar's Online Textbook of Bacteriology. www.textbookofbacteriology.net

Tokarz R., Kapoor V., Samuel J.E., Bouyer D.H., Briese T. and **Lipkin W.I. (2009):** Detection of tick-borne pathogens by MassTag polymerase chain reaction. Vector-borne and Zoonotic Diseases **9** (2):147-151

Toledo A., Olmeda A.S., Escudero R., Jado I., Valcarcel F., Casado-Nistal M.A., Rodriguez-Vargas S.M., Gil H. and **Anda P. (2009):** Tick-borne zoonotic bacteria in ticks collected from central Spain. American Journal of Tropical Medicine and Hygiene 81 (1): 67-74

Walker A.R., Bouattour A., Camicas J.L., Estrada-Pena A., Horak I.G., Latif A.A., Pegram R.G. and **Preston P.M. (2003):** Ticks of domestic animals in Africa: A guide to identification of species. Bioscience Reports, 42 Comiston drive, Edinburgh EH 5QR, Scotland, UK.

Weiss E., Dobson M.E. and **Dasch G.A. (1987):** Biochemistry of rickettsiae: Recent advances. Acta Virologica **31**:271–286

Whitman T.M., Richards, A.L., Paddock, C.D., Tamminga, C.L., Sniezek, P.J., Jiang, J., Byers, D.K. and **Sanders, J.W. (2007):** *Rickettsia parkeri* infection after tick bite, Virginia. Emerging Infectious Diseases **13** (2): 334-336

Yoshikawa H., Kimura M., Ogawa M., Rolain J. and **Raoult D. (2005):** Laboratory-confirmed Mediterranean spotted fever in a Japanese traveller to Kenya. American Journal of Tropical Medicine and Hygiene **73** (6): 1086-1089.

Zarnke R.L. (1983). Serologic survey for selected microbial pathogens in Alaskan wildlife. Journal of Wildlife Diseases **19:** 324-329

Zhang J.Z., Fan M.Y. and **Bi D.Z. (1995):** Detection of spotted fever group rickettsiae in ticks and rodents by polymerase chain reaction technique in People's Republic of China. Acta Virologica **39** (5-6): 263-267

Zhang L., Jin J., Fu X., Raoult D. and **Fournier P.E. (2006):** Genetic differentiation of Chinese isolates of *Rickettsia sibirica* by partial *ompA* Gene sequencing and multispacer typing. Journal of Clinical Microbiology **44** (7): 2465-2467

Zhu Y., Fournier P.E., Ogata H. and **Raoult D. (2005):** Multispacer typing of *Rickettsia prowazekii* enabling epidemiological studies of epidemic typhus. Journal of Clinical Microbiology **43** (9): 4708-4712

Znazen A., Khrouf F., Elleuch N., Lahiani D., Marrekchi C., M'Ghirbi Y., Jemaa M.B., Bouattour A. and **Hammami A. (2013):** Multispacer typing of *Rickettsia* isolates from humans and ticks in Tunisia revealing new genotypes. Parasites and Vectors **6**: 367-374

APPENDICES

Appendix 1: Extraction of DNA from blood

(i) Frozen blood was thawed for about one hour and mixed with a homogeniser (Velp® Scientifica, Usmate, Italy) for about 5-10 seconds to get a homogenised solution.

(ii) Using a pipette, 200 µl of each blood sample was then collected into an easy lock tube into which 20 µl of proteinase kinase enzyme was added. The manufacturer recommends 50-100 µl but this was increased to 200 µl to optimise the amount of DNA extracted. The mixture was mixed thoroughly using a vortex (Grant Incubator, Grant Instruments Ltd., Cambridgeshire, England) for 5-10 seconds to homogenise it.

(iii) 200 µl of AL buffer was then added and the mixture homogenised using a vortex. The mixture was then incubated for 20 minutes at 56°C followed by thorough mixing for 5-10 seconds to homogenise it.

(iv) 200 µl of absolute alcohol was then added and mixed thoroughly for 5-10 seconds.

(v) The homogenate was then drawn with PhysioCare Concept® pipettes (Eppendorf Nordic ApS, Horsholm, Denmark) with Multiguard Barrier® tips (Sorenson® BioScience Inc, Murray, USA) into the DNeasy® mini spin columns placed in a 2 ml collection tube and centrifuged using Nuve NF 800R® multipurpose centrifuge (Nuve Laboratory and Sterilisation Technology, Ankara, Turkey) at 8,000 rpm for one minute. The flow-through material and the collection tubes were thereafter discarded.

(vi) The DNeasy® mini spin column was then placed in a new 2 ml collection tube, 200 µl of AW1 added and centrifuged for one minute at 8,000 rpm. The flow-through material and the collection tubes were again discarded.

(vii) The DNeasy® mini spin column was again placed in a new 2 ml collection tube, 500 µl of AW2 added and centrifuged for three minute at 14,000 rpm to dry the DNeasy® membrane. The flow-through material and the collection tubes were again discarded.

(viii) The DNeasy® mini spin column was again placed in a new 2 ml collection tube and centrifuged for one minute at 14,000 rpm without addition of any buffer. This was yet another modification of the manufacturer's instructions adopted for this study in order to completely dry the membrane of the DNeasy® mini spin column since residual ethanol and buffers may interfere with subsequent reactions. This centrifugation step ensured that no residual ethanol was carried over during subsequent elution. The flow-through material and the collection tube were discarded.

(ix) The DNeasy® mini spin column was then placed into a clean 1.5 ml micro-centrifuge tube and 150 µl of AE buffer put directly onto the DNeasy® membrane. The manufacturer's recommended volume of the buffer is 200 µl but this was reduced so as to increase the concentration of the DNA extracted. This was then incubated at room temperature for one minute before being centrifuged for one minute at 800

rpm to elute (i.e. remove bound DNA from the membrane). The elute was collected and put back into the DNeasy® mini spin column and centrifuged for one minute at 8,000 rpm. This step was taken in order to increase the overall DNA yield.

(x) The quality of the extracted DNA was evaluated using the agarose gel electrophoresis protocol in which an aliquot of the extracted DNA was run on 1.2% agarose gel.

(xi) Extracted DNA was stored at -80^{o}C until use.

Appendix 2: Extraction of DNA from ticks

(i) Frozen pools of ticks were first crushed and homogenised by one minute agitation using a BioSpec Mini-BeadBeater 16® (BioSpec Products Inc, Bartlesville, UK) in 0.5 ml screw-cap tubes. This was done using 800mg of 2.0 mm and 200 mg of 0.1 mm Yttria-stabilised zirconium (YSZ) oxide beads (Glen Mills, Clifton, New Jersey, USA) and 500 µl of homogenisation media (2% L-glutamine and 15% Fetal Bovine Serum). This was followed by inverting the closed tubes ten times.

(ii) The homogenate were then short-centrifuged at maximum speed of 14,000 rpm at 4°C in an Eppendorf 5417R® bench-top centrifuge (Eppendorf Nordic ApS, Horsholm, Denmark).

(iii) Immediately thereafter, DNA was extracted from a 200 µl aliquot using the MagNA 96 Pure DNA® and Viral NA Small Volume Kit® (Roche Diagnostics Ltd, Sussex, UK) in a MagNa Pure 96® automatic extractor (Roche Diagnostics Ltd, Sussex, UK).

(iv) The quality of the extracted DNA was evaluated using the agarose gel electrophoresis protocol in which an aliquot of the extracted DNA was run on 1.2% agarose gel.

