

**SCREENING FOR TICK-BORNE ZOO NOTIC PATHOGENS IN TICKS COLLECTED
FROM LIVESTOCK IN KENYA USING REAL TIME POLYMERASE CHAIN
REACTION**

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**A thesis submitted to the Graduate School in partial fulfillment of the requirement for
Award of the Master of Science Degree in Medical Parasitology at Egerton University**

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any University.

Signature

Date.....

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SM17/3055/11

RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

To my late Dad, living Mum and other family members like Naomi Akoth, Esther Achieng, Hider Anyango, Silas Ochieng, Habakuk Okumu, Japheth Otieno, Elizabeth Akeyo, Walter Odhiambo, Beatrice Achieng, Mercy Adhiambo, and Silvia Atieno.

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ABSTRACT

Zoonoses are infectious diseases that can be transmitted (in some instances, by a vector) from animals to humans or from humans to animals (the latter is sometimes called reverse zoonosis or anthroponosis). Among these are *Babesia spp.*, *Borrelia spp.*, *Rickettsia spp.*, *Ehrlichia spp.*, *Bartonella spp.*, *Anaplasma spp.*, *Francisella tularensis*, *Coxiella burnetii* and certain encephalitis virus that are transmitted by ticks. Of the 1415 pathogens known to affect humans, 61% are zoonotic. The diseases associated with these zoonotic pathogens present more or less similar symptoms to malaria resulting in misdiagnosis and wrong prescriptions which in turn contribute to undue over treatment for malaria, which in the long run lead to development of resistance to anti-malarial drugs. This information could be used to guide diagnosis, patient management and disease control. To achieve these objectives, archived nucleic acid samples that were obtained from ticks collected from livestock were used. These abattoirs receive animals for slaughter from all over the country, (including the country's frontiers). In this study, 503 tick DNA samples that were collected from 982 cattle, 300 sheep and 379 goats from 20 counties in Kenya were screened for zoonoses using either single-plex or multiplex Real Time Polymerase Chain Reactions (RT-PCR). The assays were run in a 7500 Fast PCR machine (Applied Biosystem, USA). The data obtained was analyzed by Graphpad Prism version 5 by comparing the prevalence of each pathogen per county against the various tick species and also against the livestock from which the ticks were obtained. This study provides a catalogue of the geographical distribution of tick borne zoonoses in Kenya.

The overall prevalence showed that *Coxiella burnetii* was the most abundant 70% (74/106) of the positive samples while the lowest prevalence was in both *Anaplasma* and *Ehrlichia* <1% (1/106) although *Bartonella* was not detectable. Infections rates across the ticks species showed *Rh. pulchellus* as the most infected 38% (39/106) with highest infection of *C. burnetii* 70% (28/39) while the lowest prevalence was in *Hyalomma spp* 4% (4/106) with the highest prevalence of *Babesia* 75% (3/4). Out of the 20 counties that the animals came from, infected ticks came from 14 (70%) counties. Four of these carried the highest burden: Marsabit 25% (n=26/106), Kajiado 17% (18/106), Wajir 16% (17/106) and Narok 11% (12/106).

Based on these findings, there is need for a follow up research on humans for these zoonotic pathogens and a sensitization programme to the clinicians on existence of diseases associated with the pathogens.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACUC	Animal Care and Use Committee
BHQ	Black Hole Quencher
CCHFV	Crimean Congo Haemorrhagic Fever Virus
CDC	Centre for Disease Control
CT	Cycle threshold
ddH ₂ O	double distilled water
DEET	<i>N, N</i> -diethyl- <i>m</i> -toluamide
DEID	Department of Emerging and Infectious Diseases
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
ERC	Ethical Review Committee
GP	General Practitioner
HGA	Human Granulocytic Anaplasmosis
HE	Human Granulosa Ehrlichiosis
KEMRI	Kenya Medical Research Institute
KMC	Kenya Meat Commission
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
Q fever	Query fever
qPCR	Quantitative Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic acid

SSC	Scientific Steering Committee
TAE	Tris-Acetate-Ethylenediaminetetraacetic acid
WRP	Walter Reed Project

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Ticks are vectors of disease pathogens, making tick-borne diseases very common in the world (Morozova *et al.*, 2002). There are more than 800 species of blood sucking ticks that inhabit the planet and are second only to mosquitoes as vectors of disease pathogens. Tick developmental stages need to ingest a blood meal for complete metamorphosis (Cortinas *et al.*, 2002). They take their requisite blood meal from various classes of vertebrates (mammals, reptiles and birds). On the host's skin the tick inserts its hypostome, a central piercing element with hooks. Some ticks secrete a cementing material to fasten themselves to the host while others like the *Ixodes species* secrete pharmacological molecules that have anticoagulants, immunosuppressive and/or anti-inflammatory properties (Okuthe & Buyu, 2006).

Vector-borne infectious diseases are emerging and/or resurging as a result of changes in public health policy (e.g uncontrolled sale of antibiotics), insecticide and drug resistance, shift in emphasis from prevention to emergency response, demographic and societal changes and genetic changes in the pathogens. Effective prevention strategies can reverse this trend. Research on vaccines, environmentally safe insecticides, alternative approaches to vector control and training programs for healthcare workers are needed (Gubler, 1998). Evidence of reemergence of vector-borne diseases such malaria and dengue was first observed in the 1970s in Asia and Americas. Warnings were, however, largely ignored until recently and now it may prove to be very costly to reverse the trend. After World War II, a number of viral, protozoan and bacterial tick borne diseases were described in animals and in humans. Ticks may act not only as vectors but also as reservoirs of tick-transmitted bacteria including the spotted fever group rickettsiae, recurrent fever borreliae and *Francisella tularensis*. In these cases, the bacteria are transmitted transtadially (from stage to stage-from larvae to nymph and adults) and also transovarially-from one generation to the next via the female ovaries. Despite the information available on ticks, there is no recent review on ticks and tick-borne bacterial diseases (Parola & Raoult, 2001). The research reported here focuses on tick-borne zoonosis in Kenya.

Because of their feeding habits, ticks transmit diverse pathogens including protozoa (like *Babesia*, *Anaplasma*), bacteria (such as spirochetes, *Rickettsiae*, *Ehrlichia spp.*, *Bartonella spp.*, *Coxiella spp.*, *Borrelia spp.*) and viruses (such as Crimean Congo Haemorrhagic Fever Virus, viral encephalitis, among others) (Parola & Raoult, 2001). Some of the diseases associated with ticks include Babesiosis and Anaplasmosis (Protozoan), Lyme disease, rickettsiosis (such as African tick bite fever), Ehrlichiosis, Bartonellosis, Q fever (bacterial) while viral infections include tick borne meningoencephalitis and Crimean- Congo hemorrhagic fever (Jongejan & Uilenberg, 2004).

1.2 Statement of the Problem

Ticks are excellent vectors for diseases, making tick borne diseases to be common. Many of the tick-borne zoonoses present similar symptoms to malaria, causing a lot of misdiagnosis which results in wrong prescriptions. Livestock and human interaction make ticks to prevail in the immediate environment of humans. Traditional livestock rearing methods in Kenya promote multiplication of ticks since the livestock are rarely washed with acaricides nor treated for the pathogens they carry. These practices increase the likelihood of transmitting tick borne zoonoses. In some pathogens, once the tick is infected, it remains a carrier for life and in other instances, the infections are transmitted to fertilized eggs and subsequent developmental stages. This maintains a generation of infected ticks which again improve their chances of passing infection to humans. Very little information is available about the prevalence and distribution of these pathogens in Kenyan livestock, yet animals and humans live in close proximity. This study provides important datasets that are needed for understanding the diversity of tick borne zoonoses and their distribution in Kenya. This information could be used to guide diagnosis, patient management and disease control.

1.3 Objectives

1.3.1 General Objective

To determine the variety of tick-borne zoonoses in ticks obtained from livestock presented at abattoirs in Kenya.

1.3.2 Specific Objectives

1. To determine the prevalence of the following tick-borne zoonotic pathogens: *Coxiella burnetii*, *Babesia spp.*, *Borrelia burgdorferi* , other *Borrelia spp.*, *Anaplasma spp.*, *Ehrlichia spp.* and *Bartonella spp.*,
2. To determine the difference in infection rates of tick-borne pathogens in the different tick species.
3. Provide a catalogue of the geographical distribution of tick borne zoonotic pathogens in Kenya.

1.4 Hypotheses

1. There is no significant difference in infection rates of tick-borne pathogens in different tick species.
2. There is no significant difference in geographical distribution pattern of tick borne zoonotic pathogens in different regions of Kenya.

The first specific objective on prevalence does not require a hypothesis.

1.5 Justification

Very little information is available about the prevalence and distribution of tick borne zoonosis in Kenya. This is despite the fact that ticks are excellent vectors for diseases, making tick borne diseases to be common. Added to this is fact that the disease they cause present similar symptoms to malaria, and thereby increasing the likelihood of misdiagnosis. This study provides important datasets that are needed for understanding the diversity of tick borne zoonoses and their distribution in Kenya. This information could be used to guide diagnosis, patient management and disease control.

1.6 Limitations of the study

The number of samples for the study went down to 503 from 596 in the original study as some samples got depleted during the previous study. This also made the number of counties to come down to 20 from the initial 32. The data analysis from the small sample size may not have been the same if it would have been done from a larger sample size. This situation could not have been remedied because of the resources available and also due to time required to complete the study. Nevertheless, the data obtained will form a basis for a much larger study in future.

There was the possibility that the pathogen identified on the ticks collected from a particular host had been acquired from a totally different host species. A pathogen which was originally in a goat that was fed on by a tick which could pick the infection and drop down, later to feed on a cow and recovered while still on the cow. Such a tick was presumed to have been on a cow.

CHAPTER TWO

LITERATURE REVIEW

There is a continuing increase in the general practitioner (GP) consultations for erythema migrans caused by ticks and hospital admissions. For example, in the Netherlands 22,000 cases of tick bites were recorded (Wielinga *et al.*, 2006). The most straightforward explanation for the increase in the number of GP consultations due to tick bites is the probability due to the increase in the human recreational behavior, leading to an increased exposure to tick bites (Klasen *et al.*, 2011). In Africa, a large population of people live in close proximity to animals and are probably exposed to tick bites fairly frequently. Apart from causing inflammatory reaction at tick bite sites, ticks carry etiological agents of many protozoa (like *Babesia* and *Anaplasma*), bacteria (such as spirochetes, *Rickettsiae*, *Ehrlichia spp.*, *Bartonella spp.*, *Coxiella spp.*, *Borrelia spp.*) and viruses (such as Crimean Congo Haemorrhagic Fever Virus, viral encephalitis, among others) (Parola & Raoult, 2001). Although there are a number of studies that have documented serological evidence of human exposure to tick bite pathogens, few studies have attempted to detect infectious agents in the ticks. For example, a recent serological survey in Egypt documented the continued presence of rickettsial agents, *Bartonella*, *Coxiella* (Q fever) and *Ehrlichia*. Little data is available on the presence of these agents in the ectoparasites that might be transmitting these pathogens from animals to humans.

Babesiosis

Is a blood parasite that causes haemolytic disease called babesiosis with two common species that frequently infect humans, namely *Babesia microti* and *B. divergens* (Fritz *et al.*, 1997). *Babesia* species are close relatives of *Plasmodium* and infect the red blood cells of humans and animals worldwide. The disease causes a serious illness to wild and domesticated animals especially cattle, horses and dogs (Burri *et al.*, 2011). *Babesia spp* are transmitted by hard ticks such as *Ixodes scapularis* (black legged tick). A study on 125 non human primates in Kenya indicated a prevalence of 22% (27/125) of *B. microti* infections in free-ranging non human primates (Maamun *et al.*, 2011), a clear indication of high prevalence of this disease. The symptoms are similar to malaria taking 1-8 weeks to appear and include fever and chills, general weakness, gastrointestinal symptoms (nausea, vomiting, diarrhoea and belly pain), headache, muscle pain, joint pain, haemolytic anaemia, jaundice, dark urine, shortness of breath, swollen

spleen, arm and leg pain. Complications from severe, untreated or under-treated disease include acute respiratory distress syndrome, severe anaemia, congestive heart failure, disseminated intravascular coagulation. Confirmatory diagnosis include indirect fluorescent antibody, ELISA and PCR. Treatment involve drug combinations such as atovaquone (antiprotozoal) + oral azithromycin (antibiotic), oral quinine (antimalarial) + oral/intravenous clindamycin-antibiotic (Vannier & Krause, 2012). Control is by avoiding tick exposure through using a variety of methods and screening blood meant for transfusion for *Babesia*.