(v) Extracted DNA was stored at -80°C until use

Appendix 3: PCR-HRM conditions for SFG rickettsiae detection in ticks and wildlife

Cycle	Number of Cycle(s)	Cycle Stage	Temperature Point and Time
Initial Hold		Hold	95°C,15 minutes
Cycling 1	1	Annealing	65°C, hold 25seconds
		Extension	72°C, hold 5 seconds
Cycling 2	1	Denaturation	94°C, hold 20 seconds
		Annealing	64°C, hold 25 seconds
		Extension	72°C, hold 5 seconds
Cycling 3	1	Denaturation	94°C, hold 20 seconds
		Annealing	63°C, hold 25 seconds
		Extension	72°C, hold 10 seconds
Cycling 4	1	Denaturation	94°C, hold 20 seconds
		Annealing	62°C, hold 25 seconds
		Extension	72°C, hold 11 seconds

Cycle	Number of Cycle(s)	Cycle Stage	Temperature Point and Time
Cycling 5	1	Denaturation	94°C, hold 20 seconds
		Annealing	61°C, hold 25 seconds
		Extension	72°C, hold 12 seconds
Cycling 6	5	Denaturation	94°C, hold 20 seconds
		Annealing	60°C, hold 40 seconds
		Extension	72°C, hold 15 seconds
Cycling 7	5	Denaturation	94°C, hold 20 seconds
		Annealing	59°C, hold 40 seconds
		Extension	72°C, hold 15 seconds
Cycling 8	5	Denaturation	94°C, hold 20 seconds
		Annealing	58°C, hold 40 seconds
		Extension	72°C, hold 20 seconds
Cycling 9	5	Denaturation	94°C, hold 20 seconds
		Annealing	57°C, hold 40 seconds
		Extension	72°C, hold 25 seconds
Cycling 10	5	Denaturation	94°C, hold 20 seconds
		Annealing	56°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Cycling 11	5	Denaturation	94°C, hold 20 seconds
		Annealing	55°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Cycling 12	5	Denaturation	94°C, hold 20 seconds
		Annealing	54°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Cycling 13	1	Denaturation	95°C, hold 20 seconds
		Annealing	53°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Cycling 14	1	Denaturation	94°C, hold 20 seconds
		Annealing	50°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Cycling 15	1	Denaturation	95°C, hold 20 seconds
		Annealing	52°C, hold 50 seconds
		Extension	72°C, hold 30 seconds
Hold 2	1	Final Elongation	72°C,3 minutes
Hold 3	1	Anneal	45°C,1 minute
Melt-HRM	75°C to 90°C at 0.1°C increments with acquisitions after every 2 seconds		

Appendix 4: PCR-HRM conditions for C. burnetii detection in ticks and wildlife

Cycle	Number of Cycle(s)	Cycle Stage	Temperature Point and Time
Initial hold	1	Hold	95°C,15minutes
Cycling 1	1	Annealing	65°C, hold25seconds
		Extension	72°C, hold5seconds, acquiring
Cycling 2	1	Denaturation	94°C, hold20seconds
		Annealing	64°C, hold25seconds
		Extension	72°C, hold5seconds, acquiring
Cycling 3	1	Denaturation	94°C, hold20seconds
		Annealing	63°C, hold25seconds
		Extension	72°C, hold10seconds, acquiring
Cycling 4	1	Denaturation	94°C, hold20seconds
		Annealing	62°C, hold25seconds
		Extension	72°C, hold11seconds, acquiring
Cycling 5	1	Denaturation	94°C, hold20seconds
		Annealing	61°C, hold25seconds
		Extension	72°C, hold12seconds, acquiring
Cycling 6	3	Denaturation	94°C, hold20seconds
		Annealing	60°C, hold40seconds
		Extension	72°C, hold15seconds, acquiring
Cycling 7	1	Denaturation	94°C, hold20seconds
		Annealing	59°C, hold40seconds
		Extension	72°C, hold15seconds, acquiring
Cycling 8	1	Denaturation	94°C, hold20seconds
		Annealing	58°C, hold40seconds
		Extension	72°C, hold20seconds, acquiring
Cycling 9	1	Denaturation	94°C, hold20seconds
		Annealing	57°C, hold40seconds
		Extension	72°C, hold25seconds, acquiring
Cycling 10	1	Denaturation	94°C, hold20seconds
		Annealing	56°C, hold50secondonds
		Extension	72°C, hold30seconds, acquiring
Cycling 11	5	Denaturation	94°C, hold20seconds

Cycle	Number of Cycle(s)	Cycle Stage	Temperature Point and Time
		Annealing	55°C, hold50seconds
		Extension	72°C, hold30seconds, acquiring
Cycling 12	5	Denaturation	94°C, hold20seconds
		Annealing	54°C, hold50seconds
		Extension	72°C, hold30seconds, acquiring
Cycling 13	5	Denaturation	94°C, hold20seconds
		Annealing	53°C, hold50seconds
		Extension	72°C, hold30seconds, acquiring
Cycling 14	5	Denaturation	94°C, hold20seconds
		Annealing	52°C, hold50seconds
		Extension	72°C, hold30seconds, acquiring
Cycling 15	5	Denaturation	94°C, hold20seconds
		Annealing	51°C, hold50seconds
		Extension	72°C, hold30seconds, acquiring
Cycling 16	5	Denaturation	94°C, hold20seconds
		Annealing	50°C, hold50 seconds
		Extension	72°C, hold30 seconds, acquiring
Hold 2	1	Final Elongation	72°C,3 minutes
Hold 3	1	Anneal	45°C,1minute
Initial calibration at 75°C for 90 seconds			
Melt-HRM	75°C to 90°C at 0.1°C increments with acquisitions after every 2seconds		

Appendix 5: KAP questionnaire on SFG rickettsioses and Q fever

Introduction

Greetings. Thank you for agreeing to take part in this survey on diseases shared between wildlife, domestic animals and humans. My name is Dr. David Ndeereh and I work for the Kenya Wildlife Service. My colleagues and I are conducting a study on these diseases to better understand their presence and distribution as well as the local knowledge about them. The information obtained from this study will help to inform better management of these diseases. This survey will take only 8-10 minutes to complete and is voluntary. Be assured that all answers you provide will be kept in the strictest confidentiality for purposes of the study only. Before we start, is there anything you would like us to clarify?

Part A- Biodata

Study area: _____ GPS Location: _____

Location/Ward: _____ Village/Manyatta: _____

Enumerator: _____ Date: _____

Respondent: Name: _____ Age: _____ Sex: _____

Occupation of respondent: Pastoralist: ☐ Health Personnel: ☐ Wildlife Personnel: ☐

Other: ☐ Specify: _____

Part B- Pastoralist

Position of respondent in household: Head: ☐ Spouse: ☐ Son: ☐ Daughter: ☐

Employee: ☐ Other: ☐ Specify: _____

1. Type of livestock kept: Cattle: ☐ Goat: ☐ Sheep: ☐ Other: ☐ Specify: _____
2. Are there wildlife within your locality?: Yes: ☐ No: ☐
3. Are there times when wildlife graze with your livestock?: Yes: ☐ No: ☐
4. Do your livestock share water points with wildlife?: Yes: ☐ No: ☐
5. What type of problems do you encounter when your livestock mix with wildlife?:
   (i) Predation: ☐ (ii) Competition for pasture and Water: ☐
   (iii) Transmission of diseases: ☐ (iv) Other: ☐ Specify: _____
6. Are you aware of any diseases that wildlife can transmit to livestock? Yes: ☐ No: ☐
7. If yes to 6 above, rank the diseases in order of importance.

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____
(iv)	_____	_____
(v)	_____	_____

8. Do you think any of the diseases you have listed transmitted by Ticks?: Yes: ☐ No: ☐
9. If so, which ones?:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

10. Are any of the diseases listed in 9 above cause abortions in your livestock?:Yes: ☐ No: ☐

11. Which species of livestock are affected? Cattle: ☐ Goat: ☐ Sheep: ☐ Other: ☐
    Specify:_____
12. Do you think any of the diseases listed in 9 could affect humans?: Yes: ☐ No: ☐
13. If so, which ones?:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

14. What symptoms do you see in humans affected by the tick transmitted disease:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____
(iv)	_____	_____

15. Of the diseases listed in Q14, are the following symptoms observed in affected people?
    (i) Pneumonia: Yes: ☐ No: ☐
    (ii) Headache: Yes: ☐ No: ☐
    (iii) Fever: Yes: ☐ No: ☐
16. Do you think there are other ways the diseases listed above could be transmitted apart from ticks?
    Yes: ☐ No: ☐
17. If yes in Q16, list the ways of transmission?:
    (i) _____ (iii) _____ (v) _____
    (ii) _____ (iv) _____ (vi) _____
18. Do you think consumption and handling of animal products could cause the above diseases in humans? Yes: ☐ No: ☐
19. Do you think sharing the same house with livestock can transmitt livestock diseases to humans?:
    Yes: ☐ No: ☐

Part C- Health Personnel

Name of Medical Facility: _____ Position of Respondent: _____

Qualifications of respondent: _____Years in present facility: _____

1. Do you get cases of tick transmitted diseases in your patients? Yes: ☐ No: ☐
2. If so, list the tick transmitted diseases:
   (i) _____ (iii) _____ (v) _____
   (ii) _____ (iv) _____ (vi) _____
3. Are any of the tick transmitted diseases caused by *Rickettsia*?: Yes: ☐ No: ☐
4. What are the main clinical presentations of rickettsial diseases?
   (i) _____ (iii) _____ (v) _____
   (ii) _____ (iv) _____ (vi) _____
5. How many cases have presented with Rickettsial diseases in the last one year? _____
6. Is fever a common presentation of the diseases?: Yes: ☐ No: ☐
7. What other diseases would have similar clinical presentation?
   (i) _____ (iii) _____ (v) _____
   (ii) _____ (iv) _____ (vi) _____
8. Do you sometimes find it necessary to confirm Rickettsial diseases in your diagnosis?:Yes: ☐ No: ☐
9. If yes, what methods do you use to confirm the diagnosis?

(i) Serology (ELISA, etc):Yes: ☐ No: ☐ (ii)Molecular Diagnosis (PCR):Yes: ☐ No: ☐

Part C (2) – Q fever

10. Do you get cases of zoonotic diseases in your patients? Yes: ☐ No: ☐
11. If so, list the zoonotic diseases:
    (i) _____ (iii) _____ (v) _____
    (ii) _____ (iv) _____ (vi) _____
12. Are any of the zoonotic diseases caused by Q fever?: Yes: ☐ No: ☐
13. What are the main clinical presentations of Q fever?
    (i) _____ (iii) _____ (v) _____
    (ii) _____ (iv) _____ (vi) _____
14. How many cases have presented with Q fever in the last one year? _____
15. Is pneumonia and fever a common presentation of the disease?: Yes: ☐ No: ☐
16. What other diseases would have similar clinical presentations?
    (i) _____ (iii) _____ (v) _____
    (ii) _____ (iv) _____ (vi) _____
17. Do you sometimes find it necessary to confirm Q fever in your diagnosis?: Yes: ☐ No: ☐
18. If yes, what methods do you use to confirm the diagnosis?

    (i) Serology (ELISA, etc): Yes: ☐ No: ☐ i. (ii) Molecular Diagnosis (PCR):Yes: ☐ No: ☐

Part D: Wildlife Sector Personnel

Name of wildlife station: _____

Position of respondent: _____ Years in present station: _____

1. Does wildlife mix with livestock in your area of jurisdiction?: Yes: ☐ No: ☐
2. If yes, where does this interaction occur?:
   (i) Inside protected areas: Yes: ☐ No: ☐
   (ii) Outside protected areas: Yes: ☐ No: ☐
3. What type of problems do you encounter when wildlife mix with livestock?:
   (i) Predation: ☐ (ii) Competition for pasture and Water: ☐ (iii) Transmission of diseases: ☐
   (iv) Other: ☐ Specify: _____
4. List some diseases that wildlife can share with livestock in order of importance.

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____
(iv)	_____	_____

5. Are any of the diseases you have listed transmitted by Ticks?: Yes: ☐ No: ☐
6. If so, which ones?:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

7. Do you think any of the tick transmitted diseases listed could affect humans?: Yes: ☐ No: ☐
8. If so, which ones?:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

9. List the symptoms seen in humans affected by the tick transmitted disease:

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____
(iv)	_____	_____

10. Do you get complaints from your patrol staff bitten by Ticks?:Yes: ☐ No: ☐

11. If Yes, what types of complaints do you receive?

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

12. Among the complaints received, are there some which include the following?:
    (i) Pneumonia: Yes: ☐ No: ☐
    (ii) Headache: Yes: ☐ No: ☐
    (iii) Fever: Yes: ☐ No: ☐
13. Do you seek medical attention for these complaints?: Yes: ☐ No: ☐
14. If yes, what diagnosis is made?

	Local Name	Common Name
(i)	_____	_____
(ii)	_____	_____
(iii)	_____	_____

15. Have you ever considered taking measures to prevent tick bites in your staff?:Yes: ☐ No: ☐
16. If so, which are some of these measures?
    (i) _____
    (ii) _____
    (iii) _____
    (iv) _____
    (v) _____

Appendix 6: PCR results of SFG rickettsioses and Q fever in animals in Laikipia and Maasai Mara

Plate No.	Species	Sample ID	Study area	Latitude	Longitude	*Rickettsia*	*Coxiella*
1.	Zebra	MMZ01	Maasai Mara	36M0774089	UTM9824469	Negative	Negative
2.	Zebra	MMZ02	Maasai Mara	36M0774090	UTM9824470	Negative	Negative
3.	Zebra	MMZ03	Maasai Mara	36M0774091	UTM9824471	Negative	Negative
4.	Zebra	MMZ05	Maasai Mara	36M0762814	UTM9839831	Negative	Negative
5.	Zebra	MMZ06	Maasai Mara	36M0762815	UTM9839832	Negative	Negative
6.	Zebra	MMZ07	Maasai Mara	36M0766268	UTM9845579	Negative	Negative
7.	Zebra	MMZ08	Maasai Mara	36M0766269	UTM9845580	Negative	Negative
8.	Zebra	MMZ09	Maasai Mara	36M0766270	UTM9845581	Negative	Negative
9.	Zebra	MMZ11	Maasai Mara	36M0759902	UTM9846160	Negative	Negative
10.	Zebra	MMZ12	Maasai Mara	36M0747036	UTM9847675	Negative	Negative
11.	Zebra	MMZ13	Maasai Mara	36M0747760	UTM9843625	Negative	Negative
12.	Zebra	MMZ14	Maasai Mara	36M0747761	UTM9843626	Negative	Negative
13.	Zebra	MMZ16	Maasai Mara	36M0747762	UTM9843627	Negative	Negative

Plate No.	Species	Sample ID	Study area	Latitude	Longitude	*Rickettsia*	*Coxiella*
14.	Zebra	MMZ17	Maasai Mara	36M0747763	UTM9843628	Negative	Negative
15.	Zebra	MMZ18	Maasai Mara	36M0746984	UTM9871048	Negative	Negative
16.	Zebra	MMZ19	Maasai Mara	36M0746985	UTM9871049	Negative	Negative
17.	Zebra	MMZ20	Maasai Mara	36M0750856	UTM9862388	Negative	Negative
18.	Zebra	MMZ21	Maasai Mara	36M0748079	UTM9859414	Negative	Negative
19.	Zebra	MMZ22	Maasai Mara	36M0727761	UTM9831699	Negative	Negative
20.	Zebra	MM/ZB/01	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
21.	Zebra	MM/ZB/02	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
22.	Buffalo	OPB1	Laikipia	37N0271408	UTM0006930	Negative	Negative
23.	Buffalo	OPB2	Laikipia	37N0271408	UTM0006930	Negative	Negative
24.	Buffalo	OPB3	Laikipia	37N0271408	UTM0006930	Negative	Negative
25.	Buffalo	OPB4	Laikipia	37N0271408	UTM0006930	Negative	Negative
26.	Buffalo	OPB5	Laikipia	37N0271408	UTM0006930	Negative	Negative
27.	Buffalo	OPB6	Laikipia	37N0271408	UTM0006930	Negative	Negative
28.	Buffalo	OPB7	Laikipia	37N0261724	UTM0004581	Negative	Negative
29.	Buffalo	OPB8	Laikipia	37N0261724	UTM0004581	Negative	Negative
30.	Buffalo	OPB9	Laikipia	37N0261724	UTM0004581	Negative	Negative
31.	Buffalo	OPB10	Laikipia	37N0261724	UTM0004581	Negative	Negative
32.	Buffalo	OPB11	Laikipia	37N0261724	UTM0004581	Negative	Negative
33.	Buffalo	OPB12	Laikipia	37N0261724	UTM0004581	Negative	Negative
34.	Buffalo	OPB13	Laikipia	37N0261724	UTM0004581	Negative	Negative
35.	Buffalo	OPB14	Laikipia	37N0261724	UTM0004581	Negative	Negative
36.	Buffalo	OPB15	Laikipia	37N0261724	UTM0004581	Negative	Negative
37.	Buffalo	OPB16	Laikipia	37M0263623	UTM0003550	Negative	Negative
38.	Buffalo	OPB17	Laikipia	37M0263623	UTM0003550	Negative	Negative
39.	Buffalo	OPB18	Laikipia	37M0273557	UTM0001236	Negative	Negative
40.	Buffalo	OPB19	Laikipia	37M0273557	UTM0001236	Negative	Negative
41.	Buffalo	OPB20	Laikipia	37M0273557	UTM0001236	Negative	Negative
42.	Buffalo	OPB21	Laikipia	37M0273557	UTM0001236	Negative	Negative
43.	Buffalo	OPB22	Laikipia	37M0273557	UTM0001236	Negative	Negative
44.	Buffalo	OPB23	Laikipia	37M0273557	UTM0001236	Positive	Negative
45.	Buffalo	OPB24	Laikipia	37M0273557	UTM0001236	Negative	Negative
46.	Buffalo	OPB25	Laikipia	37M0273557	UTM0001236	Negative	Negative
47.	Buffalo	OPB26	Laikipia	37M0273557	UTM0001236	Negative	Negative
48.	Buffalo	OPB27	Laikipia	37M0273557	UTM0001236	Negative	Negative
49.	Buffalo	OPB28	Laikipia	37M0273557	UTM0001236	Negative	Negative
50.	Zebra	OPZ1	Laikipia	37N0273869	UTM0002582	Negative	Negative
51.	Zebra	OPZ2	Laikipia	37N0273869	UTM0002582	Negative	Negative
52.	Zebra	OPZ3	Laikipia	37N0273869	UTM0002582	Negative	Negative
53.	Zebra	OPZ4	Laikipia	37N0273869	UTM0002582	Negative	Negative
54.	Zebra	OPZ5	Laikipia	37N0273869	UTM0002582	Negative	Negative
55.	Zebra	OPZ6	Laikipia	37N0273869	UTM0002582	Negative	Negative
56.	Zebra	OPZ7	Laikipia	37N0273869	UTM0002582	Negative	Negative
57.	Zebra	OPZ8	Laikipia	37N0273869	UTM0002582	Negative	Negative