Anaplasmosis

Anaplasmosis in human is a tickborne disease caused by the bacterium *Anaplasma phagocytophilum*. It was previously known as human granulocytic ehrlichiosis (HGE) and has more recently been called human granulocytic anaplasmosis (HGA). HGA is transmitted to humans by tick bites primarily from the black-legged tick (*Ixodes scapularis*) and the western black-legged tick (*Ixodes pacificus*). Of the four distinct phases in the tick life-cycle (egg, larvae, nymph, adult), nymphal and adult ticks are most frequently associated with transmission of anaplasmosis to humans. The bacteria infect neutrophils, causing changes in gene expression that prolong the life of these otherwise short-lived cells (Lee *et al.*, 2008). Typical symptoms include: fever, headache, chills, and muscle aches. Usually, these symptoms occur within 1-2 weeks of a tick bite. Anaplasmosis is initially diagnosed based on symptoms and clinical presentation, and later confirmed by the use of specialized laboratory tests like PCR. The first line treatment for adults and children of all ages is doxycycline. Anaplasmosis and other tickborne diseases can be prevented (Dumler *et al.*, 2005).

Borreliosis

Lyme disease is caused by a spirochete bacterium called *Borrelia burgdorferi* and *B. afzelii* transmitted through bite of *Ixodes scapularis*. It has a global distribution and has a major public health and economic effects (Gubler, 1998). The disease was first noticed in 1975 in children who had rheumatoid arthritis but was not diagnosed until 1982 (Fritz *et al.*, 1997). The prevalence and distribution of Lyme disease in sub-Saharan Africa and in Kenya is unknown (Jowi & Gathua, 2005). The disease occurs in phases: an early localized disease with skin inflammation, disseminated disease with heart and nervous system involvement and the late

disease that include motor and sensory nerve damage, brain inflammation and arthritis. The inflammation of the skin is accompanied by a generalized fatigue, muscle and joint stiffness, swollen lymph nodes and headache. After one month without treatment, the inflammation resolves but the disease may return with involvement to all body parts. Later phases of the disease affect the heart causing inflammation of heart muscles resulting in abnormal heart rhythms and heart failure. The nervous system can develop facial muscle paralysis (Bell's palsy), abnormal sensation due to disease of peripheral nerves (peripheral neuropathy), meningitis and confusion (The International Lyme and Associated Diseases Society, 2004). Diagnosis is by finding classic rash in people who have been in endemic areas. At later stages, antibody or nucleic acid test can be done to detect Lyme bacteria using ELISA and PCR (Chang *et al.*, 1998). Treatment involves the use of antibiotics such as oral doxycycline. Prevention is by avoiding exposure to ticks and or using tick repellants. So far in East Africa, only two cases of Lyme disease have been reported in Kenya (Jowi & Gathua, 2005).

Rocky Mountain Spotted Fever

Many *Rickettsiae spp* are spread by ticks and cause mild infections, but some such as rocky mountain spotted fever (also called Tick typhus) can be lethal. The rocky mountain spotted fever is caused by *Rickettsia rickettsii* that is spread by *Dermacentor* ticks (Gubler, 1998). It remains a serious and potentially life threatening zoonotic disease today despite availability of effective treatment and advances in medical care with approximately 3-5% of patients who become ill dying from the infection. Infected ticks carry the pathogens throughout their lives, hence maintain a generation of infected ticks. In Kenya, a fatal case of spotted fever rickettsiosis was reported in a missionary from the United States who lived in a rural town (Kijabe) in the central county of Kenya, approximately 70 km north of Nairobi. Although the patient received prompt medical attention at a private hospital, her illness was initially diagnosed as malaria, and this misdiagnosis possibly contributed to the fatal outcome (Rutherford *et al*, 2004). A recent study conducted in Kenya on *Rickettsia* detection showed that several counties like Kiambu, Nandi, Meru, Machakos, Isiolo, West Pokot, Trans Nzoia, Narok, Laikipia and Elgeyo Marakwet are hot spots with a prevalence of over 20% (Mutai *et al.*, 2013). Other *Rickettsia* bacteria are transmitted by ticks such as *Dermacentor variabilis* (American dog tick), *D. andersoni* (wood tick), *Rhipicephallus sanguineus* (brown dog tick) and *Amblyomma cajennense* (Fritz *et al.*,

1997). Symptoms include fever, abnormal pain, muscle pain, flu like feeling with headache, vomiting, fatigue, loss of appetite, conjunctivitis in children, neurological signs like stiff neck and paralysis and severe case of reduced ability of blood to clot hence risk of internal bleeding (Parola *et al.*, 2005). Diagnosis involves immunohistochemistry of skin biopsy at bite site, blood test using methods such as immunofluorescence, ELISA and PCR. Treatment involves the use of antibiotics such as Doxycycline. Prevention is by avoiding areas where ticks are common, protective clothing, use of insect repellent containing DEET and careful checking of the body for ticks.

Q fever

This is a zoonosis caused by *Coxiella burnetii*, an obligate gram-negative coccobacillus intracellular bacterium. It infects various hosts including humans, ruminants and pets and in rare cases reptiles, birds and ticks. The bacterium is excreted in urine, milk, faeces and birth products (Kirkan *et al.*, 2008). These products especially birth products contain a large number of bacteria that become aerosolized after drying. In Kenya, Q fever has been reported among travelers to Maasai Mara (Loftis *et al.*, 2006). Investigation found that 4 (8%) of 50 safari travelers to Kenya contracted Q fever, 2 travelers developed overt infection, whereas 2 others developed asymptomatic illness. *C. burnetii* is highly infectious and even a few cells can cause disease). It has a life cycle that produces spores hence can remain viable and virulent for months and infection can be acquired via inhalation or skin contact or sexual transmission. The pathogen can be transmitted to humans from ticks through salivary secretions and feces (Parola & Raoult, 2001). *C. burnetii* can infect people with no known contact with animals. Incubation period is 9-40 days. Its complications may include; acute respiratory distress, thrombocytopenia, endocarditis, spontaneous abortion, meningoencephalitis, chronic fatigue syndrome. About 60% of the patients are asymptomatic (Panning *et al.*, 2008).

Ehrlichiosis

Ehrlichiosis in humans is called human monocytic ehrlichiosis, is caused by a bacterium called *Ehrlichia chaffeensis* that is transmitted by bite of an infected tick. Infected tick must attach for at least 24 hours for the disease to spread (Jongejan & Uilenberg, 2004). A survey for antibodies against *Ehrlichia chaffeensis* in human sera from eight African countries indicated that human

ehrlichiosis is common (Maamun *et al.*, 2011). Symptoms include fever, chills, muscle aches, weakness, headache, confusion, nausea, vomiting and joint pain. Infection produces mild to moderately severe illness, with high fever and headache but may occasionally be life threatening or fatal. Incubation period is 1-3 weeks after tick exposure. Laboratory tests include white blood cell count, low platelet count, abnormal liver enzyme test, ELISA and PCR (Petrovec *et al.*, 1999). Treatment involves the use of tetracycline antibiotics. Prevention requires avoiding tick bites, wearing light coloured clothes, applying repellents with DEET and removing ticks with tweezers, grasping ticks mouthparts close to skin and pulling it straight out (Fritz *et al.*, 1997).

Bartonellosis

Is an infectious disease produced by bacteria of the genus *Bartonella*. These bacteria live inside cells and can infect humans, mammals and a wide range of wild animals. The bacteria are carried by fleas, body lice and ticks (Maguina & Gotuzzo, 2000). Ticks are a serious source of infection in humans. More research needs to be done to establish the role of ticks in spreading the disease. The disease is often mild but in serious cases, it can affect the whole body. Early signs include fever, fatigue, headache, poor appetite and unusual streaked rash. Swollen glands are typical, especially around the head, neck and arms. Gastritis, lower abdominal pain, sore soles and tender subcutaneous nodules along extremities, enlarged lymph nodes and sore throat. Unusual manifestations like granulomatous conjunctivitis, neuroretinitis, atypical pneumonia or endocarditis may occur in small percentage of patients. Case fatality ratio may exceed 40% in untreated people. Bacillary angiomatosis (caused by *B. henselae* or *B. quintana*) and peliosis hepatis (caused by *B. henselae*) occur in people primarily infected with HIV. They may present as skin, subcutaneous and bone lesions (Adamska *et al.*, 2010). Diagnosis is by PCR and tissue biopsy (insensitive as standard blood tests). Most cases of bartonellosis are now diagnosed by tests based on PCR or through serological tests using specific antigens. Treatment has been done using Erythromycin and Doxycycline successfully for standard *Bartonella* but tick borne *Bartonella*, Levofloxacin is recommended or Azithromycin for children under the age of 18 years.

Meningoencephalitis

A number of meningoencephalitis are spread by tick borne flaviviruses. Though a neurological disorder, mild fever can occur and long lasting or permanent neuropsychiatric sequelae are observed in 10-20% of infected patients. The number of reported cases have been on the increase in most countries and the virus infect a wide range of hosts like ruminants, birds, rodents, carnivores, horses and humans (Burri *et al.*, 2011). Ruminants and dogs are the principal sources of infection to humans. The virus infects brain causing encephalitis or the meninges resulting in meningitis or both resulting in meningoencephalitis. Death occurs 5-7 days after onset of neurological signs. Transmission is by infected tick bite. Known vectors of the disease include *Ixodes scapularis*, *I. ricinus* and *I. persulcatus* or rarely through non pasteurized milk of infected cows. Testing is serological with ELISA. Treatment include anti inflammatory drugs like corticosteroids for relief of symptoms. There is no cure.

Crimean-Congo hemorrhagic fever (CCHF)

Is a disease caused by a tick borne virus called Nairovirus in the family Bunyaviridae spread by ixodid (hard) ticks which acts as reservoir and vector while numerous wild and domestic animals like cattle, sheep, goats and hares serve as amplifying hosts (Estrada *et al.*, 2012). Humans get infected by contact with infectious blood or body fluids or in hospitals due to improper sterilization of medical equipment, re-use of injection needles and contaminated medical supplies. In Kenya, CCHFV has been detected on only 2 occasions: in *Rhipicephalus pulchellus* ticks collected in the 1970s from a dying sheep in a veterinary laboratory in the town of Kabete outside Nairobi and from a previously healthy 25-year-old male farmer who was admitted to a mission hospital in western Kenya in October 2000 with an acute hemorrhagic illness (Sang *et al.*, 2006) . Signs and symptoms include headache, high fever, back pain, joint pain, stomach pain and vomiting. Red eyes, a flushed face, a red throat and petechiae (spots) on palate being common. Fatality rates from documented evidence range from 9%-50% (Garrison *et al.*, 2007). Laboratory diagnostic methods include antigen-capture ELISA, antibody detection by ELISA, virus isolation attempts and real time PCR (Yapar *et al.*, 2005). Treatment is primarily supportive care. Prevention require the use of insect repellents with DEET, wearing gloves and protective clothing, avoiding contact with blood and body fluids of livestock or humans that show symptoms of infection (Bente *et al.*, 2013).

2.1 Transmission of tick borne zoonotic pathogens

The infectivity of the reservoir hosts, the tick infestation rate and the host density are major variables determining epidemiology of tick transmitted diseases. These are influenced by preference for the host of the different stages of ticks, degree of tick-host contact, seasonal activity of both ticks and host, susceptibility of preferred host to the bacteria, environmental conditions and host immunity (Corrain *et al.*, 2012). The proportion of ticks that acquire bacterial infection may increase with the duration of their attachment on the reservoir host while feeding like in the case of *B. burgdorferi* infections. Transmission occurs more with increased attachment time but indirect routes of transmission are also possible like contamination of a braided skin or the eyes following crushing of ticks with fingers.

Ticks attach on people at numerous sites but are most frequently found around the head and neck and in the groin like the tick vector that transmits Rocky Mountain spotted fever disease favoured the head and neck (59%), tick vector that transmits *Ehrlichia* favoured lower extremities and buttocks and groin (54%), while *Dermacentor spp.* ticks prefer the head region (Parola & Raoult, 2001). Possible reason for the preferred sites is not known. *Ixodes* ticks do not cause pain while feeding and the immature stages are frequently not detected on people because of their sizes. Some ticks can transmit several pathogenic bacteria like the *Ixodes scapularis* a vector of *B. burgdorferi*, *Ehrlichia spp.*, agents of Ehrlichiosis and may also transmit babesiosis a protozoan disease due to *Babesia microti*. Furthermore, one tick may be infected with all three organisms, hence humans may be infected with multiple organisms if fed upon by such ticks. Such multiple infections may result in typical clinical manifestations of tick borne diseases (Bonnet *et al.*, 2013). In animals, primarily cattle, sheep and goats, *C. burnetii* can cause abortion and infertility as it localizes in the female reproductive system. High doses of *C. burnetii* have been found in birthing products of infected animals. The pathogen is shed in the urine, feces and milk of infected animals. In general, infected animals remain asymptomatic. Instead they often serve as the source of infection to humans via infective aerosols or contaminated dust. *C. burnetii* is very resistant to environmental conditions and can remain infectious for a considerable time outside the host cell (Panning *et al.*, 2008). *Babesia microti* shares a tick vector with *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, the causative agents of Lyme disease and human granulocytic anaplasmosis (HGA), respectively. Recent studies suggest that exposure to *Babesia microti* is quite common in areas endemic for Lyme

disease and anaplasmosis, so it is often prudent to test for all three diseases concurrently (Krause *et al.*, 1996). Owing to the shared tick vectors for *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Babesia microti*, and tick-borne encephalitis viruses, approximately 10% of patients have serologic evidence of co-infection, and cases of simultaneous Lyme disease or tick-borne encephalitis are well documented (Dumler *et al.*, 2007).