Plate No.	Species	Sample ID	Study area	Latitude	Longitude	*Rickettsia*	*Coxiella*
58.	Zebra	OPZ9	Laikipia	37N0273869	UTM0002582	Negative	Negative
59.	Zebra	OPZ10	Laikipia	37N0273869	UTM0002582	Negative	Negative
60.	Zebra	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
61.	Zebra	OPZ12	Laikipia	37N0252695	UTM0007978	Negative	Negative
62.	Zebra	OPZ13	Laikipia	37N0252695	UTM0007978	Negative	Negative
63.	Zebra	OPZ14	Laikipia	37N0252695	UTM0007978	Negative	Negative
64.	Zebra	OPZ15	Laikipia	37N0252695	UTM0007978	Negative	Negative
65.	Zebra	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
66.	Zebra	OPZ17	Laikipia	37N0260025	UTM0004107	Negative	Negative
67.	Zebra	OPZ18	Laikipia	37M0263623	UTM0003550	Negative	Negative
68.	Zebra	OPZ19	Laikipia	37M0263623	UTM0003550	Negative	Negative
69.	Zebra	OPZ20	Laikipia	37M0263623	UTM0003550	Negative	Negative
70.	Zebra	OPZ21	Laikipia	37M0263623	UTM0003550	Negative	Negative
71.	Zebra	OPZ22	Laikipia	37M0263623	UTM0003550	Negative	Negative
72.	Zebra	OPZ23	Laikipia	37M0263623	UTM0003550	Negative	Negative
73.	Zebra	OPZ24	Laikipia	37M0263623	UTM0003550	Negative	Negative
74.	Zebra	OPZ25	Laikipia	37M0263099	UTM0006929	Negative	Negative
75.	Zebra	OPZ26	Laikipia	37M0263099	UTM0006929	Negative	Negative
76.	Zebra	OPZ27	Laikipia	37M0263099	UTM0006929	Negative	Negative
77.	Zebra	MZ1	Laikipia	37N0237520	UTM0008097	Negative	Negative
78.	Zebra	MZ2	Laikipia	37N0237520	UTM0008097	Negative	Negative
79.	Zebra	MZ3	Laikipia	37N0252454	UTM0011656	Negative	Negative
80.	Zebra	MZ4	Laikipia	37N0252454	UTM0011656	Negative	Negative
81.	Zebra	MZ5	Laikipia	37N0252454	UTM0011656	Negative	Negative
82.	Zebra	MZ6	Laikipia	37N0247851	UTM0013969	Negative	Negative
83.	Zebra	MZ7	Laikipia	37N0245451	UTM0011794	Negative	Negative
84.	Zebra	MZ8	Laikipia	37N0245451	UTM0011794	Positive	Negative
85.	Zebra	MZ9	Laikipia	37N0252454	UTM0011656	Negative	Negative
86.	Zebra	MZ10	Laikipia	37N0252454	UTM0011656	Negative	Negative
87.	Zebra	MZ11	Laikipia	37N0252454	UTM0011656	Negative	Negative
88.	Zebra	MZ12	Laikipia	37N0252454	UTM0011656	Negative	Negative
89.	Buffalo	MB1	Laikipia	37N0252450	UTM0010973	Negative	Negative
90.	Buffalo	MB2	Laikipia	37N0252450	UTM0010973	Negative	Negative
91.	Buffalo	MB3	Laikipia	37N0247302	UTM0008954	Negative	Negative
92.	Topi	MM/TP/01	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
93.	Topi	MM/TP/02	Maasai Mara	36N0761528	UTM9821048	Positive	Negative
94.	Topi	MM/TP/03	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
95.	Topi	MM/TP/04	Maasai Mara	36N0761528	UTM9821048	Positive	Negative
96.	Topi	MM/TP/06	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
97.	Topi	MM/TP/07	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
98.	Topi	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Positive	Negative
99.	Topi	MM/TP/09	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
100.	Topi	MM/TP/10	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
101.	Topi	MM/TP/11	Maasai Mara	36N0763886	UTM9834659	Negative	Negative

Plate No.	Species	Sample ID	Study area	Latitude	Longitude	*Rickettsia*	*Coxiella*
102.	Hartebeest	MM/HB/01	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
103.	Hartebeest	MM/HB/02	Maasai Mara	36N0751613	UTM9832092	Negative	Negative
104.	Hartebeest	MM/HB/03	Maasai Mara	36N0751613	UTM9832092	Negative	Negative
105.	Hartebeest	MM/HB/04	Maasai Mara	36N0751613	UTM9832092	Negative	Negative
106.	Hartebeest	MM/HB/05	Maasai Mara	36N0751613	UTM9832092	Negative	Negative
107.	Wildebeest	MM/WB/01	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
108.	Wildebeest	MM/WB/02	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
109.	Wildebeest	MM/WB/03	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
110.	Wildebeest	MM/WB/04	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
111.	Wildebeest	MM/WB/05	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
112.	Wildebeest	MM/WB/06	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
113.	Wildebeest	MM/WB/07	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
114.	Wildebeest	MM/WB/08	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
115.	Wildebeest	MM/WB/09	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
116.	Wildebeest	MM/WB/10	Maasai Mara	36N0747904	UTM9836308	Negative	Negative
117.	Wildebeest	MM/WB/12	Maasai Mara	36N0747904	UTM9836308	Negative	Negative
118.	Impala	MM/IMP/01	Maasai Mara	36N0757755	UTM9830241	Negative	Negative
119.	Impala	MM/IMP/02	Maasai Mara	36N0757755	UTM9830241	Negative	Negative
120.	Waterbuck	OPW1	Laikipia	37M0263828	UTM0003524	Negative	Negative
121.	Waterbuck	OPW2	Laikipia	37M0263828	UTM0003524	Negative	Negative
122.	Grants gazelle	GG59	Laikipia	37N0267144	UTM0035201	Negative	Negative
123.	Grants gazelle	GG65	Laikipia	37N0267144	UTM0035201	Negative	Negative
124.	Grants gazelle	GG66	Laikipia	37N0267144	UTM0035201	Negative	Negative
125.	Grants gazelle	GG67	Laikipia	37N0267144	UTM0035201	Negative	Negative
126.	Grants gazelle	GG69	Laikipia	37N0263200	UTM0043000	Negative	Negative
127.	Grants gazelle	GG86	Laikipia	37N0263200	UTM0043000	Negative	Negative
128.	Grants gazelle	GG62	Laikipia	37N0263200	UTM0043000	Negative	Negative
129.	Wildebeest	WB2	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
130.	Wildebeest	WB9	Maasai Mara	36M0726585	UTM9831203	Negative	Negative
131.	Wildebeest	WB12	Maasai Mara	36M0726585	UTM9831203	Negative	Negative
132.	Wildebeest	WB13	Maasai Mara	36M0726585	UTM9831203	Negative	Negative
133.	Wildebeest	WB14	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
134.	Wildebeest	WB15	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
135.	Wildebeest	WB16	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
136.	Wildebeest	WB17	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
137.	Wildebeest	WB20	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
138.	Wildebeest	WB21	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
139.	Wildebeest	WB22	Maasai Mara	36M0758425	UTM9841670	Negative	Negative
140.	Wildebeest	WB24	Maasai Mara	36N0757755	UTM9830241	Negative	Negative
141.	Wildebeest	WB26	Maasai Mara	36N0757755	UTM9830241	Negative	Negative
142.	Wildebeest	WB28	Maasai Mara	36N0757755	UTM9830241	Negative	Negative
143.	Wildebeest	WB37	Maasai Mara	36N0757755	UTM9830241	Positive	Negative
144.	Wildebeest	WB39	Maasai Mara	36N0757765	UTM9830244	Negative	Negative
145.	Wildebeest	WB41	Maasai Mara	36N0757765	UTM9830244	Negative	Negative

Plate No.	Species	Sample ID	Study area	Latitude	Longitude	*Rickettsia*	*Coxiella*
146.	Wildebeest	WB42	Maasai Mara	36N0757765	UTM9830244	Negative	Negative
147.	Wildebeest	WB45	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
148.	Wildebeest	WB46	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
149.	Wildebeest	WB47	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
150.	Wildebeest	WB48	Maasai Mara	36N0747902	UTM9836306	Negative	Negative
151.	Wildebeest	WB49	Maasai Mara	36N0747902	UTM9836306	Negative	Negative
152.	Wildebeest	WB52	Maasai Mara	36N0747902	UTM9836306	Negative	Negative

Appendix 7: PCR results of SFG rickettsiae and *C. burnetii* in ticks in Laikipia and Maasai Mara

Pool No.	Tick Species	No. of Ticks	Sample ID	Study Area	Latitude	Longitude	*Rickettsia*	*Coxiella*
1.	*Rh. pulchellus*	4	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
2.	*Rh. evertsi evertsi*	5	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
3.	*Rh. evertsi evertsi*	5	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
4.	*Rh. evertsi evertsi*	6	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
5.	*Rh. appendiculatus*	5	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
6.	*Rh. appendiculatus*	6	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
7.	*Rh. appendiculatus*	6	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
8.	*Rh. appendiculatus*	5	OPZ11	Laikipia	37N0273869	UTM0002582	Negative	Negative
9.	*Rh. pulchellus*	6	OPB8	Laikipia	37N0261724	UTM0004581	Positive	Negative
10.	*Rh. evertsi evertsi*	7	OPB8	Laikipia	37N0261724	UTM0004581	Positive	Negative
11.	*Rh. appendiculatus*	7	OPB8	Laikipia	37N0261724	UTM0004581	Negative	Negative
12.	*Rh. appendiculatus*	8	OPB8	Laikipia	37N0261724	UTM0004581	Negative	Negative
13.	*Rh. appendiculatus*	7	OPB8	Laikipia	37N0261724	UTM0004581	Positive	Negative
14.	*Rh. appendiculatus*	7	OPB8	Laikipia	37N0261724	UTM0004581	Negative	Negative
15.	*Rh. appendiculatus*	7	OPB8	Laikipia	37N0261724	UTM0004581	Positive	Negative
16.	*Rh. evertsi evertsi*	7	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
17.	*Rh. evertsi evertsi*	7	OPZ16	Laikipia	37N0254829	UTM0007166	Positive	Negative
18.	*Rh. evertsi evertsi*	6	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
19.	*Rh. appendiculatus*	7	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
20.	*Rh. evertsi evertsi*	7	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
21.	*Rh. pulchellus*	2	OPZ16	Laikipia	37N0254829	UTM0007166	Negative	Negative
22.	*Rh. pulchellus*	5	OPZ17	Laikipia	37N0260025	UTM0004107	Negative	Negative
23.	*Rh. evertsi*	5	OPZ17	Laikipia	37N0260025	UTM0004107	Negative	Negative
24.	*Rh. evertsi*	5	OPZ17	Laikipia	37N0260025	UTM0004107	Positive	Negative
25.	*Rh. evertsi*	6	OPZ17	Laikipia	37N0260025	UTM0004107	Positive	Negative
26.	*Rh. evertsi*	6	OPZ17	Laikipia	37N0260025	UTM0004107	Negative	Negative
27.	*Rh. appendiculatus*	6	OPZ17	Laikipia	37N0260025	UTM0004107	Negative	Negative
28.	*Rh. appendiculatus*	5	OPB21	Laikipia	37M0273557	UTM0001236	Positive	Negative
29.	*Rh. appendiculatus*	5	OPB21	Laikipia	37M0273557	UTM0001236	Positive	Negative
30.	*Rh. appendiculatus*	5	OPB21	Laikipia	37M0273557	UTM0001236	Positive	Negative
31.	*Rh. appendiculatus*	6	OPB21	Laikipia	37M0273557	UTM0001236	Positive	Negative
32.	*Rh. Pulchellus*	5	OPB21	Laikipia	37M0273557	UTM0001236	Negative	Negative

Pool No.	Tick Species	No. of Ticks	Sample ID	Study Area	Latitude	Longitude	*Rickettsia*	*Coxiella*
33.	*Rh. appendiculatus*	6	OPB19	Laikipia	37M0273557	UTM0001236	Positive	Negative
34.	*Rh. appendiculatus*	5	OPB19	Laikipia	37M0273557	UTM0001236	Negative	Negative
35.	*Rh. appendiculatus*	5	OPB19	Laikipia	37M0273557	UTM0001236	Positive	Negative
36.	*Rh. appendiculatus*	7	OPB19	Laikipia	37M0273557	UTM0001236	Negative	Negative
37.	*Rh. Pulchellus*	1	OPB19	Laikipia	37M0273557	UTM0001236	Negative	Negative
38.	*Rh. evertsi evertsi*	5	OPZ19	Laikipia	37M0263623	UTM0003550	Negative	Negative
39.	*Rh. pulchellus*	2	OPZ19	Laikipia	37M0263623	UTM0003550	Positive	Negative
40.	*Rh. appendiculatus*	3	OPB27	Laikipia	37M0273557	UTM0001236	Negative	Negative
41.	*Rh. evertsi evertsi*	5	OPZ15	Laikipia	37N0252695	UTM0007978	Negative	Negative
42.	*Rh. pulchellus*	1	OPB13	Laikipia	37N0261724	UTM0004581	Negative	Negative
43.	*Rh. appendiculatus*	3	OPB13	Laikipia	37N0261724	UTM0004581	Negative	Negative
44.	*Rh. appendiculatus*	2	OPB13	Laikipia	37N0261724	UTM0004581	Negative	Negative
45.	*Rh. appendiculatus*	2	OPB13	Laikipia	37N0261724	UTM0004581	Negative	Negative
46.	*Rh. appendiculatus*	1	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Positive	Negative
47.	*Rh. pulchellus*	2	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
48.	*Rh. evertsi evertsi*	3	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
49.	*Rh. appendiculatus*	2	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
50.	*Rh. evertsi evertsi*	1	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
51.	*H. dromedari*	1	MM/TP/11	Maasai Mara	36N0763886	UTM9834659	Positive	Negative
52.	*Rh. evertsi evertsi*	4	OPZ18	Laikipia	37M0263623	UTM0003550	Positive	Negative
53.	*Rh. appendiculatus*	14	OPB6	Laikipia	37N0271408	UTM0006930	Positive	Negative
54.	*Rh. appendiculatus*	2	OPB6	Laikipia	37N0271408	UTM0006930	Positive	Negative
55.	*Rh. appendiculatus*	4	OPB20	Laikipia	37M0273557	UTM0001236	Positive	Negative
56.	*Rh. evertsi evertsi*	9	OPB20	Laikipia	37M0273557	UTM0001236	Positive	Negative
57.	*Rh. appendiculatus*	4	OPB16	Laikipia	37M0263623	UTM0003550	Negative	Negative
58.	*Rh. pulchellus*	3	OPZ26	Laikipia	37M0263099	UTM0006929	Positive	Negative
59.	*Rh. evertsi evertsi*	3	OPZ26	Laikipia	37M0263099	UTM0006929	Negative	Negative
60.	*Rh. evertsi evertsi*	11	OPZ25	Laikipia	37M0263099	UTM0006929	Negative	Negative
61.	*Rh. pulchellus*	5	OPZ25	Laikipia	37M0263099	UTM0006929	Negative	Negative
62.	*Rh. appendiculatus*	6	OPB4	Laikipia	37N0271408	UTM0006930	Positive	Negative
63.	*Rh. evertsi evertsi*	1	OPB4	Laikipia	37N0271408	UTM0006930	Negative	Negative
64.	*Rh. appendiculatus*	6	OPB4	Laikipia	37N0271408	UTM0006930	Negative	Negative
65.	*Rh. evertsi*	7	OPB4	Laikipia	37N0271408	UTM0006930	Negative	Negative
66.	*Rh. evertsi*	1	OPB17	Laikipia	37N0271408	UTM0006930	Negative	Negative
67.	*Rh. pulchellus*	2	OPZ3	Laikipia	37N0273869	UTM0002582	Positive	Negative
68.	*Rh. evertsi*	17	OPZ3	Laikipia	37N0273869	UTM0002582	Negative	Negative
69.	*Rh. pulchellus*	2	OPB5	Laikipia	37N0271408	UTM0006930	Negative	Negative
70.	*Rh. appendiculatus*	6	OPB5	Laikipia	37N0271408	UTM0006930	Positive	Negative
71.	*Rh. appendiculatus*	1	OPB5	Laikipia	37N0271408	UTM0006930	Positive	Positive
72.	*Rh. appendiculatus*	6	MM/WB/07	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
73.	*Rh. appendiculatus*	3	OPW1	Laikipia	37M0263828	UTM0003524	Positive	Negative
74.	*Rh. appendiculatus*	2	OPW1	Laikipia	37M0263828	UTM0003524	Negative	Negative
75.	*Rh. pulchellus*	10	OPZ13	Laikipia	37N0252695	UTM0007978	Negative	Negative