A lot of the symptoms of these tick-borne infections like Lyme disease are similar to the common diseases in Kenya like malaria; hence, if not properly diagnosed may be misconstrued for malaria resulting even in more complications as far as pathogen virulence and drug resistance are concerned. Some victims could also die mysteriously of such tick-borne diseases and presumed to be malaria related just because very little or no research has been carried out on tick-borne infections. It is hoped that, the results reported here will improve our understanding of tick borne zoonoses and their geographical distribution in diverse eco-regions of Kenya.

2.2 Diagnostic methods in ticks

The pathogens may invade and multiply in all the organs and fluids of ticks making their detection in the hemolymph and salivary glands simple. Hemolymph test is done on live ticks when the distal portion (tarsus) of the front leg is amputated. Hemolymph that appears at the site can be smeared onto microscope slide, stained and examined. Molecular methods such as PCR enable rapid detection and identification of tick borne pathogens for use in screening large number of ticks during surveys or single ticks collected from patients and suspected of having tick borne diseases (Panning *et al.*, 2008). Moreover, the use of Real Time PCR has advantages over the traditional PCR as it allows for detection of the target during early phases of reaction measuring the kinetics of the reaction in early phases. Traditional PCR uses agarose gels for detection at the final phase or end point hence time consuming as it may take days due to post PCR processing.. The results are based on size determination which may not be precise with end point variable from sample to sample. Agarose gels may not resolve these variabilities in yield but RT-PCR is sensitive enough to detect these changes. Furthermore, the gel resolution is very poor, about 10 fold, has low sensitivity, ethidium bromide for staining not very quantitative and results not expressed as numbers added to the fact that it is an environmental hazard while RT-PCR can detect as little as two-fold change, doesn't require gel and is fast in giving results.

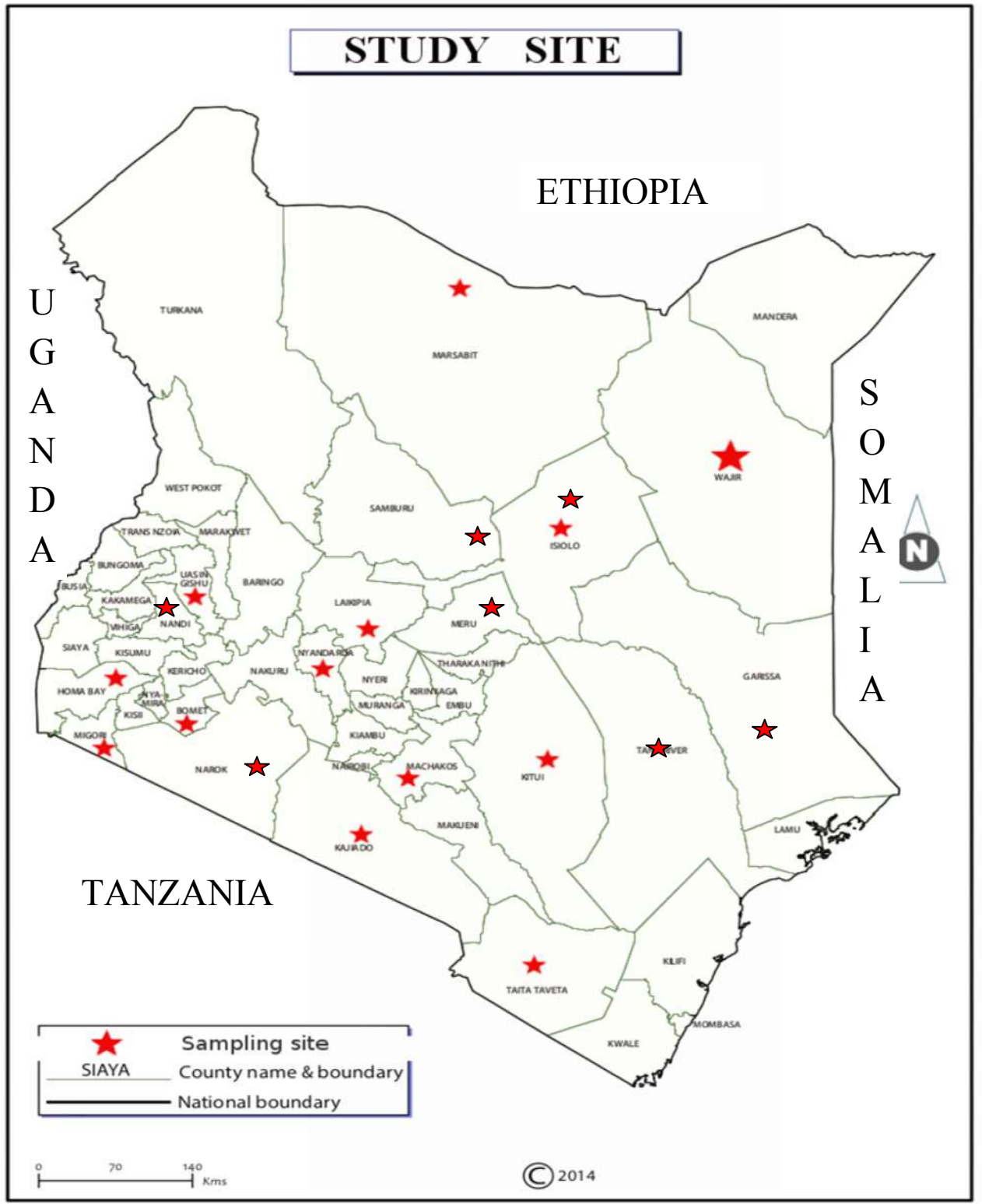
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the Kenya Medical Research Institute/Walter Reed Project (KEMRI/WRP) at Kondele Research Laboratories in Kisumu, Kenya where ticks and tick DNA samples were stored. Kenya is a country in Eastern Africa neighbouring Ethiopia to the North, Somalia to the East, Tanzania to the South and Uganda to the West and the country lies on the equator with a population of about 40 million people. The climate of Kenya varies by location from mostly cool every day, to always warm or hot. The climate along the coast is tropical hence higher rainfall and temperature throughout the year. Further inside Kenya has a climate which is more arid. The terrain is composed of low plains that rise into central highlands bisected by the Great Rift Valley and a plateau in the west of the country. The ticks had been collected from 20 counties in Kenya in a previous study that was investigating epidemiology of rickettsia. The tick samples had been taken from cattle, sheep and goats that were presented to the major slaughter houses in Nairobi and Mombasa between 2007 and 2008. For this study, the sample size used was 503 from the original 596 collected. This was because some samples were depleted during the previous study also making the number of counties to reduce to 20 from 32. Figure 3.1 summarizes the counties from which the livestock came.

Figure 3.1: Map showing the counties in Kenya from which the livestock and associated ticks came.



3.2 Study Design

The study involved extraction of DNA from tick samples followed RT-PCR to determine the presence or absence of zoonotic pathogens.

3.2.1 Ethical consideration

Scientific review and ethical approval to execute this study was sought from the animal care and use committee of Kenya Medical Research Institute (Approval reference: KEMRI SSC# 2467, see appendix 1).

3.2.2 Sampling

This study used archived DNA samples that were collected under another protocol number KEMRI SSC # 1248. Tick collection and identification under this protocol had been completed while DNA extraction for some ticks had been done while the rest had to be done. Ticks pools were collected from 1661 livestock (982 cattle, 300 sheep and 379 goats) that were presented in major slaughter houses in Nairobi (Kenya Meat Commission-KMC at Athi River and Dagoretti) and Mombasa (KMC Kibarani, Uwanja wa Ndege, Mariakani and Kasemeni), that receive livestock from all the 20 counties in the country. At least 10 adult ticks were collected from each animal with ticks. The 503 adult ticks that were available for this study are shown in Table 3.1. *Rhipicephalus pulchellus* formed the largest percentage 29% (144/503) followed by *Rh. appendiculatus* 20% (100/503) then *Amblyomma gemma* 12% (62/503), *Boophilus annulatus* 12% (61/503), *Hyalomma truncatum* 9% (47/503), *A. hebraeum* 8% (41/503), *Hyalomma spp.* 5% (24/503) , *Amblyomma spp* 3% (13/503) with *Amblyomma variegatum* being the least 2% (11/503).

Table 3.1: Tick species collected.

Tick species	Tick Collected
<i>Rhipicephallus pulchellus</i>	144
<i>Rhipicephallus appendiculatus</i>	100
<i>Boophilus annulatus</i>	61
<i>Amblyomma gemma</i>	62
<i>Amblyomma hebraeum</i>	41
<i>Amblyomma variegatum</i>	11
<i>Amblyomma spp.</i>	13
<i>Hyalomma truncatum</i>	47
<i>Hyalomma spp</i>	24
Total	503

The sample size was calculated using the Daniel (1999) formula, according to the average tick borne diseases (TBD) prevalence rates of 50% reported in Kenya, (Okuthe & Buyu, 2006). The ticks had already been identified by taxonomic characteristics. Ticks of the same species and from the same domestic animals were pooled prior to tick DNA extraction. Nine tick species (*Rhipicephalus appendiculatus*, *R. pulchellus*, *Boophilus annulatus*, *Amblyomma gemma*, *A. hebraeum*, *A. variegatum*, *Amblyomma sp.*, *Hyalomma truncatum* and *Hyalomma rufipes*.) belonging to four genera were identified based on the taxonomic key as described by (Okello-Onen *et al.*, 1999).

3.2.3 DNA Extraction

Some of the tick DNA had already been extracted in the earlier study (Mutai *et al.*, 2013) while the rest were extracted during this study in which stored ticks were removed from the 70%

ethanol and allowed to dehydrate in 3 mL of PBS buffer (pH 7.4) for five minutes. Pools of the tick samples from each animal were ground together in a mortar then the lysate transferred to 1.5 mL Eppendorf tubes. The tick DNA was extracted from the lysate as described by the QIAamp MinElute Virus Spin kit after tick. The DNA extraction involved pipetting 25µL QIAGEN Protease into a 1.5 mL microcentrifuge tube then adding 200 µL of the lysate into the microcentrifuge tube. To this 200 µL Buffer ATL was added and pulse-vortexed for 15 seconds. The tube containing the mixture was incubated at 56 °C for 15 minutes in a heating block before being centrifuged briefly to remove drops from the inside of the lid. 250 µL of ethanol (96-100%) was then added and mixed thoroughly by pulse vortexing for 15 seconds before incubating for 5 minutes at room temperature (15-25 °C). The 1.5 mL tube was centrifuged to remove drops from inside of the lid and then all the lysate was carefully transferred to the QIAamp MinElute column and centrifuged at 8000 rpm for 1 minute then placing the column in a clean 2 mL collection tube while discarding the filtrate. 500 µL of Buffer AW1 was added and centrifuged at 8000 rpm for 1 minute then the column transferred to clean 2 mL collection tube before adding Buffer AW2 and centrifuging at 8000 rpm and then transferring the column to a new tube. 500 µL of ethanol was added to the column and centrifuged at 8000 rpm for 1 minute and placed in a new tube while discarding the filtrate. The column membrane was dried completely by centrifuging at 14,000 rpm for 3 minutes and further dried by opening the lid and incubating it at 56 °C for 3 minutes. Finally the column was placed in a clean 1.5 mL microcentrifuge tube and 110 µL of Buffer AVE added and incubated at room temperature for 1 minute before centrifuging at full speed of 14,000 rpm for 1 minute. The resulting purified tick DNA was stored in 70% ethanol at -80°C for use.