Pool No.	Tick Species	No. of Ticks	Sample ID	Study Area	Latitude	Longitude	*Rickettsia*	*Coxiella*
76.	*Rh. appendiculatus*	1	OPZ13	Laikipia	37N0252695	UTM0007978	Positive	Negative
77.	*Rh. evertsi*	10	OPZ13	Laikipia	37N0252695	UTM0007978	Negative	Negative
78.	*Rh. pulchellus*	2	MZ11	Laikipia	37N0252454	UTM0011656	Negative	Negative
79.	*Rh. evertsi*	2	MZ11	Laikipia	37N0252454	UTM0011656	Positive	Negative
80.	*Rh. evertsi*	16	MZ11	Laikipia	37N0252454	UTM0011656	Negative	Negative
81.	*Rh. pulchellus*	1	OPB18	Laikipia	37M0273557	UTM0001236	Negative	Negative
82.	*Rh. evertsi*	3	OPZ27	Laikipia	37M0273557	UTM0001236	Positive	Negative
83.	*Rh. appendiculatus*	1	MM/WB/01	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
84.	*Rh. appendiculatus*	2	MM/WB/11	Maasai Mara	36N0747904	UTM9836308	Negative	Negative
85.	*Rh. appendiculatus*	3	MM/HB/02	Maasai Mara	36N0751613	UTM9832092	Positive	Negative
86.	*Rh. appendiculatus*	1	MM/HB/03	Maasai Mara	36N0751613	UTM9832092	Negative	Negative
87.	*D. rhinocerinus*	4	B/RHINO	Laikipia	37N0271407	UTM0006934	Negative	Negative
88.	*Rh. appendiculatus*	2	OPZ18	Laikipia	37M0263623	UTM0003550	Negative	Negative
89.	*Rh. pulchellus*	2	OPB18	Laikipia	37M0263623	UTM0003550	Positive	Negative
90.	*Rh. appendiculatus*	5	OPB18	Laikipia	37M0263623	UTM0003550	Negative	Negative
91.	*Rh. pulchellus*	1	OPB28	Laikipia	37M0273557	UTM0001236	Positive	Negative
92.	*Rh. appendiculatus*	5	OPB28	Laikipia	37M0273557	UTM0001236	Negative	Negative
93.	*Rh. pulchellus*	4	OPB3	Laikipia	37N0271408	UTM0006930	Negative	Negative
94.	*Rh. appendiculatus*	7	OPB3	Laikipia	37N0271408	UTM0006930	Negative	Negative
95.	*Rh. evertis evertsi*	5	MM/WB/08	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
96.	*Rh. pulchellus*	4	OPZ14	Laikipia	37N0252695	UTM0007978	Negative	Negative
97.	*Rh. everti evertsi*	7	OPZ14	Laikipia	37N0252695	UTM0007978	Negative	Positive
98.	*Rh. pulchellus*	2	OPZ20	Laikipia	37M0263623	UTM0003550	Negative	Negative
99.	*Rh. evertsi evertsi*	5	OPZ20	Laikipia	37M0263623	UTM0003550	Negative	Negative
100.	*Rh. pulchellus*	1	OPB1	Laikipia	37N0271408	UTM0006930	Negative	Negative
101.	*Rh. appendiculatus*	9	OPB1	Laikipia	37N0271408	UTM0006930	Negative	Negative
102.	*Rh. appendiculatus*	5	B/RHINO	Laikipia	37N0271409	UTM0006931	Negative	Negative
103.	*Rh. evertsi evertsi*	5	OPZ21	Laikipia	37M0263623	UTM0003550	Negative	Negative
104.	*Rh. evertsi evertsi*	6	OPZ6	Laikipia	37N0273869	UTM0002582	Negative	Negative
105.	*Rh. evertsi evertsi*	6	OPZ7	Laikipia	37N0273869	UTM0002582	Negative	Negative
106.	*Rh. appendiculatus*	3	OPZ7	Laikipia	37N0273869	UTM0002582	Negative	Negative
107.	*Rh. appendiculatus*	4	OPB17	Laikipia	37M0263623	UTM0003550	Negative	Negative
108.	*Rh. appendiculatus*	6	OPB9	Laikipia	37N0261724	UTM0004581	Negative	Negative
109.	*Rh. appendiculatus*	3	OPB9	Laikipia	37N0261724	UTM0004581	Negative	Negative
110.	*Rh. pulchellus*	2	OPZ10	Laikipia	37N0273869	UTM0002582	Negative	Negative
111.	*Rh. evertsi evertsi*	5	OPZ10	Laikipia	37N0273869	UTM0002582	Negative	Negative
112.	*A. gemma*	5	OPB2	Laikipia	37N0271408	UTM0006930	Negative	Negative
113.	*Rh. evertsi evertsi*	5	OPB2	Laikipia	37N0271408	UTM0006930	Negative	Negative
114.	*Rh. evertsi evertsi*	3	MM/WB/10	Maasai Mara	36N0747904	UTM9836308	Negative	Negative
115.	*Rh. appendiculatus*	3	OPW2	Laikipia	37M0263828	UTM0003524	Negative	Negative
116.	*Rh. evertsi evertsi*	4	OPZ24	Laikipia	37M0263623	UTM0003550	Negative	Negative
117.	*H. albiparmatum*	2	MM/ZB/02	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
118.	*Rh. evertsi evertsi*	4	MM/ZB/02	Maasai Mara	36N0763886	UTM9834659	Negative	Negative