3.2.4 Detection of Tick-borne zoonotic pathogens

PCR allows amplification of DNA fragments from genomic DNA by selective amplification of a specific region of DNA. The DNA from the tick samples was amplified using two primer sets and a probe in a real time PCR as shown in Table 3.3. The assay was run either as single-plex or multiplex PCR designs based on the annealing temperatures. The reaction mix (10 µL) for the assays had sterile 0.6 µL double distilled H₂O, 5.0 µL Quantitec Master Mix (California, USA), 0.5 µL forward primer, 400 nMoles of reverse primer, 300 nMoles of probe labeled with either FAM, VIC, CY3 or CY5 dye, and 3µL DNA template. The three µL of the DNA extracted from

the ticks was evaluated by quantitative real time PCR (qPCR) assays for the pathogens in the 10 μ L reaction mix. The assays were run in a 7500 Fast PCR machine (Applied Biosystem, USA).

The first step in the PCR reaction involved heating the reaction mix at 95°C for 15 minutes to denature the double stranded DNA into single stranded DNA followed by 45 cycles of 95°C for 15 seconds each and annealing at either 60°C for 1 minute for *Babesia/Anaplasma/Borrelia burgdorferi* (panel of three pathogens), *Coxiella/Ehrlichia* (panel of two pathogens) or annealing at 56°C for a minute for *Bartonella/Borrelia recurrentis* (panel of two pathogens) or annealing at 55°C for a minute for *Babesia microti* (singleplex). This step allowed the target specific forward primer (Table 3.3) to anneal to the target sequence then Taq polymerase would extend the primers sequence. A reverse primer and one of the BHQ probes (which contain reporter dye molecules –Table 3.3) would anneal to the newly replicated strand. The polymerase extends and cleaves the bound probe from the target strand causing the reporter to be no longer quenched by its proximity to the BHQ dye and fluorescence is released. Each replication resulted in cleavage of a probe and as a result the fluorescence signal would increase proportionally to the amount of amplification product and with the colour that corresponds to the filter dye hence an increase in the fluorescent intensity. The results of the run was reflected in real time on the PCR machine through a graphical plot whereby the x axis represented the cycle number while y axis represented the fluorescence of the reporter signal. For quality control of the assays, the runs had both positive and negative control samples. The controls had pathogens at different concentrations (neat, 1:10 dilution and 1:100 dilution) to ensure that the target could be detected over a wide range of concentrations. The positive controls were purchased from Fuller Laboratories, (California). The Fuller Laboratories develop clinical and veterinary assays for the diagnosis of vector-borne and zoonotic diseases. An amplification would be considered positive if it was above the threshold (exceeding background fluorescence). The faster an amplification crossed the threshold, the more the target were present. The negative control used was PCR water. The choice of PCR water as the negative control was based on the fact it was the diluents for extracted DNA. Thus, was ideal for evaluating contamination in the extraction process. Buffer used in the DNA extraction process could not be used as they contain inhibitors of PCR amplification.

Table 3.2: The oligonucleotide sequence.

Organism	Primers and Probe	Oligonucleotide Sequences 5' - 3'
<i>Borrelia burgdorferi</i>	Bb Forward	CGAGTCTTAAAAGGGCGATTTAGT
	Bb Reverse	GCTTCAGCCTGGCCATAAATAG
<i>Anaplasma phagocytophilum</i>	Bb Probe	[NED]AGATGTGGTAGACCCGAAGCCGAGTG[BHQ2]
	APMSP2 Forward	ATGGAAGGTAGTGTGGTTATGGTATT
	APMSP2 Reverse	TTGGTCTTGAAGCGCTCGTA
<i>Ehrlichia chaffeensis</i>	APMSP2 Probe	[HEX]TGGTGCCAGGGTTGAGCTTGAGATTG[BHQ1]
	ECH16S Forward	GCGGCAAGCCTAACACATG
<i>Ehrlichia chaffeensis</i>	ECH16S Reverse	CCCGTCTGCCACTAACAATTATT
	ECH16S Probe	[CY5]AGTCGAACGGACAATTGCTTATAACCTTTTGGT [BBQ]
<i>Babesia microti</i>	BAB Forward	GGAAGTCTCGTGAACCCTTATCACTTAAA
	BAB Reverse	GTAGGTGAACCTGCAGAAGGATCAAGC
	BAB Probe	[CY5]GGAAGGAGAAGTCGTAACAAGGTTTCC[BBQ]
<i>Bartonella henselae</i>	BARTICS Forward	TGCTTCGACTCCACTGTACGTC
	BARTICS Reverse	CACCTGCTGCAATACATGCAAATG
	BARTICS Probe	TTGCAGCTTCATCAGGTGCTAATC
<i>Coxiella burnetii</i>	ICD-439 Forward	CGTTATTTTACGGGTGTGCCA
	ICD-513 Reverse	CAGAATTTTTCGCGGAAAATCA
	Probe	[6FAM]CATATTCACCTTTTCAGGCGTTTTGACCG[BH Q-1]

These are the sequence of forward primers, reverse primers and probes that were used in PCR during detection of pathogens *C. burnetii*, *A. phagocytophilum*, *E. chaffeensis*, *Babesia* and *B. hensellae*.

3.3 Data analysis

The study generated data that described the number and species of ticks that carry important tick-borne zoonotic pathogens and also described the pathogen prevalence and distribution in the

country. The generated data was routinely entered into a database created in Microsoft access. Continuous data was tested for Normality and descriptive statistics performed where appropriate. Prevalence was calculated against variables which constituted the tick type, livestock type from which the ticks were obtained (cattle, sheep and goat) and the geographical region of origin. Computational analysis was performed with Graphpad prism 5 statistical software (GraphPad Software Inc. San Diego CA).

CHAPTER FOUR

RESULTS

4.1 Animal origin and tick samples

Five hundred and three tick pools were collected in 20 counties in Kenya from 1661 livestock (982 cattle, 300 sheep and 379 goats, Fig. 3.1). Although a lot of ticks were collected, the sample size of 982 cattle, 300 sheep and 379 goats was based on another study that evaluated tick borne rickettsioses. The data analysis from this small sample size may not have given the same representation as that from a larger sample size, hence could have affected the actual prevalence obtained. Nearly 50% of the ticks collected were of genus *Rhipicephallus* (246/503, 49%). The other genus were *Amblyomma* 127/503 (25.2%), *Hyalomma* 68/503 (13.5%) and *Boophilus* 62/503 (12.3%), Table 3.1. They were screened for tick borne zoonotic pathogens in the genus *Coxiella*, *Babesia*, *Borrelia*, *Bartonella*, *Anaplasma*, and *Ehrlichia* as shown in the Table 3.2.

4.2 PCR amplification plots for identifying pathogen carriage in ticks.

Figures 4.1-4.6 show typical computer generated PCR amplification plots, with fluorescence displayed against cycle thresholds (C_T) for each of the pathogens tested. Each pathogen is shown with its representative amplification plot; *C. burnetii*, *Babesia spp.*, *B. burgdorferi*, *Borrelia spp.*, *A. phagocytophilum* and *E. chaffeensis*. The cycle threshold was the cycle number at which the fluorescent intensity of a given reaction crossed threshold (the cut-off for background fluorescence of the negative control samples). Samples with C_T values above the threshold were reported as positive while those that failed to cross the threshold were reported as undetectable. The arrow shows negative control sample while arrowhead shows the threshold line. The circles shows two positive control samples while the rectangles show tick samples that tested positive. The negative control used was PCR grade water.

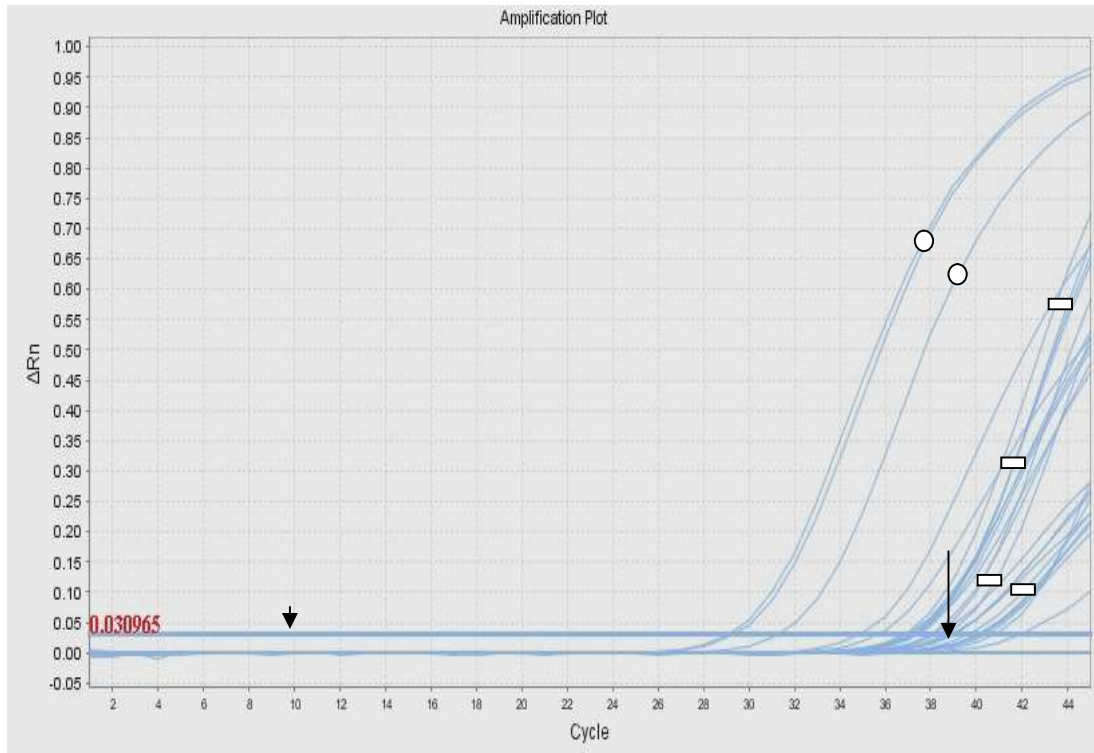


Figure 4.1: Typical multiplex real time PCR amplification plot of zoonotic pathogen *C. burnetii* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles for *C. burnetii* control and positive tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was be considered positive. The circles show the positive control while the rectangles show the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

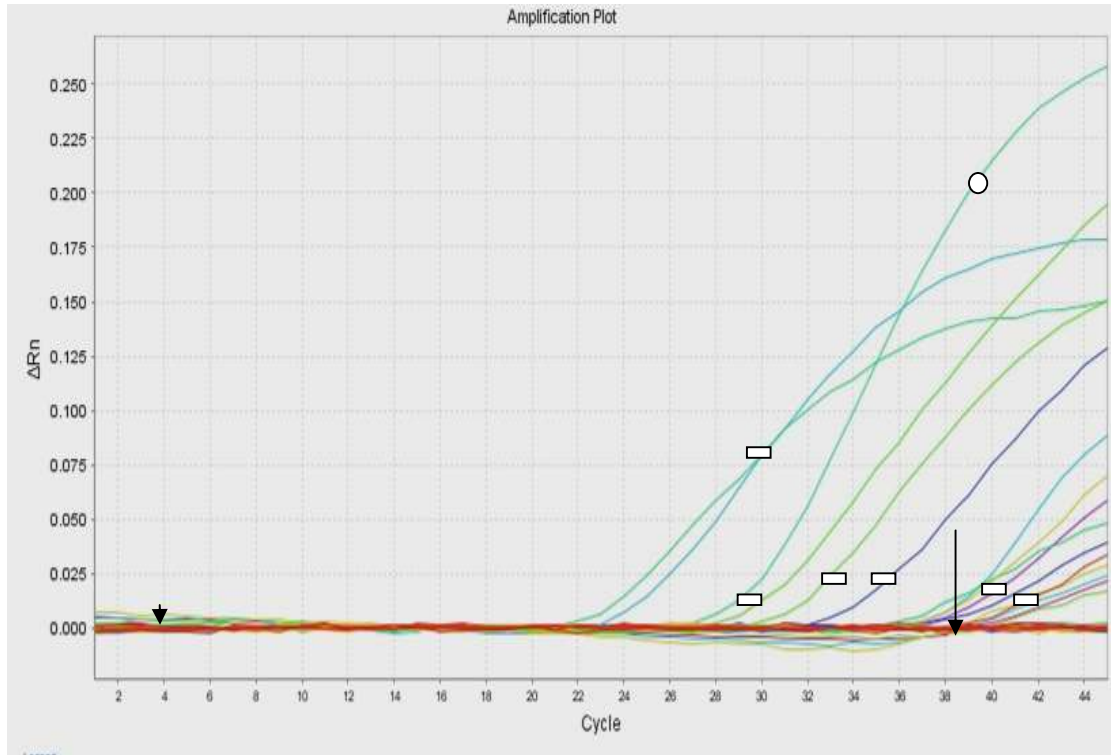


Figure 4.2: Typical multiplex real time PCR amplification plot of zoonotic pathogen *Babesia spp* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles *Babesia spp* control and tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was considered positive. The circle shows the positive control while the rectangles show the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