Pool No.	Tick Species	No. of Ticks	Sample ID	Study Area	Latitude	Longitude	*Rickettsia*	*Coxiella*
119.	*Rh. evertsi evertsi*	3	MM/WB/12	Maasai Mara	36N0747904	UTM9836308	Negative	Negative
120.	*Rh. pulchellus*	4	OPZ9	Laikipia	37N0273869	UTM0002582	Negative	Negative
121.	*Rh. evertsi evertsi*	3	OPZ9	Laikipia	37N0273869	UTM0002582	Negative	Negative
122.	*Rh. evertsi evertsi*	8	MM/WB/09	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
123.	*H. albiparmatum*	2	MM/ZB/01	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
124.	*Rh. evertsi evertsi*	7	MM/ZB/01	Maasai Mara	36N0761528	UTM9821048	Negative	Negative
125.	*Rh. evertsi evertsi*	7	OPZ8	Laikipia	37N0273869	UTM0002582	Negative	Negative
126.	*Rh. pulchellus*	9	OPZ12	Laikipia	37N0252695	UTM0007978	Negative	Negative
127.	*Rh. evertsi evertsi*	14	OPZ12	Laikipia	37N0252695	UTM0007978	Negative	Negative
128.	*Rh. pulchellus*	4	OPB7	Laikipia	37N0261724	UTM0004581	Negative	Negative
129.	*Rh. appendiculatus*	15	OPB7	Laikipia	37N0261724	UTM0004581	Negative	Negative
130.	*Rh. pulchellus*	1	OPZ22	Laikipia	37M0263623	UTM0003550	Negative	Negative
131.	*Rh. evertsi evertsi*	4	OPZ22	Laikipia	37M0263623	UTM0003550	Negative	Negative
132.	*Rh. evertsi evertsi*	3	MM/WB/02	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
133.	*Rh. appendiculatus*	3	MM/WB/08	Maasai Mara	36N0747904	UTM9836300	Negative	Negative
134.	*Rh. pulchellus*	4	OPB15	Laikipia	37N0261724	UTM0004581	Negative	Negative
135.	*Rh. appendiculatus*	12	OPB15	Laikipia	37N0261724	UTM0004581	Negative	Negative
136.	*Rh. pulchellus*	3	OPB16	Laikipia	37M0263623	UTM0003550	Negative	Negative
137.	*Rh. appendiculatus*	5	OPB16	Laikipia	37M0263623	UTM0003550	Negative	Negative
138.	*Rh. pulchellus*	4	OPB24	Laikipia	37M0273557	UTM0001236	Negative	Negative
139.	*Rh. appendiculatus*	7	OPB24	Laikipia	37M0273557	UTM0001236	Negative	Negative
140.	*Rh. evertsi evertsi*	10	MZ7	Laikipia	37N0245451	UTM0011794	Negative	Negative
141.	*Rh. evertsi evertsi*	5	MZ7	Laikipia	37N0245451	UTM0011794	Negative	Negative
142.	*Rh. evertsi evertsi*	6	MM/WB/04	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
143.	*Rh. evertsi everts*	5	MB2	Laikipia	37N0252450	UTM0010973	Negative	Negative
144.	*Rh. appendiculatus*	4	MM/TP/08	Maasai Mara	36N0763886	UTM9834659	Negative	Negative
145.	*A. variegatum*	2	MM/WB/05	Maasai Mara	36N0801852	UTM9865338	Positive	Negative
146.	*A. truncatum*	2	MM/WB/05	Maasai Mara	36N0801852	UTM9865338	Positive	Negative
147.	*Rh. evertsi evertsi*	5	MM/WB/02	Maasai Mara	36N0801852	UTM9865338	Negative	Negative
148.	*Rh. pulchellus*	1	MM/WB/09	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
149.	*Rh. appendiculatus*	7	MM/WB/09	Maasai Mara	36N0744202	UTM9838955	Negative	Negative
150.	*Rh. evertsi evertsi*	2	MZ8	Laikipia	37N0245451	UTM0011794	Negative	Negative
151.	*Rh. evertsi evertsi*	12	MB1	Laikipia	37N0252450	UTM0010973	Negative	Negative
152.	*Rh. pulchellus*	4	MB1	Laikipia	37N0252450	UTM0010973	Negative	Negative
153.	*Rh. appendiculatus*	7	OPB2	Laikipia	37N0271408	UTM0006930	Negative	Negative
154.	*Rh. evertsi evertsi*	3	OPB2	Laikipia	37N0271408	UTM0006930	Negative	Negative
155.	*Rh. pulchellus*	2	MZ12	Laikipia	37N0252454	UTM0011656	Negative	Negative
156.	*Rh. evertsi evertsi*	14	MZ12	Laikipia	37N0252454	UTM0011656	Negative	Negative
157.	*Rh. evertsi evertsi*	4	MZ2	Laikipia	37N0237520	UTM0008097	Negative	Negative
158.	*Rh. evertsi evertsi*	10	MZ10	Laikipia	37N0252454	UTM0011656	Negative	Negative
159.	*Rh. appendiculatus*	3	MZ10	Laikipia	37N0252454	UTM0011656	Negative	Negative
160.	*Rh. evertsi evertsi*	10	OPZ23	Laikipia	37M0263623	UTM0003550	Negative	Negative
161.	*Rh. pulchellus*	10	OPB12	Laikipia	37N0261724	UTM0004581	Negative	Negative

Pool No.	Tick Species	No. of Ticks	Sample ID	Study Area	Latitude	Longitude	*Rickettsia*	*Coxiella*
162.	*Rh. evertsi evertsi*	3	OPB12	Laikipia	37N0261724	UTM0004581	Negative	Negative
163.	*Rh. appendiculatus*	10	OPB12	Laikipia	37N0261724	UTM0004581	Negative	Negative
164.	*Rh. pulchellus*	10	OPZ4	Laikipia	37N0273869	UTM0002582	Negative	Positive
165.	*Rh. appendiculatus*	10	OPZ4	Laikipia	37N0273869	UTM0002582	Negative	Positive
166.	*Rh. evertsi evertsi*	20	OPZ4	Laikipia	37N0273869	UTM0002582	Negative	Negative
	Total	**851**						

Appendix 8: Sequences of detected SFG Rickettsia species in wildlife and ticks

No.	Accession numbers	Sample ID/ Host Species	Gene	Sequence
1.	KX244606	T8 /Topi (*Damaliscus korrigum*)	*glt*A partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA
2.	KX421818	OPB8 (Tick Pool 10)/ *Rh. evertsievertsi*	*glt*A partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA

3.	KX421822	OPB8 (Tick Pool 15)/ *Rh. appendiculatus*	*glt*A partial gene	CGTCGGCTTCGTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGA ACTTACCGCTATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCT ATAGGACAACCGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGA TGTTTGCAACGCCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGA TATTTATCCTACATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTC ATCCGGAGCTAACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCAC GGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCT AAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGT GTATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAG GAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCT CTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTA TCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGT AGGCTGGATGGCACAATGGAAAGAATC
4.	KX686602	OPB8 (Tick Pool 17)/ *Rh. evertsi evertsi*	*omp*B partial gene	TTCTAATACCCCTGGTACAGTTTATGGCTTAGGCACAGGTATTGGTGCTTCAAAGTTCAAGCAA GTAACGTTTACTACAGACTATAACAATTTAGGTAATATTATTGCAACTAACGCAACAATTAATG ATGGTGTAACTGTTACTACAGGCGGTATAGCCGGAATAGGTTTTGACGGTAAAATTACTCTTG GAAGTGTTAACGGTAACGGTAATGTAAGATTTGTTGACGGTATATTGTCTAATTCTACAAGTAT GATTGGTACTACTAAAGCTAATAATGGTACTGTAACTTATTTAGGTAATGCATTCGTCGGTAAT ATAGGTGATTCAGATACCCCTGTAGCTTCTGTTAGATTTACAGGTAGTGATGGTGGTGCAGGAT TACAAGGAAATATTTATTCACAAGTCATAGACTTTGGTACTTATAACTTAGGTATTTTAAATTC TAATGTAATTTTAGGCGGCGGTACTACTGCTATTAACGGTAAAATCAATCTTCTTACAAATACT TTAACATTTGCAAGTGGTACTTCAACATGGGGAAACAATGCTTCTATTGAAACTACTTTAACAT TAGCAAACGGTAATATAGGTAACATCGTTATTTTGGAAGGTGCGCAAGTTAATGCAACAACCA CAGGAACTACAACCATTAAAGTACAAGATAATGCCAATGCAAATTTCAGTGGTACACAAACTT ATACTTTAATCCAAGGTGGTGCTAGATTTAACGGTACTTTAGGAGGTCCCA
5.	KX421818	OPZ17 (Tick Pool 24) /(*Rh. evertsi*	*glt*A partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA

6.	KX421819	OPB21 (Tick Pool 29)/ *Rh. appendiculatus*	*glt*A partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA
7.	KX421820	OPB18 (Tick Pool 52)/ *Rh. evertsi evertsi*	*gltA* partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA
8.	KX421821	OPB6 (Tick Pool 53)/ *Rh. appendiculatus*	*gltA* partial gene	GTTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGAACTTACCGCT ATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCTATAGGACAAC CGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGATGTTTGCAACG CCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGATATTTATCCTAC ATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTCATCCGGAGCTA ACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCACGGCGGGGCTAA TGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAAATATATAGC TAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATAAAAA CTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATG AATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTATCTATAAAGC TATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGTAGGCTGGAT GGCACAATGGAAAGAA

9.	KX686600	MZ11/Tick Pool 79 (*Rh. evertsi*)	*ompA* partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG
10.	KX686599	OPZ27/Tick Pool 82 (*Rh. evertsi*)	*omp*A partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG
11.	KX686595	OPB18 (Tick Pool 89)/ *Rh. pulchellus*	*omp*A partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG
12.	KX686597	Opb18 (Tick Pool 91)/ *Rh. pulchellus*	*omp*A partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG

13.	KX421822	MM/WB/50 (Tick Pool 145)/ *A. variegatum*	*glt*A partial gene	CGTCGGCTTCGTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGA ACTTACCGCTATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCT ATAGGACAACCGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGA TGTTTGCAACGCCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGA TATTTATCCTACATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTC ATCCGGAGCTAACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCAC GGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCT AAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGT GTATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAG GAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCT CTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTA TCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGT AGGCTGGATGGCACAATGGAAAGAATC
	KX686596		*omp*A partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG
	KX686601		*omp*B partial gene	GGGGTACCACTCACCGCAGCGATATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTG TTATTGCTACTAATAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGAT AACGGCTGCAGGGGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTAC TTACGGTGGTGATTATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTT GCGGGTACTACTCCCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGG GAGGTAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGC TGTTGCTGCAAATCATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCT TTAGGGGGAGCGAATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCA GGCAATATAAATGGAGGAGGTTATAAAACCTGT