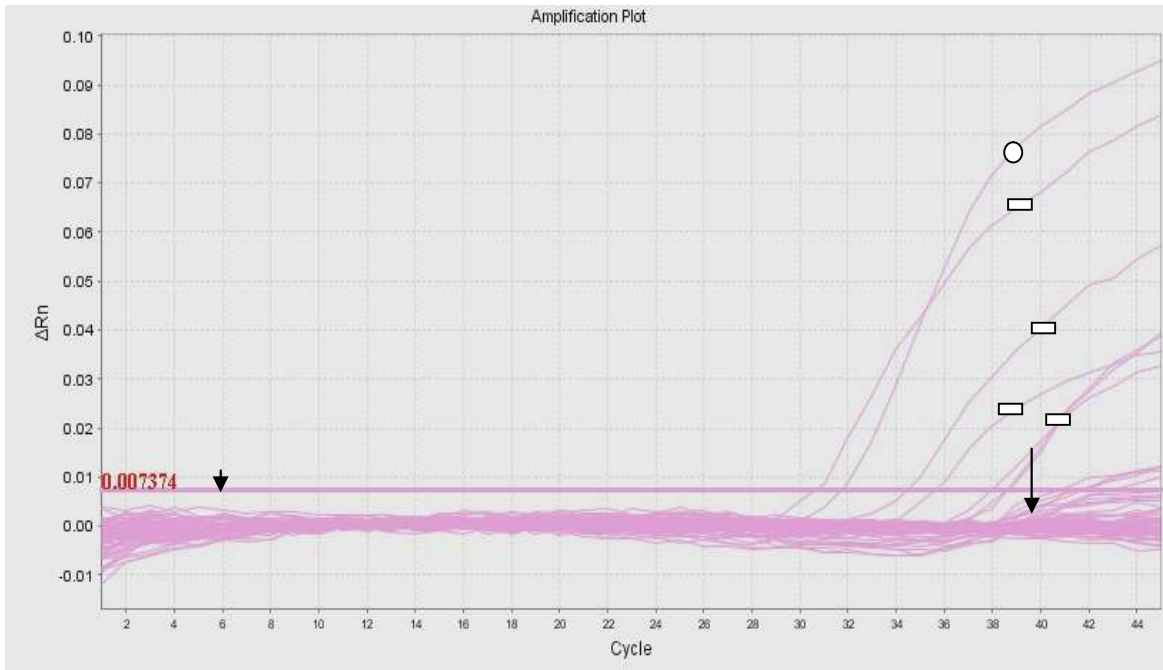


Figure 4.3: Typical multiplex real time PCR amplification plot of other *Borrelia spp* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles for *Borrelia* control and tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was considered positive. The circle shows the positive control while the rectangles show the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

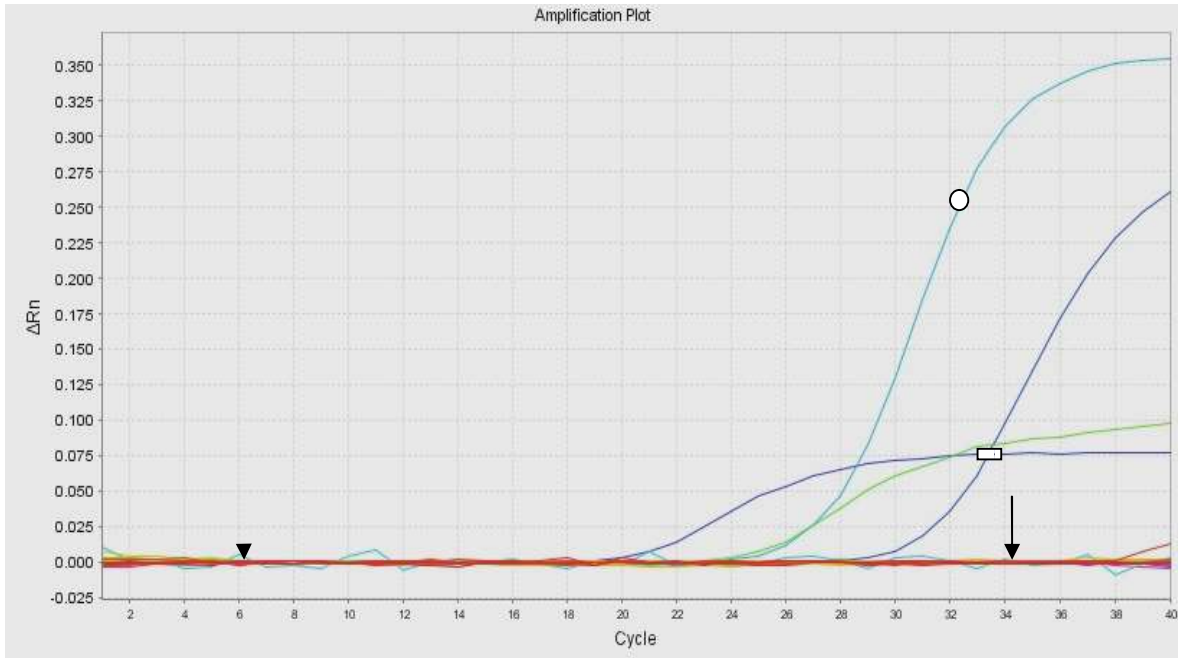


Figure 4.4: Typical multiplex real time PCR amplification plot of zoonotic pathogen *B. burgdorferi* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles for *B. burgdorferi* control and tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was considered positive. The circle shows the positive control while the rectangle shows the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

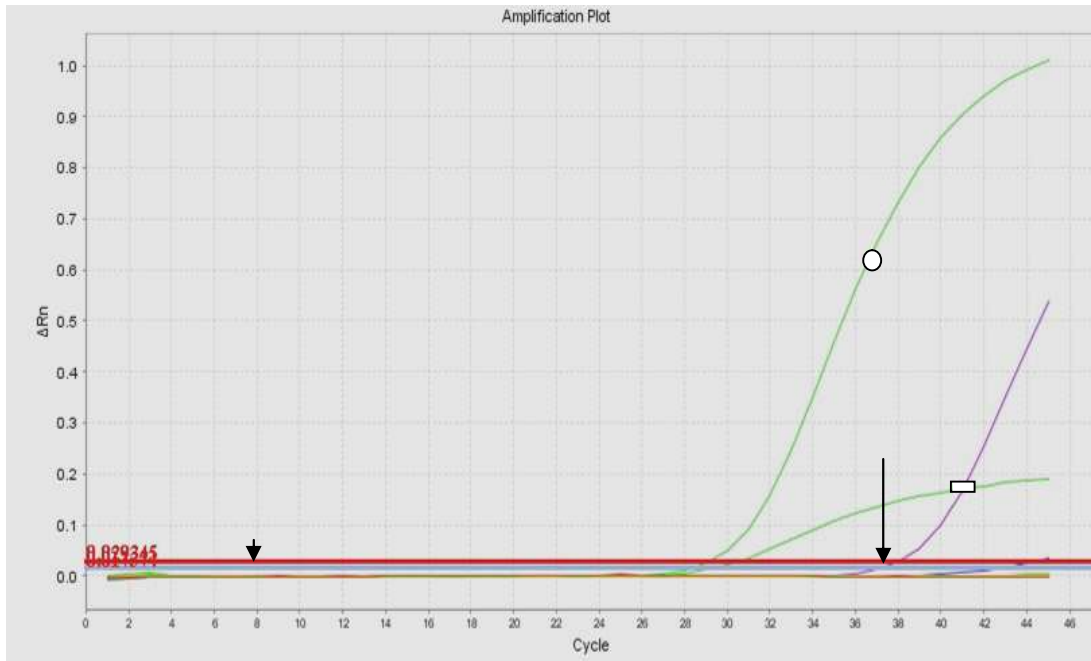


Figure 4.5: Typical multiplex real time PCR amplification plot of zoonotic pathogen *A. phagocytophylum* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles for *A. phagocytophylum* control and tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was considered positive. The circle shows the positive control while the rectangle shows the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

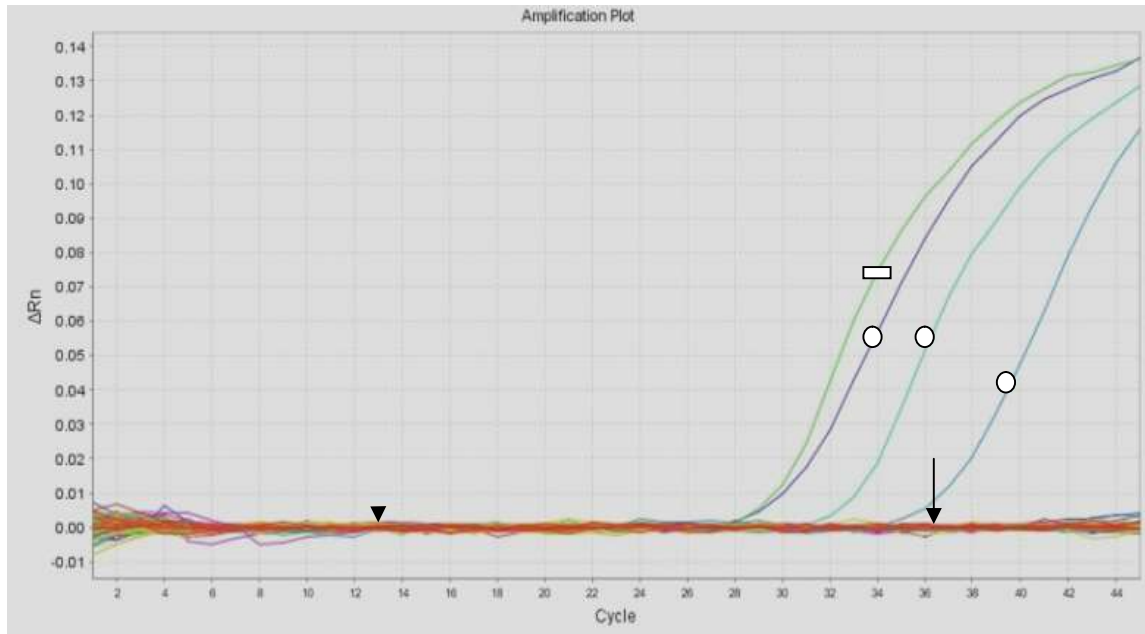


Figure 4.6: Typical multiplex real time PCR amplification plot of zoonotic pathogen *E. chaffeensis* performed in a 7500 Fast real time thermocycler (Applied Biosystems, USA). Amplification profiles for *E. chaffeensis* control and tick samples. The x axis represents the cycle number while y axis represents the fluorescence of the reporter signal. The arrow shows negative control, while arrow head shows threshold line above which any amplification was considered positive. The circles show the positive control while the rectangles show the positive samples. The negative control used was PCR grade water (double distilled and nuclease free).

4.3 Overall Zoonoses prevalence rates

Out of 503 ticks collected, 106/503 (21%) were positive for at least one pathogen. Of the 6 pathogens tested, *Bartonella henselae* was not detected. Of the 106 positive samples (Figure 4.7) the pathogen prevalence rates were as follows: *C. burnetii* 70% (74/106) was the most abundant of which 28/74 (38%) were detected in *Rhipicephalus pulchellus* ticks, 8/74 (11%) in *R. appendiculatus*, 5/74 in *Boophilus annulatus*, 9/74 in *Amblyomma gemma*, 11/74 (15%) in *A. hebraeum*, 6/74 (8%) in other *Amblyomma species*, 6/74 (8%) in *Hyalomma truncatum* and 1/74 (1%) in other *Hyalomma species*. Non-human *Babesia species* was the second most abundant at 17% (18/106) with 6/18 (33%) in *R. pulchellus* ticks, 2/18 (11%) in *R. appendiculatus*, 5/18 (28%) in *B. annulatus*, 2/18 (11%) in *H. truncatum* and 3/11 (17%) in other *Hyalomma species*. None of the *Babesia* were human pathogens (*B. microti* was not detected), 5% (5/106) had *B.*

burgdorferi of which 1/5 (20%) was in *R. pulchellus* ticks, 3/5 (60%) in *B. annulatus* ticks and 1/5 (20%) in *A. hebraeum*. 7% (7/106) of the pathogens were other *Borrelia* species of which 2/7 (29%) were in *R. pulchellus*, 1/7 (14%) in *B. annulatus* and 4/7 (57%) in *A. gemma*. <1% (1/106) had *A. phagocytophylum* that was detected in *R. pulchellus* while <1% (1/106) had *E. chaffeensis* that was detected in *R. pulchellus*. This shows that *C. burnetii* was the most abundant of all the pathogens detectable while the human infective *Babesia* (*B. microti* and *B. henselae*) were not detected. Other important human zoonoses that were detected include *B. burgdorferi* that causes Lyme disease, *A. phagocytophylum* that causes human granulocytic anaplasmosis and *E. chaffeensis* that causes ehrlichiosis.

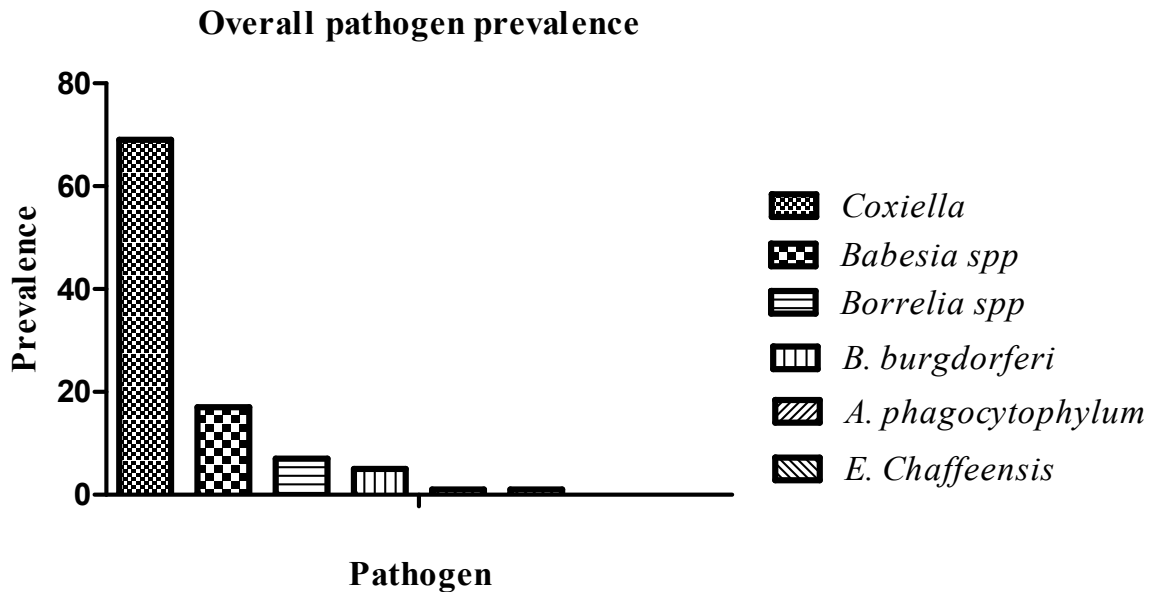


Figure 4.7: Overall zoonotic pathogen prevalence rates. *C. burnetii* had the highest prevalence of 70% (74/106), *Babesia* species 17% (18/106), *Borrelia* species 7% (7/106), *B. burgdorferi* 5% (5/106), *A. phagocytophylum* <1% (1/106), *E. chaffeensis* <1% (1/106).

4.4 Tick infection rates

Table 4.1 shows the tick infection rates. Out of 503 ticks collected (Table 3.1), 106/503 (21%) were positive for at least one pathogen. 49/246 (20%) *Rhipicephallus* spp were infected. Of these, 39/49 (80%) were *R. pulchellus*, 10/49 (20%) *R. appendiculatus*. Of the 31/127 (24%) infected *Amblyomma* spp, 13/31 (42%) were *A. gemma*, 12/31 (39%) were *A. hebraeum* and 6/31 (19%) were other *Amblyomma* spp. 12/68 (18%) *Hyalomma* spp were infected, of which 8/12

(67%) were *H. truncatum* and 4/12 (33%) were other *Hyalomma spp* and *B. annulatus* 14/62 (23%).

Table 4.1: Tick *spp* infection rates.

Tick species	<i>Coxiella</i>	<i>Babesia</i>	<i>B.</i> <i>burgdorferi</i>	<i>B.</i> <i>recurrentis</i>	<i>Anaplasma</i>	<i>Ehrlichia</i>	Total
<i>Rhipicephalus pulchellus</i>	28(38%)	6(33%)	1(20%)	2(28%)	1(100%)	1(100%)	39
<i>Rhipicephallus appendiculatus</i>	8 (11%)	2(11%)	0(0%)	0(0%)	0(0%)	0(0%)	10
<i>Boophilus annulatus</i>	5(7%)	5(28%)	3(60%)	1(14%)	0(0%)	0(0%)	14
<i>Amblyomma gemma</i>	9 (12%)	0(0%)	0(0%)	4(57%)	0(0%)	0(0%)	13
<i>Amblyomma hebraeum</i>	11(15%)	0(0%)	1(20%)	0(0%)	0(0%)	0(0%)	12
<i>Amblyomma species</i>	6(8%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	6
<i>Hyalomma truncatum</i>	6 (8%)	2(11%)	0(0%)	0(0%)	0(0%)	0(0%)	8
<i>Hyalomma species</i>	1 (1%)	3(17%)	0(0%)	0(0%)	0(0%)	0(0%)	4
Total	74(100%)	18(100%)	5(100%)	7(100%)	1(100%)	1(100%)	106

Rh. pulchellus ticks had the highest infection rate 37% (39/106) and were mainly infected with *C. burnetii* 28/39 (72%) and *Babesia* 15% (6/39). Infection rate in *B. annulatus* was at 13% (14/106) with the highest infestation being by *C. burnetii* 36% (5/14) and Non-human *Babesia* 36% (5/14). Infection rate in *A. gemma* was 12% (13/106) mainly with *C. burnetii* 69% (9/13) and *B. recurrentis* 31% 4/13. *A. hebraeum* infection was 11% (12/106) and 9% for *Rh. appendiculatus* (10/106). Lowest prevalence was detectable in *Hyalomma spp* 4% (4/106), *Amblyomma spp* 6% (6/106) and *H. truncatum* 8% (8/106).

C. burnetii

C. burnetii infections in the different ticks are shown in Figure 4.8. The detection was based on isocitrate dehydrogenase (ISD) gene of the pathogen. Out of the nine tick species examined, *C. burnetii* was most prevalent in *Rh. pulchellus* 38% (28/74) compared to 11% (8/74) in *Rh. appendiculatus*, 12% (9/74) in *A. gemma*, 15% (11/74) in *A. hebraeum*, 8% (6/74) in *Amblyomma spp*, 8% (6/74) in *H. truncatum*, 1% (1/74) in *Hyalomma spp* and 7% (5/74) in *B. annulatus*. Infections were not detected in *A. variegatum*. The high prevalence of *C. burnetii* in *Rh. pulchellus* was significantly different from the other tick species ($p < 0.05 = 0.0009$).

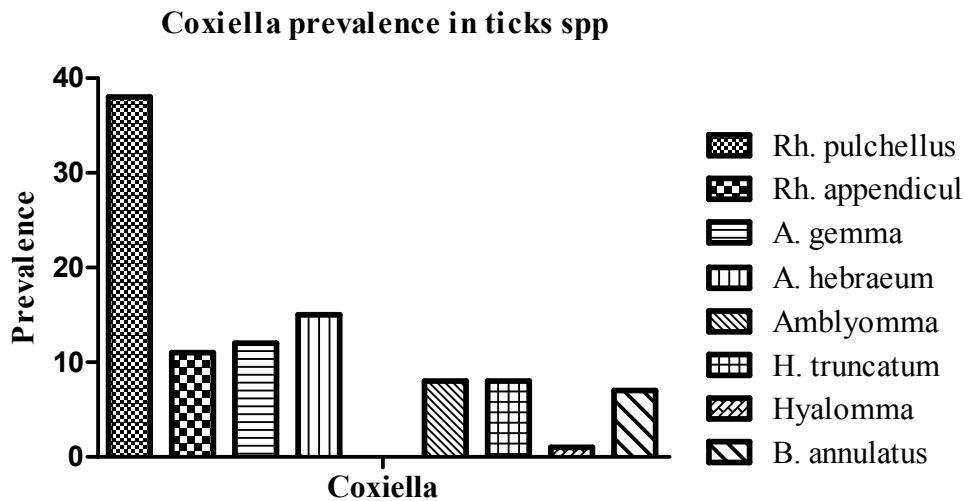


Figure 4.8: Infection rates of *Coxiella burnetii* in ticks. Infection rate in *Rh. pulchellus* was 38%, *Rh. appendiculatus* 11%, *A. gemma* 12%, *A. hebraeum* 15%, *Amblyomma spp* 8%, *H. truncatum* 8%, *Hyalomma spp* 1%, and *B. annulatus* 7%. Infection was not detected in *A. variegatum*

Babesia

Fig. 4.9 shows *Babesia* infections rates in different tick species. *Babesia* was detected based on 18S rRNA gene and the infection rate was 17% (18/106), however none had the human infectious species (*B. microti*) were detected. As shown in Fig. 4.2, Fig. 4.9 and Table 4.1 the genus *Babesia* was detected in all tick species except the *Amblyomma*. Highest prevalence was detected in *Rh. pulchellus* 33% (6/18) and *Boophilus annulatus* ticks with lowest prevalence in

Hyalomma spp 17% (3/18), *Rh. appendiculatus* and *Hyalomma truncatum* each at 11% (2/18). There was no significant difference in the prevalence among the various tick species ($p= 0.0606$).

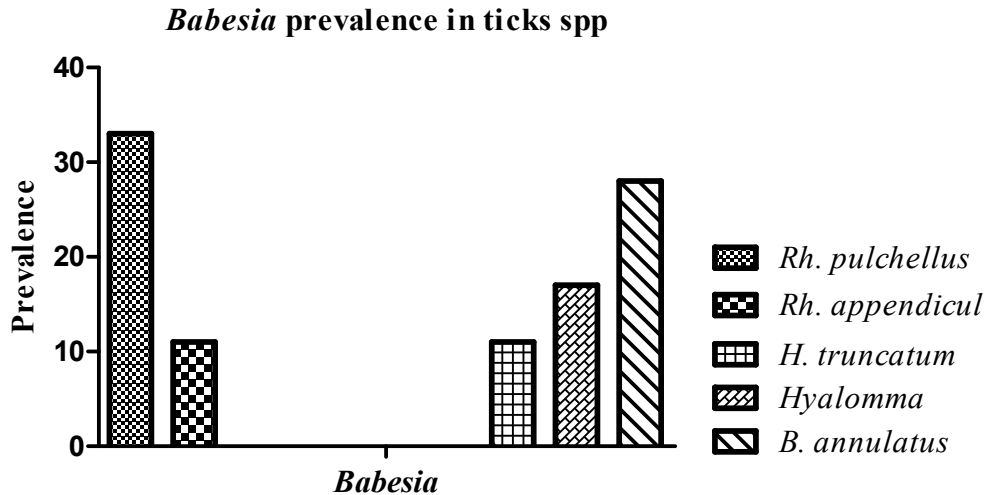


Figure 4.9: Prevalence rate of *Babesia* in different tick species. *Babesia* prevalence rate in *Rh. pulchellus* was 33%, *Rh. appendiculatus* 11%, *H. truncatum* 11%, *Hyalomma spp* 17%, and *B. annulatus* 28%. Infections were not detected in *A. gemma*, *A. hebraeum*, *A. variegatum* and *Amblyomma spp*.

Borrelia burgdorferi

Human *Borrelia* which causes lyme disease was detected based on flagellin gene and had an overall prevalence of 5% (5/106). Of these, 60% (3/5) was detected in *Boophilus annulatus* ticks, 20% (1/5) in *A. hebraeum* and 20% (1/5) in *Rh. pulchellus* ticks with no significant difference in the infection among the various tick species ($p = 0.1235$). On the other hand, there were other *Borrelia* species detected with a prevalence of 7% (7/106) of which 57% (4/7) were detected in *A. gemma* ticks, 29% (2/7) in *Rh. puchellus* while 14% (1/7) were detected in *Boophilus annulatus* ticks with no significant difference in infection among tick species, $P = 0.1235$ (Figures 4.4 and 4.10).