14.	KX421823	MM/WB/50 (Tick Pool 146)/ *A. truncatum*	*gltA* partial gene	CGTCGGCTTCGTCTCTTTCGGCATTTTATCCTGATTTATTGAATTTTAAGGAAGCAGATTACGA ACTTACCGCTATTAGAATGATTGCTAAGATACCTACCATCGCTGCAATGTCTTATAAATATTCT ATAGGACAACCGTTTATTTATCCTGATAATTCGTTAGATTTTACCGAAAATTTTCTGCATATGA TGTTTGCAACGCCTTGTACGAAATATACAGTAAATCCAATAATAAAAAATGCTCTTAATAAGA TATTTATCCTACATGCCGATCATGAGCAGAATGCTTCTACTTCAACAGTCCGAATTGCCGGTTC ATCCGGAGCTAACCCTTTTGCTTGTATTAGCACGGGTATTGCCTCACTTTGGGGACCTGCTCAC GGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCT AAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGT GTATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAG GAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCT CTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCCGGTATTA TCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACGGTACTTTTTGCAATAGCAAGAACTGT AGGCTGGATGGCACAATGGAAAGAATC
	KX686595		*ompA* partial gene	CGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTATTGCTACTAA TAATAATGCAACATTTAGTGATAATGTTGGCAATAATAATTGGAATGAGATAACGGCTGCAGG GGTAGCTAATGGTGCTCCTGCTGGCGGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGAT TATACTATCACTGCAGATGCAGCCGATCGTATTATTACGGCTATAAATGTTGCGGGTACTACTC CCGTAGGTCTAGATATTGCTCAAAATACCGTTGTTGGTTCGATTATAACGGGAGGTAACTTGTT GCCTGTTACTATTACTGCCGGCAAAAGCTTAACTTTAAACGGTAATAATGCTGTTGCTGCAAAT CATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAGCTTTAGGGGGAGCG AATGCTGCACTAATTATACAATCTGCAGCCCCGGCAAAGATAACACTTGCAGGCAATATAAAT GGAGGAG
	KX686601		*ompB* partial gene	CCAATGCGGTCATCTATACCCCTGGTACAGTTTATGGCTTAGGCACAGGTATTGGTGCTTCAAA GTTCAAGCAAGTAACGTTTACTACAGACTATAACAATTTAGGTAATATTATTGCAACTAACGC AACAATTAATGATGGTGTAACTGTTACTACAGGCGGTATAGCCGGAATAGGTTTTGACGGTAA AATTACTCTTGGAAGTGTTAACGGTAACGGTAATGTAAGATTTGTTGACGGTATATTGTCTAAT TCTACAAGTATGATTGGTACTACTAAAGCTAATAATGGTACTGTAACTTATTTAGGTAATGCAT TCGTCGGTAATATAGGTGATTCAGATACCCCTGTAGCTTCTGTTAGATTTACAGGTAGTGATGG TGGTGCAGGATTACAAGGAAATATTTATTCACAAGTCATAGACTTTGGTACTTATAACTTAGGT ATTTTAAATTCTAATGTAATTTTAGGCGGCGGTACTACTGCTATTAACGGTAAAATCAATCTTC TTACAAATACTTTAACATTTGCAAGTGGTACTTCAACATGGGGAAACAATGCTTCTATTGAAAC TACTTTAACATTAGCAAACGGTAATATAGGTAACATCGTTATTTTGGAAGGTGCGCAAGTTAAT GCAACAACCACAGGAACTACAACCATTAAAGTACAAGATAATGCCAATGCAAATTTCAGTGGT ACACAAACTTATACTTTAATCCAAGGTGGTGCTAGATTTAACGGTACTTTAGGAGGTCCCAACT TTACCCCCCGAGAAA

Appendix 9: GenBank BLAST hit results of *C. burnetii* isolated in ticks

No	Strain	Genbank accession number	Identity	E value	Host species	Country
1.	Cb175	HG825990.3	100%	1E-58	Human	Guyana
2.	Namibia	CP007555.1	100%	1E-58	Goat	Namibia
3.	3345937	CP014354.1	100%	1E-58	Goat	Netherlands
4.	3262	CP013667.1	100%	1E-58	Goat	Netherlands
5.	EVC13	KT391020	100%	1E-58	Goat	France
6.	EVC282	KT391019.1	100%	1E-58	Goat	France
7.	EVC286	KT391018.1	100%	1E-58	Goat	France
8.	EVC477	KT391017.1	100%	1E-58	Goat	France
9.	EVC13 WGPS 7	KT381466.1	100%	1E-58	Goat	France
10.	EVC13 WGPS 21-22-23	KT381467.1	100%	1E-58	Goat	France
11.	Z3055	LK937696.1	100%	1E-58	Sheep	Germany
12.	CbuK Q154	CP001020.1	100%	1E-58	Human	USA
13.	CbuK Q212	CP001019.1	100%	1E-58	Human	Canada
14.	RSA 331	CP000890.1	100%	1E-58	Human	Italy
15.	WAV IS1111A	DQ882629.1	100%	1E-58	Human	USA
16.	WAV IS1111A	DQ882624.1	100%	1E-58	Human	USA
17.	WAV IS1111A	DQ882623.1	100%	1E-58	Human	USA
18.	Q238 WAV IS1111A	DQ882614.1	100%	1E-58	Human	USA
19.	Q195 WAV IS1111A	DQ882613.1	100%	1E-58	Goat	Idaho, USA
20.	Q195 WAV IS1111A	DQ882611.1	100%	1E-58	Goat	Idaho, USA
21.	Q195 WAV IS1111A	DQ882610.1	100%	1E-58	Goat	Idaho, USA
22.	Q195 WAV IS1111A	DQ882609.1	100%	1E-58	Goat	Idaho, USA
23.	PAV Q173 IS1111A	DQ882605.1	100%	1E-58	Human,	California, USA
24.	PAV Q173 IS1111A	DQ882601.1	100%	1E-58	Human,	California, USA
25.	PAV Q173 IS1111A	DQ882600.1	100%	1E-58	Human	California, USA
26.	KAV Q154 IS1111A	DQ882589.1	100%	1E-58	Human	Oregon, USA

No	Strain	Genbank accession number	Identity	E value	Host species	Country
27.	KAV Q154 IS1111A	DQ882586.1	100%	1E-58	Human	Oregon, USA
28.	KAV Q154 IS1111A	DQ882585.1	100%	1E-58	Human	Oregon, USA
29.	KAV Q154 IS1111A	DQ882584.1	100%	1E-58	Human	Oregon, USA
30.	RSA 343 IS1111A	DQ882575.1	100%	1E-58	Human	Italy
31.	RSA 343 IS1111A	DQ882574.1	100%	1E-58	Human	Italy
32.	RSA 493	AE016828.2	100%	1E-58	*Dermacentor andersoni* (Tick)	Montana, USA
33.	RSA 493 close 7 IS1111A	M80806.1	100%	1E-58	*Dermacentor andersoni* (Tick)	Montana, USA

Appendix 10: Sequences of detected *C. burnetii* in ticks collected in Laikipia

No.	Sample ID/Host Species	Gene	Sequence	Accession Number
1.	OPB5 (Tick Pool 71)/ *Rh. appendiculatus*	IS *1111a* Partial sequence	GCTCCTCCACACGCTTCCATCACCACGCAGCCCACCTTAAGACTGGCTACG GTGGATACATACTGAGCACGCTTAACCCGTCTCGTGTAGATCACTTTACCC CACTCATCAATTCCACACAGTTGAACC	KU994893
2.	OPZ14 (Tick Pool 97)/ *Rh. evertsi evertsi*	IS *1111a* Partial sequence	GCTCCTCCACACGCTTCCATCACCACGCAGCCCACCTTAAGACTGGCTACG GTGGATACATACTGAGCACGCTTAACCCGTCTCGTGTAGATCACTTTACCC CACTCATCAATTCCACACAGTTGAACC	
3.	OPZ4 (Tick Pool 164)/ *Rh. pulchellus*	IS *1111a* Partial sequence	GCTCCTCCACACGCTTCCATCACCACGCAGCCCACCTTAAGACTGGCTACG GTGGATACATACTGAGCACGCTTAACCCGTCTCGTGTAGATCACTTTACCC CACTCATCAATTCCACACAGTTGAACC	