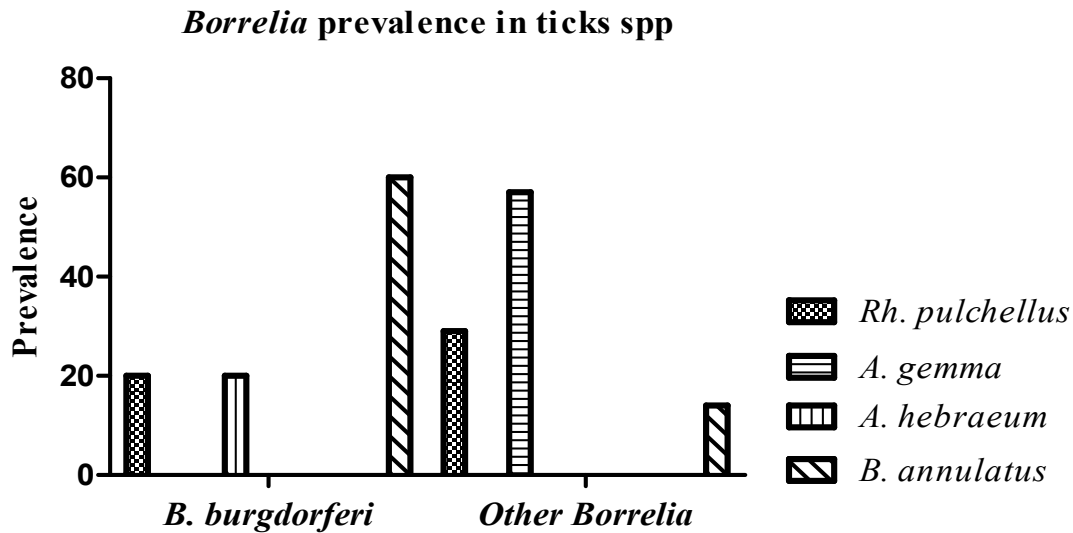


Figure 4.10: Prevalence rate of *B. burgdorferi* and other *Borrelia* in different tick species. For *B. burgdorferi* only *Rh. pulchellus*, *A. hebraeum* and *B. annulatus* were prevalent at 20%, 20% and 40% respectively while other *Borrelia* had prevalence in *Rh. pulchellus* at 29%, *A. gemma* at 57% and *B. annulatus* at 14%.

A. phagocytophylum

The pathogen was detected based on major surface protein 2 (msp2) and the overall prevalence was <1% (1/106) and only in *Rh. pulchellus* tick. There was no significant difference in the infection among the tick species ($p = 0.09621$, Table 4.1).

Ehrlichia chaffeensis detection was based on 16S rRNA gene and it had an overall prevalence of <1% (1/106) and was only detected in a *Rh. pulchellus* tick with no significant difference in the infection among the tick species ($p = 0.09621$, Table 4.1).

4.5 Pathogen detection in relation to the livestock the ticks came from

Table 4.2 shows pathogen distribution in relation to the livestock the ticks came from. Cattle had the highest infestation with infected ticks 61% (65/106), compared to sheep 26% (27/106) and goats 13% (14/106). Specifically, 58% (43/74) of *C. burnetii* were detected in ticks that came from cattle, compared to 30% (22/74) for sheep and 12% (9/74) in goats. *Babesia* infections were found in ticks that came from cattle at 55% (10/18) compared to sheep 22% (4/18) and goat 22% (4/18). *B. burgdorferi*, *A. phagocytophylum* and *E. chaffeensis* were only associated with

cattle, while other *Borrelia* originated more in cattle 72% (5/7) than sheep 14%(1/7) or goat 14% (1/7).

Table 4.2: Pathogens in relation to the livestock from which ticks were obtained.

	Cattle	Sheep	Goats	Total
<i>Coxiella burnetii</i>	43	22	9	74
<i>Babesia spp.</i>	10	4	4	18
<i>Borrelia burgdorferi</i>	5	0	0	5
<i>Other Borrelia</i>	5	1	1	7
<i>Anaplasma</i>	1	0	0	1
<i>Ehrlichia</i>	1	0	0	1
Total	65	27	14	106

Ticks from cattle had the highest prevalence of infected ticks 61%, (65/106) compared to ticks from sheep 26% (27/106) while those from goats had the lowest 13% (14/106). Most *C. burnetii* infections were detected in ticks from cattle at 58% (43/74), compared to sheep 30% (22/74) and goats at 12% (9/74). Most non-human *Babesia* were detected in ticks that came from cattle at 55% (10/18) compared to sheep 22% (4/18) and goat 22% (4/18). *B. burgdorferi*, *A. phagocytophylum* and *E. chaffeensis* were only detected in ticks originating from cattle. More *Borrelia* were detected in ticks from cattle 72% (5/7) compared to sheep 14%% (1/7) or goat 14% (1/7).

4.6 Co-infection in the ticks

Of the 503 ticks that were screened, only 106 had pathogens. Overwhelming majority, 95/106 (90%) had mono-infections. Ten percent (11/106) had multiple pathogens of either two (10/11) or three (1/11). Five tick species (*Rh. pulchellus*, *Boophilus annulatus*, *Amblyomma gemma*, *Amblyomma hebraeum* and *Hyalomma spp.*) had co-infection of *C. burnetii*, *Babesia*, *Borrelia burgdorferi* and unspiciated *Borrelia*. *Rh. pulchellus* tick was the most co-infected 64% (7/11), such that one *Rh. pulchellus* tick was infected with three pathogens (*C. burnetii*, *Babesia* and unspiciated *Borrelia*) and the remaining six infected with two pathogens each. The remaining

four tick species were each co-infected with two pathogens as shown in Table 4.3. All co-infections included *C. burnetii* with either *Babesia* or *B. burgdorferi* or unspiciated *Borrelia*

Table 4.3: Co-infection in ticks.

Tick No.	Sample Identity	Pathogen Co-infection	Tick species
34	R2G/015/T	<i>C. burnetii</i> , <i>Babesia</i> , other <i>Borrelia</i>	<i>Rh. pulchellus</i>
30	R2G/019/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>Rh. pulchellus</i>
33	R2G/016/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>Rh. pulchellus</i>
201	R2B/013/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>Rh. pulchellus</i>
368	R1S/006/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>Rh. pulchellus</i>
52	R2B/008/T	<i>C. burnetii</i> , other <i>Borrelia</i>	<i>Rh. pulchellus</i>
57	R2B/006/T	<i>C. burnetii</i> , <i>B. burgdorferi</i>	<i>Rh. pulchellus</i>
217	R1B/008/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>B. annulatus</i>
140	R2B/013/T	<i>C. burnetii</i> , other <i>Borrelia</i>	<i>A. gemma</i>
60	R2B/005/T	<i>C. burnetii</i> , <i>B. burgdorferi</i>	<i>A. hebraeum</i>
215	R1B/006/T	<i>C. burnetii</i> , <i>Babesia</i>	<i>Hyalomma sp.</i>

4.7 Pathogen distribution in the Kenyan Counties

As shown in Table 4.4, out of the twenty counties sampled, only fourteen had incidences of ticks borne pathogens and all are in pastoral areas. Marsabit County had the highest prevalence, 25% (26/106), followed by Kajiado with 17% (18/106), Wajir 16% (17/106) and Narok, 11% (12/106). The lowest prevalence rate was recorded in Bomet and Uasin Gishu counties at 1% (1/106) each. Tick borne zoonoses were most prevalent in the pastoral counties: Marsabit 25% (26/106), Kajiado 17(18/106), Wajir16% (16/106) and Narok 11% (12/106) while those with the lowest prevalence rate were Bomet 1% (1/106), Uasin Gishu 1% (1/106), Ewaso Nyiro 2 (2/106) and Homabay 2% (2/106). *C. burnetii* was the most common and was most prevalent in Marsabit 27% (20/74), Kajiado 20% (15/74) and Wajir 16% (12/74) but lowest in Bomet 1% (1/74), Samburu 1% (1/74), Migori 1% (1/74), Uasin Gishu 1% (1/74). *Babesia* species were most prevalent in Narok 39% (7/18) and Wajir 22% (4/18) with many areas reporting less than 1%. *B.*

burgdorferi was only detected in three counties: Marsabit 40% (2/5), Nyandarua 40% (2/5) and Migori 20% (1/5) while the unspciated *Borrelia* were detected in Marsabit 43% (3/7), Nyandarua 29% (2/7) with most areas recording less than 1%, Kajiado 14% (1/7) and Wajir 14% (1/7). Incidence of *A. phagocytophilum* and *E. chaffeensis* was low, each at <1% and were detected in Narok (1/106) and Marsabit (1/106) respectively. Overall, Marsabit had the highest variety of pathogens detected namely *C. burnetii* (20/106), *B. burgdorferi* (2/106), *unspciated Borrelia* (3/106) and *Ehrlichia* (1/106). Non-pastoral areas had very low prevalence of *C. burnetii*.

Table 4.4: Pathogen prevalence rates in the Kenyan Counties.

County	livestock sampled	B.		Other			<i>Ehrlichia</i>	Total
		<i>Coxiella</i>	<i>Babesia</i>	<i>burgdorferi</i>	<i>Borrelia</i>	<i>Anaplasma</i>		
Bomet	40	1(1%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1 (1%)
Samburu	31	1(1%)	1(6%)	0(0%)	0(0%)	0(0%)	0(0%)	2(2%)
Kajiado	161	15(20%)	2(11%)	0(0%)	1(14%)	0(0%)	0(0%)	18(17%)
Machakos	75	2(3%)	1(6%)	0(0%)	0(0%)	0(0%)	0(0%)	3(3%)
Migori	21	1(1%)	1(6%)	1(20%)	0(0%)	0(0%)	0(0%)	3(3%)
Marsabit	47	20(27%)	0(0%)	2(40%)	3(43%)	0(0%)	1(100%)	26(25%)
Kitui	19	4(5%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	4(4%)
Laikipia	20	4(5%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	4(4%)
Narok	35	4(5%)	7(39%)	0(0%)	0(0%)	1(100%)	0(0%)	12(11%)
Nyandarua	10	3(4%)	0(0%)	2(40%)	2(29%)	0(0%)	0(0%)	7(6%)
Homabay	19	0(0%)	2(11%)	0(0%)	0(0%)	0(0%)	0(0%)	2(2%)
Taita								
Taveta	40	6(8%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	6(6%)
Uasin								
Gishu	20	1(1%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(1%)
Wajir	21	12(16%)	4(22%)	0(0%)	1(14%)	0(0%)	0(0%)	17(16%)
Totals		74(100%)	18(100%)	5(100%)	7(100%)	1(100%)	1(100%)	106

CHAPTER FIVE

DISCUSSION

Ticks

Information on tick-borne zoonoses in Kenya is scanty. However, reports of human cases with zoonoses among international travelers after visiting sub Saharan Africa indicate the increasing significance of these neglected diseases. This is probably being fueled by increasing contacts between humans and animals (Burke *et al.*, 2012). A number of factors make ticks amenable to infections with a variety of pathogens. First, ticks have a life cycle associated with a multitude of wild and domestic animals that make it difficult to eliminate or to control as opposed to lice and fleas that tend to be host specific. Second, ticks may carry infections for life, either by transtadial and transovarial transmission. No wonder that ticks may be infected with a variety of pathogens picked from diverse hosts. Given this background, a study was conducted to determine the prevalence of 6 tick borne zoonoses and their regional distribution. The number of ticks collected, their taxonomic assignment and geographical distribution have been reported before (Mutai *et al.*, 2013) and tick origins are shown in Figure 3.1. The pathogens evaluated were *C. burnetti*, the causative agent of Q-fever, *Babesia spp* that cause a host of diseases in animals and *B. microti* that causes human babesiosis, *A. phagocytophilum* the causative agent for human granulocytic anaplasmosis, *Borellia burgdorferi*, *Borrelia recurrentis*, that cause Lyme disease and relapsing fever in humans respectively, *Bartonella henselae* that causes bartonellosis, and *E. chaffeensis* that causes human ehrlichiosis.

RT-PCR

Detection of these pathogens was by real time PCR (RT-qPCR) that allows selective amplification of target genes as indicated in Table 3.2. Real Time PCR allows for detection of PCR amplification during early phases of reaction, has very high sensitivity, high resolution and very high precision as opposed to the traditional PCT which has problems of poor resolution, low sensitivity, low resolution, size based discrimination, results not expressed in numbers, post PCR processing and ethidium bromide for staining not being quantitative all added to the fact that the agarose gel results are only obtained at the end of the reaction hence time consuming making results to take days. This makes RT-PCR to be way above the traditional PCR. To allow multiplexing, the assays were done in panels of three pathogens (*Babesia/A. phagocytophilum/B.*

burgdorferi) and two pathogen pair (*E.chaffeensis/C. burnetii*) and *B. hensellae/B. recurrentis*) while single-plex assay was done for *B. microti*. The pathogen grouping was based on consideration such as annealing temperatures compatibility and absence of cross inhibition. As shown in Figure 4.1-4.6, the assay conditions allowed unambiguous detection of target pathogens. The tick-borne zoonoses that were detected include *C. burnetii*, non-human *Babesia spp.*, *Borrelia spp.*, *A. phagocytophilum* and *E. chaffeensis* while *B. hensellae* and *B. microti* were not detected in any of the samples (Figure 4.1-4.6). The results show that ticks of the genus *Rhipicephalus* and *Amblyomma* were the dominant carriers of pathogens with detection rate of 46% (49/106) and 29% (31/106) respectively (Table 4.1). This is consistent with the findings of Mutai *et al.* (2013) in which the two species were the dominant carriers of rickettsiae.

Coxiella burnetii

C. burnetii is responsible for Q (Query) fever, a zoonotic disease endemic worldwide (Bonnet *et al.*, 2013). It is transmitted through inhalation of contaminated aerosols, infected faeces and birth products or through a tick vector. It can also be transmitted sexually through sperm cells (Thompson *et al.*, 2012). As shown in Figure 4.7, *C. burnetii* infection rates was the highest (70%, 74/106) compared to 17% for *Babesia spp.*, 7% for *Borrelia spp.*, 5% for *B. burgdorferi*, <1% for *A. phagocytophilum* and <1% for *E. chaffeensis*. Among the tick species the highest prevalence of the *C. burnetii* was recorded in *Rh. pulchellus* 38% (28/74). This tick was also the highest carrier of other pathogens tested. It is possible that the significant differences in *C. burnetii* detection rates ($p < 0.0009$) between tick species is based on the ticks mating system. Lockhart *et al.*, (1996) suggested that tick species with a more promiscuous mating system like *Rh. pulchellus* could have a higher prevalence of infection as the infected individuals are more likely to come in contact with and infect the previously uninfected ticks through the sperms.

As shown in Table 4.2, *C. burnetii* infection rates were highest in ticks obtained from cattle 58% (43/74) compared to 30% in sheep (22/74) and was lowest in ticks from goats 12% (9/74). *C. burnetii* was found to be more prevalent in Wajir, Marsabit and Kajiado counties with prevalence rates of 28% (21/74), 27% (20/74) and 20% (15/74) respectively (Table 4.4). Wajir and Marsabit counties are found in North- Eastern Kenya and they border Isiolo (to the West and South respectively), which has several game reserves like Shad, Buffalo and other private game ranches (Mati *et al.*, 2005). In Kenya the game reserves allow for communal grazing of livestock

and wild animals coupled with temporary human settlements by the nomadic communities hence allowing habitat sharing, thereby increasing the chances of transmission. Cases of domestic canines frequenting forests and returning infested with ticks and suffering from tick borne rickettsioses have been reported (Rutherford *et al.*, 2004). Also, the geographical location of Wajir makes it to have links with Ethiopia, Somali and Southern Sudan. These countries have high densities of livestock and they have a constant migration of livestock across their borders (Njoroge *et al.*, 2000). Kajiado County borders Nakuru county to the North in which the economic activities are agrarian and animal husbandry. Livestock rearing takes place in the lowland areas of the county characterized by poor soils and unreliable rainfall. To the North-West of Kajiado is Narok which is inhabited by the nomadic pastoral community. Narok has the Mau Narok forest reserve which has been encroached by humans resulting in fragmentation on previous forest areas and cultivation on the forest margins (Lambrechts *et al.*, 2007). In Mau Narok, forest reserve encroachment and fragmentation has increased agricultural activity, human settlement and animal husbandry. Kajiado also borders Makueni and Machakos to the North-East which are semi-arid areas with intense livestock rearing. The constant migration of domestic animals across county boundaries in search of pastures and water could lead to the spread of tick borne zoonoses. In essence these are traditionally pastoral regions with free livestock interaction and little or no tick control measures. This might have contributed to the high prevalence of the pathogen in these three districts. According to CDC (2008) fact sheet on Q fever in Missouri, contact with cattle and sheep are the main sources of naturally-occurring *C. burnetii* infections, hence herd to herd contact between animals from this country could lead to spread of *Coxiella* infections. Another factor could be lower priority given to livestock rearing in the peri urban regions like Kajiado where livestock rearing only provides a fraction of the family income as employment and business given a higher priority hence a relatively a higher proportion of irregular tick control (Okuthe & Buyu, 2006) which in turn increases the probability of *Coxiella* transmission. In the United States, Q fever outbreaks have resulted mainly from occupational exposures involving veterinarians, meat processing plant workers, sheep and dairy workers, livestock farmers, and researchers at facilities housing sheep. These modes of transmission in these countries could also apply in these regions in Kenya.

Babesia

Human babesiosis is caused by microscopic intraerythrocytic protozoal parasites called *B. microti* that infect red blood cells and is one of the most frequently reported infections of free-living and domestic animals (Zobba *et al.*, 2011). *B. microti* was not detectable in the tick DNA samples analyzed. This suggests that there is no significant risk of human babesia infections in Kenya.

On the other hand, non-human *Babesia spp* were more prevalent in *Rh. pulchellus*, 33% (6/18), *Boophilus annulatus* 28% (5/18) ticks than *Hyalomma spp* 17 (3/18), *H. truncatum* 11% (2/18) and *Rh. appendiculatus* 11% (2/18) ticks but none was detectable in *Amblyomma* ticks (Table 4.1 and Fig. 4.9). Like in *C. burnetii*, *Babesia* infections were more prevalent in ticks obtained from cattle (56%, 10/18) than sheep and goats, each with a prevalence of 22% (4/18).

Narok had the highest number of ticks infected with other *Babesia*, 39% (7/18), followed by 22% in Wajir (4/18), 11% in Homabay (2/18), 11% in Kajiado (2/18), 5% in Migori (1/18), 5% in Machakos (1/18) and Ewaso Nyiro 5% (1/18). The higher prevalence of non-human *Babesia* in Narok and Wajir is probably as a result of free livestock movement and absence of tick control measures. This enhances the spread of tick borne pathogens (Lambrechts *et al.*, 2007).

Borrelia

B. burgdorferi are gram-negative microaerophilic mobile spirochetes which cause Lyme disease. The prevalence and distribution of Lyme disease in Kenya is unknown (Jowi & Gathua, 2005). In this study, the pathogen for human borreliosis, *B. burgdorferi* had a prevalence of 5% (5/106) while other *Borrelia* species had a prevalence of 7% (7/106), Figure 4.10. This data suggests that there is some considerable level of risk to *B. burgdorferi* in humans in Kenya.

Anaplasma phagocytophylum

Are gram-negative intracellular rickettsial organisms transmitted by Ixodid ticks (Stuen, 2007). In this study, only one sample had detectable *A. phagocytophylum* (Table 4.1). A similar research conducted in public parks in Italy did not detect any DNA of *A. phagocytophylum* in the tick samples screened (Corrain *et al.*, 2012). This could suggest that the human pathogen is rare in Kenya.

E. chaffeensis

This is an obligate intracellular small gram-negative cocci. In vivo, they mainly infect leukocytes, where they occur within membrane-bound vacuoles. The intraphagosomal ehrlichia divide by binary fission to produce a cluster of organisms called morula. The first *E. chaffeensis* to be isolated was in United States in 1991 (Parola & Raoult, 2001). In this study, there was only one tick DNA sample that contained *E. chaffeensis*, suggesting that the pathogen is very rare, hence of very low risk.

Bartonella hensellae

There was no *B. hensellae* detectable in the samples and studies show that this pathogen is rarely found in *Ixodes* ticks (Sanogo *et al.*, 2003). This suggests that there is insignificant risk of the pathogen in Kenya.

Co-infection

As shown in Table 4.3, about 10% of ticks examined had co-infection. This implies that ticks could potentially transmit multiple organisms to humans. In the study, 11 out of 106 tick DNA samples were detectable with multiple infections and they originated from five tick species namely *Rh. pulchellus*, *B. annulatus*, *Amblyomma gemma*, *Amblyomma hebraeum* and *Hyalomma sp.* A study done in the United States at Westchester County site (New York) showed that *Ixodes scapularis* is a vector of *B. burdorferi sensu stricto* and the agent of human granulocytic ehrlichiosis (HGE) and may also transmit babesiosis a protozoan disease due to *Babesia microti*. Patients with antibodies against *B. burdorferi sensu stricto* and or *B. microti* were reported (Parola & Raoult, 2001), although having antibodies to the two pathogens does not necessarily imply that they were transmitted together. Such multiple infections may result in typical clinical manifestations of tick-borne diseases.

Prevalence of zoonoses in the counties

This study found high prevalence of the tick-borne zoonoses in four counties of Kenya, namely Marsabit, Kajiado, Wajir and Narok. These counties also have large scale traditional livestock rearing activities and pastoralists that freely mix their animals. Mixing of animals enhances the chance of ticks getting transferred to different animals and in turn increases the chances of

transfer of the pathogens. Routine tick control measures are not carried out in these areas hence tick population keep increasing and in turn promote the population of these pathogens (Okuthe & Buyu, 2006). These counties are also contiguous to National parks with high wildlife populations. Sharing of habitats with wildlife could increase sharing of tick borne pathogens.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

Conclusions

A number of zoonoses were detected in ticks evaluated, among them *C. burnetii*, non-human *Babesia spp.*, *Borrelia spp.*, *A. phagocytophilum* and *E. chaffeensis*. *Bartonella hensellae* and *Babesia microti* were not detected in any of the samples. Of these pathogens, *Coxiella burnetii* was the most common.

Infection rate of pathogens was highest in *Rhipicephalus* and *Amblyomma* ticks but lowest in *Boophilus* and *Hyalomma* ticks. 90% of the infections detected in ticks were mono-infections. Of the 106 ticks with pathogens, 10% (11/106) had multiple infections.

The study found high prevalence of tick-borne zoonoses in four counties of Kenya: Marsabit, Kajiado, Wajir and Narok. These are counties with large scale traditional livestock rearing activities.

Recommendations

Because the current study was done on samples drawn from another previous study, and also because of inadequate time and funds available, it is recommended that a bigger study be initiated to expand these current findings with more conclusive datasets.

A follow-up human case study should be conducted to determine prevalence of *C. burnetii*, *Borrelia spp.*, *A. phagocytophilum* and *E. chaffeensis* among human population in the country. This would allow for a comparative analysis to be made between humans and livestock and to establish if there is a correlation in the prevalence.

Such studies should be concentrated in the four Counties (Marsabit, Kajiado, Wajir and Narok) identified to have the highest prevalence of tick borne zoonoses.

Clinicians should be sensitized about the possibility of these zoonotic diseases among patients with febrile illnesses so that they do not only restrict diagnosis and treatment to malaria but to widen their scope while handling patients and avoid misdiagnosis.

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APPENDICES

Appendix 1: Letter of ethical approval from ACUC to conduct the research.



KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O. Box 54628- 00200 NAIROBI - KENYA,
Tel: (254) (020) 2722541, 2713349; 0722-205901, 0733-400003; Fax (254) (020) 2726115
E-mail: cvr@nairobi.mimcom.net, Website: www.kemri.org

7th May 2007,

John Waitumbi,
Kenya Medical Research institute
US Army Medical Research Unit – Kenya
P.O Box 54, Kisumu
Kenya.

Dear Dr Waitumbi,

RE: (SSC 1248) Zoonotic Diseases surveillance of Rickettsia Infections in Domestic animals presented at Dagoreti and Athi River slaughter Houses, Nairobi Kenya

Your above referenced proposal was received by the KEMRI Animal Care and Use Committee (ACUC) for review.

The committee established that all aspects of animal care and use have been addressed appropriately in your proposal. Thus, it recommends that you proceed with your work after obtaining all the other necessary approvals.

The ACUC expects you to adhere to all animal handling procedures as described in your proposal and wishes you success in your work.

Yours sincerely


Dr. Peter Borus
Chairman ACUC

Appendix 2: Letter of approval from ERC



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website: www.kemri.org

KEMRI/RES/7/3/1

July 23, 2013

TO: **MISHAEL OSWE,
PRINCIPAL INVESTIGATOR**

THROUGH : **DR. RASHID JUMA
DIRECTOR, CCR,
NAIROBI,**

*Pls do
1/8/2013*

Dear Sir,

RE: **SSC PROTOCOL NO. 2467– REVISED (*RE-SUBMISSION*): TICK BORNE ZONOSIS IN ARCHIVED BLOOD SAMPLES OBTAINED FROM DOMESTIC ANIMALS AT DAGORETTI, ATHI RIVER AND COASTAL REGION SLAUGHTER HOUSES, KENYA.**

Reference is made to your letter dated May 10th, 2013. The ERC Secretariat acknowledges receipt of the Revised Study Protocol – version 4 dated 17 April 2013.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the initial review have been adequately addressed.

The study is granted approval for implementation effective this **23rd day of July 2013**. Please note that authorization to conduct this study will automatically expire on **22nd July 2014**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **10th June 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

**DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE**