EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF *ANAPLASMA* AND *EHRLICHIA* SPECIES INFECTING DAIRY CATTLE IN SMALLHOLDER FARMS IN PERI-URBAN NAIROBI, KENYA

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A thesis submitted in fulfillment of requirements for Doctor of Philosophy Degree in Veterinary Clinical Studies (Medicine)

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November 2020

DECLARATION

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

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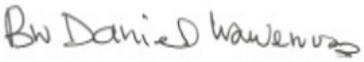
Signature  Date 19th November 2020

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DEDICATION

I dedicate this thesis to my late mother Peyasei Kuraru may her soul rest in peace, my father Peter Simintei, my husband Polycarp Matara and my daughters; Anatolia Nyaboke and Victoria Nanyori for the incredible love, patience and encouragement they showed me through my PhD journey.

ACKNOWLEDGEMENT

Every good and perfect gift comes from above; it comes down from the father of all light with Him there is no such thing as alteration or shadow caused by change (James 1:17). Trusting and claiming His Word, I give all glory and thanksgiving to the Almighty and powerful God for the special graces and favour He has given me through this PhD journey.

Special gratitude to my supervisors; Prof. D.W. Gakuya, Dr. G.O. Aboge, Prof. N. Maingi and Prof. C.M. Mulei who believed in me and dedicated their valuable time and wisdom to the success of this research. I appreciate your great counsel and guidance without which this research work would not have been a success.

I appreciate Dr. Dennis Makau who patiently guided me through the epidemiological analysis of my research data. You did not tire in the many times we had to repeat the analysis. Special appreciation to Hellen Kariuki and Dr. Esther Kanduma, who articulately and patiently took me through the DNA sequence analysis for this research. You were definitely God sent ladies, may you be blessed abundantly. To my soul-mate and friend Dr. Christine Mbindyo thank you for sharing every challenge and success I experienced through this doctoral journey.

I am grateful for the support I received from my colleagues at the Department of Clinical Studies, especially Prof. James Mwangi-Nguhiu, who constantly encouraged me and believed I would deliver quality research and even when things got tough, he took to my side to guard my work. Additionally, I appreciate the laboratory assistance from Richard Omulo Otieno-Veterinary Parasitology Laboratory, Nixon Simani and Alice Kinyua-Hematology and Biochemistry Laboratory respectively and Alfred Mainga-Molecular Laboratory.

Sincere appreciation to the University of Nairobi for waivering my school fees and providing a conducive environment that enabled me complete my PhD.

I acknowledge the financial and mentorship support from CARTA (Consortium for Advanced Research Training in Africa) and Deutscher Akademischer Austauschdienst (DAAD). The special model of pedagogy taught through the CARTA program molded the researcher I am today through the guidance of the wonderful faculty across Africa and the North. I am forever indebted to their support and guidance. Special thanks to CARTA-cohort 8 fellows for creating a cheerful team of doctoral students that made research so exciting. To my mentor Prof. Peter Ngure, thank you for believing in me and challenging me to explore my full potential.

To my wonderful and loving husband Polycarp Matara, you were there to listen and bear the heaviness of my challenges and the joy of my successes all through this doctoral journey, I sincerely appreciate you. Thank you for running errands on our behalf as I worked on this PhD. Special thanks to my beautiful PhD girls; Anatolia Nyaboke and Victoria Nanyori for the radiant love you always showed me despite being away from you for long hours and at times for months. To my baby-minder, Elizabeth Nashipae, thank you for being the sister that I never had.

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LIST OF ABBREVIATIONS

A, T, G, C	Nucleotides in DNA: Adenine, Thymine, Guanine and Cytosine
BLAST	Basic Local Alignment Search Tool
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
ECF	East Coast Fever
EDTA	Ethlene-diamine-tetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
OIE	World Organization for Animal Health
PI	Persistent infection
PCR	Polymerase Chain Reaction
RPM	Revolutions per minute
SFG	Spotted Fever Group
TBD	Tick borne disease

ABSTRACT

Livestock production contributes 12% of the Kenyan Gross Domestic Product (GDP) and 40 % of the Agricultural GDP. Milk is the main livestock product and urban and peri-urban areas provide a ready market, which has led to proliferation of smallholder dairy farms in these areas. Among the major threats to optimum production on the dairy farms are tick-borne diseases. Among these diseases, anaplasmosis and ehrlichiosis caused by *Anaplasma* and *Ehrlichia* species respectively, are only second to East Coast Fever in their deleterious effects. Information on these infections in cattle in smallholder farms in peri-urban Nairobi County has been scanty and unconfirmed. In the recent past, there has been rising cases reported from these peri-urban areas to the University of Nairobi Veterinary Hospital and diagnosed on microscopy as ehrlichiosis. Confirmation of the infections and identification of the species involved was not done, mainly because the clinical presentations were unspecific and the microscopy used had a low sensitivity. The objectives of this study were to investigate the species of *Anaplasma* and *Ehrlichia* infecting dairy cattle in peri-urban Nairobi, assess the risk factors associated with these infections and the tick vectors that harbour the pathogens.

A cross-sectional study was undertaken in peri-urban Nairobi County, where four sub-counties; Kasarani, Lang'ata, Dagorretti and Westlands were purposively selected for the study. A total of 314 apparently healthy dairy cattle from 109 farms were randomly recruited. Whole blood, serum samples, and all ticks found attached to the cattle were collected. A pre-tested questionnaire was used to collect data on potential risk factors for the infections on the farms. Giemsa-stained blood smears were screened under a microscope for *Ehrlichia* and *Anaplasma* pathogens and antigen Enzyme-linked immunosorbent assay (ELISA) used to screen for the presence of *Ehrlichia ruminantium* in the sera. Whole blood DNA was

extracted and tested for presence of *Anaplasma* and *Ehrlichia* DNA through amplification of the 16S rRNA gene using Polymerase Chain Reaction (PCR). *Anaplasma* and *Ehrlichia* species confirmation was done by sequencing of the PCR amplicons. Morphological identification of the ticks was done and DNA extracted from individual ticks and then analysed for *Anaplasma* and *Ehrlichia* pathogens through PCR amplification of 16S rRNA gene using same primers as used on blood. Genetic identities of the pathogens from both blood and ticks were confirmed through BLASTn analysis and phylogenetic reconstruction.

On microscopy, 249 of the 314 (79.3%) sampled cattle had *Ehrlichia*-like inclusion bodies in their white blood cells but only 55 of 292 (18.6%) serum samples tested positive for *E. ruminantium* on the Ag-ELISA. On PCR, out of 306 blood DNA samples analysed 61 (19.9%) and 10 (3.3%) were positive for *Anaplasma* and *Ehrlichia* species, respectively. Sequencing of representative samples; 54.1% (33/61) for *Anaplasma* species and 60% (6/10) for *Ehrlichia* species revealed infections with *Anaplasma platys, A. marginale, A. bovis* and *Ehrlichia minasensis*.

Location of farms in Lang'ata Sub-county (p=0.009) and presence of ticks on cattle (p=0.007) were factors significantly associated with sero-positivity to *E. ruminantium* in cattle. The higher prevalence in Lang'ata Sub-county could be because it borders the Nairobi National Park and wild animals are reservoirs for tick-borne diseases including *E. ruminantium.* Transmission by ticks is also the major route for *E. ruminantium* infections in susceptible cattle. Cleaning of cowsheds fortnightly compared to cleaning every day was significantly associated with sero-positivity to *E. ruminantium* (p=0.008) and occurrence of *Anaplasma*

and *Ehrlichia* on PCR (p=0.034). Accumulated slurry compromises animal welfare increasing stress to the animals and predisposing them to diseases.

Sixty-six (21%) of the 314 cattle examined had ticks. A total of 94 adult ticks were found on the cattle and of these 63(67.0%), 18(19.1%), 13(13.8%) were in the genera *Rhipicephalus, Amblyomma* and *Hyalloma,* respectively. Although *R. (boophilus) decoloratus* was the most prevalent 24.5% (23/94) the re-emerging *R. (b) microplus* 6.4% (6/94) were also identified. Reports of *R (b) microplus* in Kenya, which is a highly invasive species, have previously only been in the coastal region and its identification in other parts of the country implies possible emergence of infestations and the associated diseases. *Rickettsia* were found in the ticks, with *Rickettsia (R.) conorii* in *H. rufipes* and *A. variegatum*, *R. aeschlimanii* in *R. (b) microplus* and *H. rufipes* and *E. ruminantium* and *E. canis* in *A. variegatum.* These *Rickettsia* have all previously been reported in the tick vectors, but the zoonotic importance as well as economic impact these pathogens in the smallholder farms needs to be investigated.

Although clinical disease was not evident in the study cattle, control measures remain paramount since relapse of clinical disease due to *Anaplasma* and *Ehrlichia* is possible especially when animals are stressed. This study provides the first report of *A. platys* and *E. minasensis* infections in dairy cattle in Kenya. These are emerging pathogens, with *A. platys* being considered potentially zoonotic. Further studies to characterise these pathogens including their transmission and pathogenicity in cattle are recommended. There is need for countrywide studies to determine the extent of spread of *R. microplus* tick so as to pre-empt possible infections of cattle with pathogens it vectors such as *B. bovis* and *E. minasensis*.

CHAPTER 1: INTRODUCTION

1.1 Background

Agriculture accounts for 26% of the Kenyan Gross Domestic Product (GDP) and of these, 40% is from livestock (FAO, 2020). Consequently, livestock production as a sub-sector contributes 12% of the Kenyan Gross Domestic Product (GDP) (Behnke and Muthami, 2011). Milk is by far the most important of the livestock products estimated to be four times more important than meat (Kenya Markets Trust, 2019; De Jong *et al.,* 2015). Approximately 80% of milk in Kenya is produced by smallholder dairy farms commonly practicing integrated livestock and crop production (MoALF, 2019; Odero-Waitituh, 2017).

There is high concentration of milk production in the urban and peri-urban areas of Kenya owing to the product marketing influence and convenience of such areas (Thorpe *et al.,* 2000). This has been observed in Nairobi County where the high population of approximately 4.3 million people (Kenya National Bureau of Statistics, 2019) has raised the demand for milk and other animal products thereby resulting in increased peri-urban farming (Kaitibie *et al.,* 2010). Despite the high potential and dominance of the dairy sector, its overall production levels are lower than expected due to constraints of high cost of farm inputs and equipment, low milk prices, suboptimal nutrition and diseases. Among the most important diseases that contribute to this suboptimal dairy production are the tick-borne diseases (TBDs) (Rademaker *et al.,* 2016; Maingi and Njoroge, 2010; Wesonga *et al.,* 2010).

East Coast Fever (ECF), anaplasmosis, babesiosis and ehrlichiosis are the major tick-borne diseases of cattle in Kenya (Adjou Moumouni *et al.,* 2015; Wesonga *et al.,* 2017). Anaplasmosis is only second to ECF in its impact on the smallholder dairy farms in peri-

urban, Nairobi (Gakuya and Mulei, 2005). Anaplasmosis and ehrlichiosis are caused by the bacteria in the genera *Anaplasma* and *Ehrlichia* respectively. In Kenya, anaplasmosis due to *Anaplasma marginale* is the most widely reported, with few reports of *A. bovis* (Adjou Moumouni *et al*., 2015; Njiiri *et al*., 2015). *Anaplasma centrale* has not been reported in Kenya possibly because of the less pathogenic nature of the organism thus clinical disease is rarely reported (Kocan *et al.,* 2010a). The severity of disease condition due to *A. marginale* is related to the extent of destruction of red blood cells and sudden deaths can occur in naïve cattle (Aktas and Özübek, 2017; Kocan *et al*., 2010a). Elsewhere cattle have been documented to be infected with *A. phagocytophilum* causing tick borne fever (Stuen, 2007; Silaghi *et al*., 2018; Atif, 2015) and *A. platys* causing undescribed disease (Dahmani *et al*., 2015; Ben Said *et al*. 2017). Noting the wide pathogen range that can potentially cause anaplasmosis in cattle, it was paramount to investigate the specific pathogens in circulation in dairy cattle in Kenya.

On the other hand, ehrlichiosis (heart water) is caused by *Ehrlichia ruminantium* which is mainly a cattle pathogen (Allsopp, 2010). The disease causes severe economic losses in Africa where approximately 150 million animals are at risk of infection (Allsopp, 2010; de la Fuente, *et al*. 2008). The estimation of these losses in endemic areas such as Kenya is complicated by the fact that farmers do not provide regular reports, definitive diagnoses are hardly delivered (Allsopp, 2010) and infections often coincide with other TBDs such as anaplasmosis and East Coast Fever (Allsopp, 2015). Despite this, the few economic studies that have been carried out have pointed to quite substantial economic losses. A study in Tanzania recorded economic losses of USD 22.6 million/year with cattle mortality accounting for 8.8 million USD (Kivaria, 2006) while Melaku *et al*. (2014) reported losses from

mortalities, cost of acaricides and antibiotics, losses in milk and meat at approximately USD 7884.67 million/year in Ethiopia.

There have been few reports of ehrlichiosis in ruminants (Njiiri *et al*., 2015; Wesonga *et al*., 2006) as well as its detection in ticks in Kenya (Omondi *et al*., 2017). The difficulty in diagnosing ehrlichiosis due to its non-pathognomonic clinical signs has led to underestimation of the economic losses, especially in endemic countries like Kenya (Allsopp, 2015). Furthermore, its high economic importance, as reported in Tanzania and Ethiopia (Kivaria, 2006 and Melaku *et al.* (2014), necessitates continued investigation into the existence of this pathogen so as to put in place appropriate control measures.

Tick-borne diseases are vectored by a number of tick species that are wide spread in Kenya (Keesing *et al.,* 2018; Oswe *et al.,* 2018; Kariuki *et al.,* 2012). Following the structural adjustment policy affecting delivery of animal health services in Kenya in late 1980s, tick control was greatly affected leading to an upsurge of tick borne diseases (Mutavi *et al.,* 2018). Thereafter, tick control and disease management was left in the hands of communities who had little knowledge on control measures especially handling of acaricides (Mutavi *et al.,* 2018; Mugambi *et al.,* 2012). The mishandling and misuse of acaricides (Sungirai *et al.,* 2016; Mugambi *et al.,* 2012) has resulted in increased health risks to the environment, animals and people (De Meneghi *et al.,* 2016). Since then, tick-borne diseases have consistently been a threat to the livestock sector in Kenya where it is estimated that nearly 80% of the animals are affected and is estimated that one animal dies of TBD each second in Kenya (Kanduma, 2018).

The spatial distribution of tick vectors is related to the occurrence of the specific diseases they transmit (Doudier *et al.,* 2010). The problem of TBDs is enhanced by the dynamic population of the tick-vectors and the increased susceptibility of exotic cattle breeds and their crosses, which are commonly kept by the smallholder dairy farmers (Behnke and Muthami, 2011; Kaitibie *et al.,* 2010;). Additionally, the inevitable climate change, increase in human population and the constantly evolving changes in land use patterns in Kenya especially urban and peri-urban areas, are some of the reasons that have also led to changes in the epidemiology and diversity of TBDs (Keesing *et al.,* 2018; Rademaker *et al.,* 2016; Mureithi and Mukiria, 2015; Kilpatrick and Randolph, 2012). This has resulted in challenges of predicting outbreaks of tick-borne disease and mapping of tick vectors (Keesing *et al.,* 2018) to guide in the control efforts. Other than climate change, the development in diagnostic techniques and increased public health interest have resulted in detection of emerging pathogens causing infections in both animals and human (Kilpatrick and Randolph, 2012; Randolph, 2010).

Tick-borne diseases (TBDs) cause both economic losses and public health risks. Although the exact economic impact has not been comprehensively evaluated and quantified in Kenya, they have been shown to cause colossal losses through mortalities, chemotherapy, acaricide application and decreased production through decreased weight gain and reduced milk yield (Mugambi *et al.,* 2012; Wesonga *et al.,* 2010; Kivaria, 2006; Muraguri *et al.,* 2005). McLeod and Kristjanson (1999) approximated the economic losses associated with tick borne pathogens in Kenya to be approximately Kshs. 30 billion (USD 281 million). In a case study in Njiru Sub-county in Nairobi County, the livestock farmers reported that tick infestation and tick-borne diseases have contributed to nearly 30% of food insecurity in the area (Mureithi

and Mukiria, 2015). This implies that households that depend on livestock lose their income and animal proteins sources which have been shown to substantially reduce stunting in children (Choudhury and Headey, 2018). Overall, there is increased poverty levels on the livelihoods of livestock-dependent communities due to loss of income from production and their sources of animal protein (Kanduma, 2018). Additionally, domestic animals have been reported as potential reservoir for zoonotic infections (Ybañez and Inokuma, 2016; Sen *et al.*, 2011), thereby posing health risk to animal owners who are in constant contact with them (Chien *et al.*, 2019; Mtshali *et al.*, 2015; Parola and Raoult, 2001).

Smallholder dairy farming in peri-urban Nairobi, Kenya has been estimated to produce 4.3 million kilograms of milk annually with an annual rise in this value (Alarcon *et al.*, 2017). Despite the importance of the smallholder dairy farms, they are faced with unique challenges mainly because of extensive land sub-division in the urban and peri-urban areas of the city. This implies that there are small land sizes for cattle housing and minimal spaces for growing of fodder (Alarcon *et al.*, 2017). This coupled with minimal knowledge on animal welfare concerns have resulted in farmers practising sub-optimal animal husbandry consisting of dilapidated cattle houses, lack of roofing, inadequate walk alleys, rugged concrete or stone floors with pot-holes that hurt the cattle hooves and accumulated slurry that prevent cattle from lying down (Nguhiu-Mwangi *et al.*, 2013; Aleri *et al.*, 2012). These stressful conditions decrease the immunity of the animals predisposing them to increased infections (Garry, 2008).

Moreover, the cattle are commonly housed close to the people's houses and at times sharing houses with people further posing health risk to the human occupants in the presence of

zoonotic infections (Alarcon *et al.,* 2017). With the outsourcing of fodder and hay from roadsides and in tick-infested areas (Kiambi *et al.,* 2018; Rademaker *et al.,* 2016), tick-borne diseases remain a great threat to these production systems. It remains paramount to understand the tick-borne pathogens circulating in these unique production systems with the aim of mitigating their effects through appropriate control measures.

Therefore, this study was carried out to investigate the causative agents of tick-borne diseases in the *Anaplasma* and *Ehrlichia* genera, assess the associated risk factors associated with their occurrence as well as analyze *Anaplasma* and *Ehrlichia* spp. harbored by ticks infesting dairy cattle in smallholder dairy farms in peri-urban Nairobi.

1.2 Problem statement

East Coast Fever, anaplasmosis and babesiosis have been considered to be the most important tick borne diseases in Kenya (Rademaker *et al.,* 2016; Adjou Moumouni *et al.,* 2015; Wesonga *et al.,* 2010; Gakuya and Mulei, 2005). However, in the recent past (since 2014), a number of dairy cattle examined by clinicians from the University of Nairobi Veterinary Hospital (Ambulatory Services) have revealed infection of cattle with suspected ehrlichiosis (unpublished data). Clinical presentation of the cases has been varied and unspecific with some cattle appearing clinically healthy, others presenting with unthriftness, lymphadenopathy, gradual loss in body condition with others succumbing to the infection. The clinical presentation observed in the clinical cases is not typical of the commonly reported tick-borne diseases in Kenya. On further microscopic examination in the laboratory, moruli were observed in the white blood cells of the affected animals (unpublished data). The

pathogens involved in these infections remained unclear, hence the need for further investigation.

Additionally, a retrospective review of the trend of tick-borne diseases screened microscopically from the blood samples submitted to the hematology laboratory of Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi (2010-2019) revealed a rise in the incidence of bovine ehrlichiosis as other tick-borne diseases decreased (unpublished data) (Figure 1.1). Using the percentage of infections calculated against the total cases of TBDs reported in that period, the trends indicated a spike in the cases diagnosed as ehrlichiosis in the recent past (2013-2019) (Figure 1.1). The broad diagnosis of all these cases as ehrlichiosis raised the need to further understand the diversity of the pathogen involved and to characterize potentially zoonotic pathogens that may be in circulation.

Anaplasmosis and ehrlichiosis have been overlooked for a long time in Kenya. Although these diseases do not cause high mortalities as compared to East Coast Fever (Muraguri *et al.,* 2005), the decreased productivity (Kocans *et al.,* 2010a) and associated increased susceptibility to other tick borne infections (Woldehiwet, 2010) cannot be underestimated. Furthermore, they constitute a high number of zoonotic pathogens in the *Anaplasmataceae* family (Eremeeva and Dasch, 2011). Investigation into the causative agents of anaplasmosis and ehrlichiosis in smallholder dairy cattle in peri-urban Nairobi and the risk factors associated with their occurrence will shed light into the disease burden they contribute to both livestock and human populations.

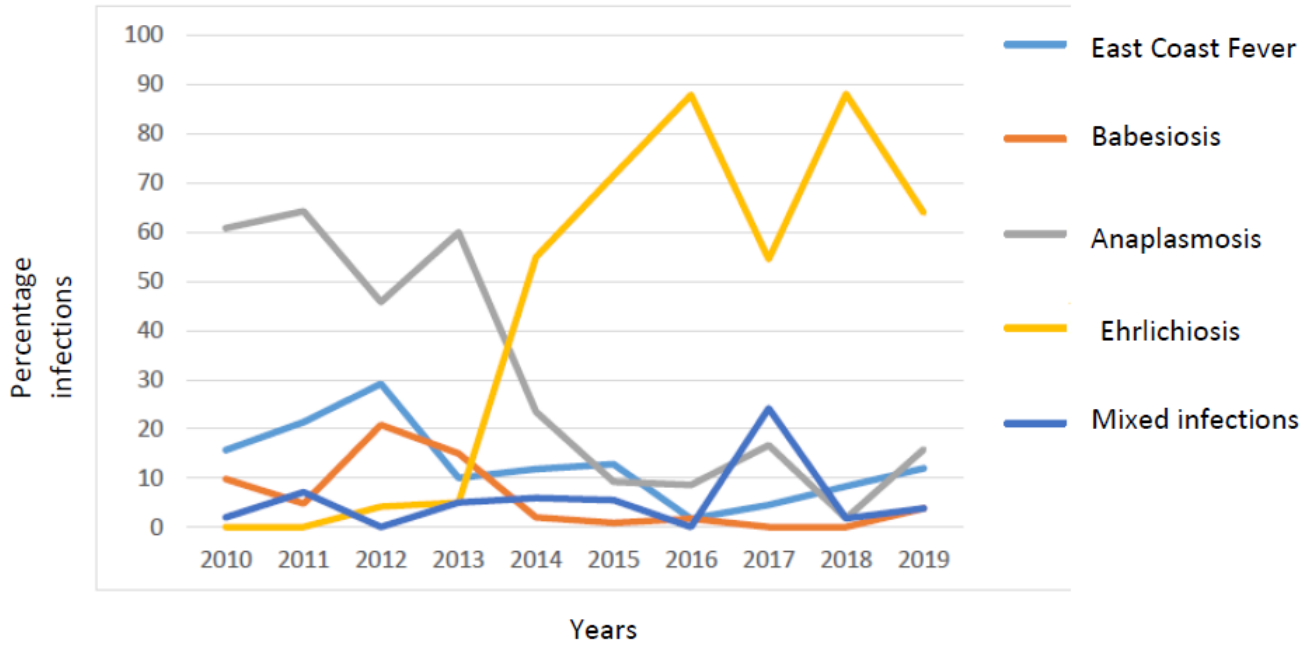

Figure 1.1: Trends in the percentage of cases of cattle diagnosed with various tick borne diseases in the last 10 years using records from Hematology Laboratory, Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.

1.3 Objectives

1.3.1 Overall objective

To determine the prevalence, risk factors, molecular epidemiology and the tick vector identification of *Anaplasma* and *Ehrlichia* species infecting dairy cattle in the smallholder farms in peri-urban sub-counties of Nairobi County, Kenya.

1.3.2 Specific objectives

1. To determine the prevalence of *Anaplasma* and *Ehrlichia* pathogens infecting dairy cattle in smallholder farms in peri-urban Nairobi.
2. To assess the risk factors associated with the occurrence of *Anaplasma* and *Ehrlichia* pathogens in smallholder dairy farms in peri-urban Nairobi.
3. To detect and characterize the specific species of *Anaplasma* and *Ehrlichia* infecting dairy cattle in the study area using molecular markers.
4. To identify ticks infesting dairy cattle in peri-urban Nairobi and the species of *Anaplasma* and *Ehrlichia* they harbor.

1.3.3 Hypothesis

1. There is low prevalence of *Anaplasma* and *Ehrlichia* pathogens infecting dairy cattle in smallholder farms in peri-urban Nairobi.
2. A varied number of household, management, animal level factors are responsible for infection of dairy cattle with *Anaplasma* and *Ehrlichia* pathogens in the study area.
3. A wide range of *Anaplasma* and *Ehrlichia* pathogens including the zoonotic ones are infecting dairy cattle in the study area.
4. Different genera and species of hard ticks infest dairy cattle in the study area and they harbor various species of *Anaplasma* and *Ehrlichia* pathogens.

1.4 Justification

Despite the well-known economic and public health importance of tick borne diseases worldwide and even in Kenya, there is limited information on the molecular epidemiology of these diseases, especially those caused by parasites in the *Anaplasma* and *Ehrlichia* genera. Some of the species in these genera are of zoonotic potential hence posing a health risk to people when cattle are infected. The ambiguity of the clinical presentation of the reported cases at the University of Nairobi, as well as unavailability of confirmatory tests being

conducted at the laboratory, presented with a challenge of understanding the specific pathogens infecting the dairy cattle, the risk factors of infections and the tick vectors.

The mentioned diagnosis of ehrlichiosis using presence of moruli in white blood cells is ambiguous since a number of pathogens in the Family Anaplasmataceae can present with moruli in bovine white blood cells (Aguiar *et al.,* 2019; Cabezas-Cruz *et al.,* 2019; Iqbal *et al.,* 2019; Priyanka *et al.,* 2017; Kasari *et al,* 2010). Moreover, the reorganization of pathogens between the *Anaplasma* and *Ehrlichia* genera by Dumler *et al.* (2001) indicated the close relatedness of these pathogen implying that definitive diagnosis should rely on molecular diagnosis other than morphological identification.

This study therefore aimed at understanding and clarifying the tick-borne pathogens infecting dairy cattle in the peri-urban areas of Nairobi using molecular techniques as well as assessing farm management factors that are associated with their occurrence. Additionally, the study analyzed ticks infesting these animals to determine the pathogens that they harbor. In knowing the tick-borne pathogens in the *Anaplasma* and *Ehrlichia* genera infecting cattle, veterinarians will be guided to consider these infections among their differential diagnosis, prompting early diagnosis and treatment. Furthermore, by comprehending these infections and the risk factors involved, appropriate control measures will be put in place thereby ensuring healthy cattle. Consequently, this will contribute to food security, leading to poverty eradication as envisioned in Sustainable Development Goal (SDG) 2. Moreover, healthy cattle will have high production of both milk and meat hence increasing livestock's contribution to national GDP, thereby the attainment of two-digit economy as envisioned in Kenyan Vision 2030.

CHAPTER 2: LITERATURE REVIEW

2.1 Etiology and transmission of anaplasmosis and ehrlichiosis

Anaplasma and *Ehrlichia* pathogens causing anaplasmosis and ehrlichiosis respectively are obligate intracellular gram negative bacteria of the Family Anaplasmataceae and are of both veterinary and public health concern (Eremeeva and Dasch, 2011; Rymaszewska and Grenda, 2008). The terms anaplasmosis and ehrlichiosis have been used loosely to refer to diseases caused by pathogens in the genus *Anaplasma* and *Ehrlichia* respectively (The Center for Food Security and Public health, 2013; Mcquiston *et al.,* 2003).

The Family *Anaplasmataceae* has been of interest due to the growing emerging species of zoonotic interest (Silaghi *et al.,* 2017). Pathogens in this family multiply in intracellular vacuoles called moruli found in the cytoplasm of cells in both vertebrates and invertebrates (Kocan *et al.* 2010a). Reclassification of various species from the two genera has been undertaken concluding that the genus *Anaplasma* has five species; *A. marginale, A. centrale, A. platys, A. bovis* and *A. phagocytophilum* while the genus *Ehrlichia* has the species *E. ruminantium, E. chaffeensis, E. canis* and *E. ewingii* (Dumler *et al.,* 2001).

Anaplasma species documented to infect domestic ruminants including cattle are *Anaplasma marginale (A. maginale), A. centrale, A. ovis, A. bovis, A. phagocytophilum* and more recently *A. platys* (Park *et al.,* 2018; Dahmani *et al.,* 2015) while *Ehrlichia ruminantium (E. ruminantium)* is the main species of *Ehrlichia* known to infect cattle (Allsopp, 2015). *Ehrlichia minasensis* is an emerging *Ehrlichia* species that has been shown to infect cattle and the disease condition it causes in cattle remains under study (Cabezas-Cruz *et al.,* 2019; Zobba *et al.* 2014). The Persistent Infection (PI) is a common state in animals infected with

Anaplasma or *Ehrlichia* species where they remain asymptomatic but can act as source of these infections to susceptible animals (Brown and Barbet, 2016; Kocan *et al.,* 2010a; Garry, 2008).

Human disease has been associated with *Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia chaffeensis, E. ewingii, E. canis* and *E. ruminantium* (Bakken and Dumler, 2015; Maggi *et al.,* 2013; Louw *et al*., 2005). Despite close contact with animals infested with ticks being identified as a risk factor for human infections with *Anaplasma* and *Ehrlichia* species (Li *et al.,* 2011; Vorou *et al.,* 2007), this practice is still common in Kenya (Alarcon *et al*., 2017; Ndeereh *et al.,* 2016). There is need to investigate the occurrence and identity of these pathogens in cattle so as to control them and prevent potential infections in humans.

The tick bites are the main mode of transmission of *Anaplasma* and *Ehrlichia* species (Baneth, 2014). In addition to bacteria, ticks can also transmit rickettsia, protozoa and viruses (Rajput *et al.,* 2006; Shyma *et al.,* 2013). Ticks are only second to mosquitoes in transmission of important animal and human diseases (Parola and Raoult, 2001). In addition to transmission of pathogens, they can also cause tick toxicosis and tick worry (Shyma *et al.,* 2013). The ability of hard ticks to feed for long hours and painless bites facilitate the transmission of pathogens (Stuen *et al.,* 2013). While few ticks such as *Ixodes* spp are attracted to human and can transmit pathogens (Atif, 2015; Stuen *et al.,* 2013), others like *Rhipicephlus microplus* do not feed on people (Parola *et al.,* 2003). Generally, ticks feed on a variety of hosts, both wild and domestic animals but humans are usually a coincidental host (Alberti *et al.,* 2005).

Anaplasma marginale is transmitted by *Rhipicephalus (boophilus) microplus* (Aguirre *et al.*, 1994) although other ticks' species can still transmit (Kocan *et al.,* 2004). The closely related *Anaplasma centrale* is transmitted by *Rhipicephalus simus* (Potgieter and van Rensburg, 1987) while *A. bovis* has been detected in a number of *Rhipicephalus* and *Ambylomma* species (Omondi *et al.,* 2017). *Anaplasma phagocytophilum* is transmitted by *Ixodes* ticks in the temperate regions (Stuen *et al.,* 2013), but multiplicity of ticks and mites and possibly tick-infested migratory birds transmit the organisms in other areas (Stuen, 2007). *Rhipicephalus sanguineous* is the vector known to transmit *A. platys* (Pesapane *et al.,* 2019; Ybañez *et al.,* 2016). *Amblyomma* ticks are the known vectors that transmit *E. ruminantium* (Allsopp, 2010) but biting insects and blood transfusion could also transmit these organisms (Al-badrani, 2013). Ticks have been shown to develop a persistent infected state to allow them transmit these pathogens to hosts (Kocan *et al.,* 2003).

In addition to tick vectors, other modes of transmission have been documented. Mechanical transmission through fomites and biting flies is common for *Anaplasma marginale* (Kocan *et al.,* 2010a) while iatrogenic transmission has been observed at the peak of routine procedures in farms (Garry, 2008). Vertical transmission of *E. ruminantium* in small ruminants (Bonto Faburay *et al.,* 2007a) and cattle (Deem *et al.,* 1996) has also been reported. Migratory birds and cattle egrets can provide alternative routes of transmission for these pathogens across continents (Atif, 2015; Bjöersdorff *et al.,* 2001; Stuen 2007) resulting in temperate pathogens being detected in the tropics. There being a wide range of potentially pathogenic *Anaplasma* and *Ehrlichia* species, clarifying the specific species involved in the infections in dairy cattle in peri-urban areas of Nairobi is paramount so as to develop targeted control measures.

2.2 Prevalence of anaplasmosis and ehrlichiosis

Assessment of prevalence of tick-borne pathogens influences the need to put in place control measures (Uilenberg, 1995). *Anaplasma marginale* is an important pathogen that has been detected widely in Africa (Ben Said *et al.,* 2018; Hove *et al.,* 2018; Mekonnen *et al.,* 2002). The prevalence reported is varied depending on geographic location; East Africa at 50% (Adjou Moumouni *et al.,* 2015), Southern Africa 97.3% (Fernandes *et al.,* 2019), North Africa 19.4% (Dahmani *et al.,* 2019) and West Africa 39.1% (Lorusso *et al.,* 2016). This wide spread distribution of *A. marginale* may be associated with the tropical and sub-tropical climate common in Africa which supports the vector ticks *Rhipicephalus (boophilus)* species (Constable *et al*., 2017).

Reports of *A. bovis* infection in cattle have been relatively low ranging between 0.4 and 4.4% (Rjeibi *et al.,* 2018; Belkahia *et al.,* 2015; Ceci *et al.,* 2014; Muhanguzi *et al.,* 2010) except for a study in western Kenya that reported 39% in indigenous calves ((Njiiri *et al.,* 2015). Although the clinical significance of *A. bovis* is low when evaluated singly, the overall effect of infections with multiple pathogens needs to be investigated.

Low prevalence of *A. phagocytophilum* has been reported in Africa as observed by Teshale *et al.* (2018) in Ethiopia and Muhanguzi *et al.* (2010) in Uganda where they recorded similar prevalence of 2.7% in cattle. Contrary to this, Dahmani *et al.* (2015) reported a high prevalence of 41% in cattle in Algeria. This may be explained by the use of a new marker gene to detect *Anaplasma* species contrary to the one used by the previous authors.

There has been relatively low prevalence (based on molecular techniques) of *E. ruminantium* in Africa including Kenya; 0.6% in Ethiopia (Teshale *et al.,* 2018), 0.4% in Kenya (Njiiri *et al.,* 2015), 1.1% in Nigeria (Lorusso *et al.,* 2016) and 1.7% in Uganda (Byaruhanga *et al.,* 2015).

Co-infections are a consistent finding by many studies investigating *Anaplasma* and *Ehrlichia* species (Ben Said *et al.,* 2018; Rjeibi *et al.,* 2018; Adjou Moumouni *et al.,* 2015; Belkahia *et al.,* 2015; Njiiri *et al.,* 2015). This is explained by multiple pathogens that can be present in a tick at the same time (Iweriebor *et al.,* 2017; Mtshali *et al.,* 2015), facilitating multiple infections (Raileanu *et al.,* 2018; Eremeeva and Dasch, 2011).

2.3 Risk factors for anaplasmosis and ehrlichiosis

Abiotic and biotic factors influence the occurrence of anaplasmosis and ehrlichiosis. The abiotic factors include seasonality, geographic location, climatic conditions and management practices while biotic factors are age, breed, sex, tick infestation and physiological status (Ben Said, *et al*., 2018). Some of the abiotic factors influence distribution of the tick vectors and thereby the associated pathogens (Tembo *et al.,* 2018; Walker *et al.,* 2014a). Important management factors in smallholder dairy farms include housing, sources of feeds, parasite control and biosecurity measures (Sivakumar *et al.,* 2017; Nguhiu-Mwangi *et al.,* 2013; Aleri *et al.,* 2012).

Management factors especially stressors due to housing and nutrition have been shown to influence the animals' immunity thereby the response to infection (Abuelo, 2020). Acaricide application, frequency of application as well as the presence of the tick vector have an

influence on the occurrence of *Anaplasma* and *Ehrlichia* infection (De Meneghi *et al.,* 2016; Mugambi *et al.,* 2012; Swai *et al.,* 2008). Indeed, Belkahia *et al.* (2015) reported increased *Anaplasma* infections in farms with poor management. Animal movement and presence of wildlife are also potential risk factors for the infection (Adjou Moumouni *et al.,* 2015).

Host factors affects development and severity of *Anaplasma* and *Ehrlichia* infections. Younger animals up to 2 years tend to be resistant to *Anaplasma marginale* infections or may develop mild signs (Constable *et al.,* 2017; Kocan *et al.,* 2010a). This resistance has been associated with passive immunity passed through colostrum although other unexplained factors tend to play a role (Constable *et al.,* 2017; Garry, 2008). Despite this, when stressors such as starvation, transportation or extreme weather conditions are present, the animals still come down with the clinical disease (Garry, 2008). On contrary, younger animals are more susceptible to *E. ruminantium* infections than adults (Melaku *et al.,* 2014). Similarly, *E. minasensis* has been reported to cause severe disease in calves than adults with the later commonly being persistently infected and acting as sources of infections to the calves (Aguiar, 2017).

The exotic dairy breeds of cattle and their crosses which are commonly kept in peri-urban Nairobi due to their high milk production (Rademaker *et al.,* 2016), have higher susceptibility to tick-borne diseases (Mattioli *et al.,* 2000). Some physiological states of the cattle such as pregnancy and lactation suppress immunity predisposing cattle to infection (Leblanc, 2020; Kocan *et al.,* 2010b). The variability in the management practices of cattle kept in peri-urban areas of Nairobi necessitates the need to assess important risk factors that predispose cattle to infection by *Anaplasma* and *Ehrlichia* species.

2.4. Pathogenicity and clinical signs of anaplasmosis and ehrlichiosis

The pathogenicity and disease caused by *Anaplasma* and *Ehrlichia* species varies with the specific pathogen and the host affected (Eremeeva and Dasch, 2011; Rar and Golovljova, 2011; Gajadhar *et al*., 2010). *Anaplasma marginale* causes anaplasmosis in domestic and wild ruminants. Kocan *et al.* (2010a) describes the disease associated with this pathogen to be either paracute where animals die without any prior signs, occurring in highly susceptible animals or acute form which is common in endemic areas. In the acute form, animals present with fever, pale mucous membranes, drop in milk production, abortion in pregnant animals and infertility in males, gastrointestinal signs associated with dehydration with few animals presenting with neurologic deficits (Aktas and Özübek, 2017).

Anaplasma centrale is a closely related pathogen to *A. marginale* although they differ in their morphology and virulence (Rymaszewska and Grenda, 2008). Some authors even think that it is a naturally attenuated strain of *A. marginale* (Rar and Golovljova, 2011). *Anaplasma centrale* causes a mild disease to cattle but its infection results in immunity to *A. marginale* (Kocan *et al.,* 2003). On this basis live vaccines against *A. marginale* have been developed (Kocan *et al.,* 2010a).

Anaplasma bovis is a monocytic pathogen infecting cattle, goats, wild deer (Yang *et al*., 2015; Ceci *et al*., 2014; Liu *et al*., 2012) and sometimes dogs (Sakamoto *et al.,* 2010). The infection is commonly asymptomatic but when the clinical disease occurs, it presents with fever, lymphadenopathy, pale mucous membranes and reduced weight gain (Noaman and Shayan, 2010). Although *A. bovis* has been detected in cattle in Kenya (Njiiri *et al.,* 2015), little

emphasis has been placed on it as an economically important pathogen. Co-infections with this pathogen are common (Rjeibi *et al.,* 2018; Belkahia *et al.,* 2015) therefore its role in enhancing the pathogenicity of other *Anaplasma* species needs to be investigated (De Waal, 2000).

Anaplasma phagocytophilum is a zoonotic species known to infect white blood cells of a wide range of hosts (Eremeeva and Dasch, 2011; Stuen, 2007). Its indiscriminate host range has been associated with high adaptability and its capacity to circumvent host immune pathways (Silaghi *et al.,* 2018; Stuen, *et al.,* 2013). It is among the most important tick-borne zoonosis causing life threatening disease to humans (Bakken and Dumler, 2015). Since livestock and in particular cattle have been documented as potential domestic reservoirs (Atif, 2015; Noaman and Shayan, 2009; Stuen *et al.,* 2013), caution is needed in the various management practices to minimize potential transmission. In cattle, *A. phagocytophilum* mainly affects the weak animals and presents with unspecific clinical signs such as fever, abortion and reduced weight gain (Stuen *et al.,* 2013). The greatest challenge is the immunosuppression caused by this pathogen thereby predisposing animals to secondary bacterial infection (Woldehiwet, 2010).

Anaplasma platys has been known to be a dog pathogen that causes canine infectious cyclic thrombocytopenia (Pesapane *et al.,* 2019). Zobba *et al.* (2014) in their study investigating *Anaplasma* pathogens in Mediterranean region, detected pathogens closely related to *A. platys* in cattle and goats. These pathogens were however detected in the neutrophils and had high sequence identity of 93% to the platelet associated organisms. A suggestion that a cell tropism may have occurred. Subsequently a number of other authors have detected *A. platys*-like pathogens in cattle (Chien *et al.,* 2019; Dahmani *et al.,* 2019; Fernandes *et al.,* 2019; Dahmani

et al., 2017a). This could be explained by the diverse evolutionary adaptations of the pathogens in the anaplasmataceae family enabling them to infect different cells of the hematopoietic system as well as endothelial cells (Eremeeva and Dasch, 2011). The implication in the diagnosis needs to be considered noting a possible cross reaction with other neutrophil associated pathogens like *Anaplasma phagocytophilum*.

Ehrlichia ruminantium is the major *Ehrlichia* pathogen causing ehrlichiosis in cattle. It has been associated with great economic losses in cattle in Africa and Carribean Islands (Allsopp, 2010). Due to its serious socio-economic impact on the livelihood of communities, *Ehrlichia ruminantium* infection has been OIE (World Organization for Animal Health) listed as a notifiable disease (OIE, 2018a). Clinical disease due to *E. ruminantium* is commonly overt and is characterized by fever, emaciation, pale mucous membranes and lymphadenopathy (Melaku *et al.,* 2014; Njiiri, 2012). The indiscriminate nature of this pathogen allows it to infect a range of other animals including dogs and wild animals (Peter *et al.,* 2002; Allsopp and Allsopp, 2001). The zoonotic potential of this pathogen has been suspected following the death of 3 children in South Africa who presented with neurologic symptoms and on post-mortem the moruli were observed in endothelial cells of the brain and *E. ruminantium* DNA detected in their tissues (Louw *et al.,* 2005). The continued investigation into this pathogen would deepen the understanding into its pathogenicity and possible reservoir hosts.

The clinical disease associated with the emerging *E. minasensis* is characterized by fever, depression and lymphadenopathy (Aguiar *et al.,* 2019; 2014). Although the acute form of the disease leading to fatality has been reported in a calf in Brazil (Aguiar *et al.,* 2019), the common clinical disease is asymptomatic and closely related to chronic canine ehrlichiosis

(Aguiar *et al.,* 2017). An overlap in the clinical signs of *E. ruminantium* and *E. minasensis* such as fever and lymphadenopathy has been reported but postmortem findings are very different (Aguiar *et al.,* 2019). While the gross findings in *E. ruminantium* infection are associated with increased permeability of endothelial cells therefore hydrothorax, hydro pericardium, edema in lungs and brain (Allsopp *et al.,* 2015; Melaku *et al.,* 2014) those of *E. minasensis* are mainly diffuse swelling of the lymph nodes especially the mesenteric ones with no lesions in other organs (Aguiar *et al.,* 2014).

Ehrlichia chaffeensis causes disease in dogs and humans. The human disease manifests with varying symptoms; fever, headaches, muscle pains, malaise, nausea, abdominal pain, non-productive coughs with some severe cases of renal failure and neurological problems (Ismail *et al.,* 2010; Li *et al.,* 2011; Aktas *et al.,* 2010). Majority of the cases have been related with occupation, indicating higher incidence among the peasants keeping livestock (Stuen *et al.,* 2013; Li *et al.,* 2011). Recently, Jagero *et al.* (2016) and Kitaa (2014) detected *E. chaffeensis* in buffalo and dog respectively for the first time in Kenya. The epidemiological role of ruminants as reservoirs for *E. chaffeensis* is not known since there is no documented natural infection apart from the experimental infection of calves by Delos Santos *et al.* (2007). *Ehrlichia canis* which is typically a canine pathogen has also been reported to cause human disease (Perez *et al.,* 2006). The strain involved in this infection appeared different since the patients did not mount immune response typical to *E. canis* infection in dogs. Confirmation of the species of the pathogen circulating in a given geographic area would aid in early diagnosis and intervention.

2.5. Diagnosis of anaplasmosis and ehrlichiosis

The clinical diagnosis of anaplasmosis and ehrlichiosis is very challenging due to the ambiguity in the clinical signs or subclinical presentation in both animals and humans thus necessitating the use of laboratory techniques (Battilani *et al.,* 2017; Silaghi *et al.,* 2017; Jin *et al.,* 2012). The available diagnostic techniques are microscopy, serology and molecular based (Ybañez *et al.,* 2014; Gokce *et al.,* 2008). The decision to use one or the other or a combination of these techniques, depends on the degree of sensitivity required, the stage of the infection (Jin *et al.,* 2012) and the availability of the test.

Presumptive diagnosis of *Anaplasma* or *Ehrlichia* pathogens can be made on microscopic observation of moruli in the cytoplasm of white blood cells or erythrocytes. Microscopy has routinely been used since it is cheap and easily availability (Al-badrani, 2013; Noaman and Shayan, 2009; Dumler *et al.,* 2005). The results depend on one's experience and at times artefacts can be confused for moruli, hence the low sensitivity that has been associated with this technique (Teshale *et al.,* 2018, 2016; Eremeeva and Dasch, 2011; Walker *et al.,* 2004). Moreover, the method cannot differentiate the different species of the organisms (Paddock and Childs, 2003). Nevertheless, Atif (2015) emphasizes its usefulness in acute phase of the infections but its accuracy declines rapidly thereafter.

Several serological tests have been developed to detect infections with *Anaplasma* and *Ehrlichia* pathogens; these include competitive enzyme-linked immuosorbent assay (cELISA), indirect ELISA, card agglutination test and indirect fluorescent test (IFAT) (OIE, 2008). Competitive enzyme-linked immuosorbent assay has been particularly used to detect carrier animals and those that are persistently infected (Brown and Barbet, 2016). Serological

tests have been used to detect exposure to *Anaplasma* and *Ehrlichia* infection but not necessarily an active infection (Njiiri *et al.,* 2015; Muhanguzi *et al.,* 2010). Despite their high sensitivity compared to microscopy (Milner and van Beest, 2013; Da Costa *et al.,* 2005), cross-reactivity between species remains a major challenge (Atif, 2015; Dreher *et al*., 2005; Semu *et al*., 2001). This has been associated with genetic relatedness of the pathogens (Dumler *et al.,* 2001). The challenges of cross reactivity and the fact that hosts are usually sero-negative at the initial stage of the infection implies that direct methods; mainly molecular needs to be used for diagnosis (Silaghi *et al.,* 2017).

Molecular techniques are the most sensitive methods for diagnosis of *Anaplasma* and *Ehrlichia* infections and are used as confirmatory tests (M'Ghirbi *et al.,* 2016; Dahmani *et al.,* 2015; Njiiri *et al.,* 2015). They involve the use of marker genes to detect pathogen's DNA in whole blood or buffy coat (Dumler *et al.,* 2001; Sen *et al.,* 2011). They overcome challenges of crossreactivity with closely related species in additon to detection of strain variation of pathogens, thereby their differences in virulence (Al-Khedery and Barbet, 2014; Dunning Hotopp *et al.,* 2006). Moreover, these techniques allow for sequencing and phylogenetics so as to predict future patterns of invasion and diversity (Eremeeva and Dasch, 2011; Ogden and Rosenberg, 2006).

The conserved gene 16S rRNA has been extensively used as a sensitive tool for detection and phylogenetic classification of *Anaplasma* and *Ehrlichia* species (Dumler *et al.,* 2001; Lewis 2001). Other authors have used it in combination with other genes such as heat shock protein (groEL), citrate synthase (gltA), 23S rDNA and major surface protein 4 gene (msp4) (Belkahia *et al.,* 2015; Dahmani *et al.,* 2015; Ybañez *et al.,* 2014). Combined gene assays are

used to enhance sensitivity since genes with multiple copies such as membrane surface proteins (MSP) are more sensitive for detection of Anaplasmataceae while more conserved genes (16S rRNA) are useful for database cross matching and sequence comparisons (Silaghi *et al.,* 2017). Nevertheless, when detecting species in the genera *Anaplasma, Ehrlichia* and *Rickettsia* simultaneously, 16S rRNA has been used singly (Soares *et al.,* 2020). Therefore, the current study utilized 16S rRNA gene to confirm the *Anaplasma* and *Ehrlichia* species in circulation among dairy cattle in the study area as well as pathogens harboured by the ticks.

2.6. Treatment and control of anaplasmosis and ehrlichiosis

Long-acting tetracycline is the main drug used both for treatment and prophylaxis of anaplasmosis and ehrlichiosis in livestock (Dinkisa, 2018; Hove *et al.,* 2018; Melaku *et al.,* 2014; Stuen *et al.,* 2013). However, rifamycin and sulphonamides have also been shown to be effective (Dinkisa, 2018). Enofloxacin has been used to clear the carrier state which is commonly not cleared by tetracycline (Kocan *et al.,* 2010). On the other hand, doxycycline is the recommended drug for *Anaplasma* and *Ehrlichia* infections in dogs and humans (Kitaa *et al.,* 2014; Botelho-Nevers *et al.,* 2012; Chapman *et al.,* 2006).

Current disease prevention and control strategies in domestic animals are based on the reduction of tick-infestation by chemical acaricides such as organophosphates, carbamates or pyrethrines through dipping or with variety of pour-on applications (Kanduma 2018; Minjauw and Mcleod, 2003). This is not always effective when the aspects of controlling pathogens in the wildlife are not incorporated (Walker *et al.,* 2014b; Kariuki *et al.,* 2012). Moreover, the widespread acaricide resistance especially among the one host tick reduces the effectiveness of this method (Vudriko *et al.,* 2016; Jonsson, 2006). Other factors such as method of

application, spraying at the tick predilection sites and correct dilution of acaricide have been thought to influence its effectiveness (Wesonga *et al.,* 2017). To reduce environmental contamination by acaricides that can potentially pose health risk to animals and humans, use of footbath dips have been advocated (Stachurski, 2000). A multiple strategy approach involving compulsory dipping of animals, movement restriction, isolation of sick animals and restricting transportation has been encouraged to eradicate some of the important tick borne diseases (Walker, 2011).

Killed and live attenuated vaccines have been developed to control clinical signs associated with *Anaplasma* and *Ehrlichia* infections (Kocan *et al.,* 2003; Palmer *et al.,* 1999). These however, do not protect against persistent infected state which is largely responsible for mechanical and vector transmission (Kocan *et al.,* 2010b), while others do not provide adequate protection against the clinical disease (Eskeland *et al.,* 2019). Re-infection remains a challenge when animals are exposed to areas with different strains of the pathogen (Faburay *et al.,* 2007a). Despite this vaccination has been thought to be economical in comparison to the huge economic losses incurred due to tick-borne diseases (De Waal, 2000). Alternatively, infect and treat regimes in calves infected with *A. marginale* have been attempted although post-inoculation reactions have been observed (Kocan *et al.,* 2010b). Identifying the major pathogens in a given geographic area and evaluating the available control strategies will guide on choosing the appropriate action.

2.7. Molecular classification of *Anaplasma* and *Ehrlichia* pathogens

Anaplasma and *Ehrlichia* pathogens are intracellular gram negative bacteria that reside as moruli in eukaryotic cells (Ismail and McBride, 2017). They consist of a small genome (0.8-

1.5Mb) thereby relying mostly on the host cell for survival (Dunning Hotopp *et al.,* 2006). On molecular analysis of the 16S rRNA and heat shock protein (groEl) gene, the Anaplasmataceae family consists of *Anaplasma, Ehrlichia, Neorickettsia* and *Wolbachia* genera (Dumler *et al.,* 2001). This reclassification was based on their molecular characteristics rather than the previously used criterion of their morphology, ecological, epidemiological and clinical presentation (Dumler *et al.,* 2001).

The phylogenetic trees constructed using 16S ribosomal RNA (rRNA) gene yielded 4 distinct clades. These were; *Anaplasma* (including formerly *Ehrlichia phagocytophila* group, *Ehrlichia platys* and *Ehrlichia bovis*), *Ehrlichia* (including *Cowdria ruminantium*), *Wolbachia* and *Neorickettsia* (including *Ehrlichia sennetsu* and *Ehrlichia risticii*) (Ybañez *et al.,* 2014; Dumler *et al.,* 2001). *Ehrlichia phagocytophilum, E. equi* and an agent causing human granulocytic ehrlichiosis were reclassified as one pathogen *Anaplasma phagocytophilum* (Ismail *et al.,* 2010; Woldehiwet, 2010). The 16S rRNA gene can be used for confirmation of species where 97% similarity has been recommended as cutoff for similar species (Janda and Abbott, 2007). The close relatedness of these pathogens has influenced diagnosis where cross-reactivity remains a great challenge especially when using serology.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study was conducted in the peri-urban areas of Nairobi County in which Nairobi, the capital city of Kenya is located (highlighted in red in the location of Nairobi County within the map of Kenya, with the magnified map of the study area indicated by a blue arrow in Figure 3.1).

The county lies at 1.28333 latitude and 36.81667 longitude and 1795 m above sea level. The average temperature of Nairobi County is 19^{o} C while the annual rainfall is 869mm. The average It consists of 17 sub-counties, the highest number of administrative units in a single county in Kenya. The city has a population of 4.3 million people (KNBS, 2019). Livestock keeping is practised at the peri-urban areas of the city and the main livestock kept are dairy cattle, small ruminants (sheep and goats), pigs, broilers, layers, broilers and indigenous chicken (Alarcon *et al.,* 2017). The smallholder dairy production systems constitute nearly 80% of Nairobi peri-urban dairy farming and these have been established to meet the high demand for the milk within small land sizes. The only previous study that investigated TBDs in peri-urban areas of Nairobi reported an incidence of 7.7% (Gakuya and Mulei, 2005).

For purposes of data collection, the county was mapped into four quadrants, taking the central business district (CBD) as the center. The north quadrant bordered by Thika road and Waiyaki Way (A), the east quadrant bordered by Mombasa road and Thika road (B), south quadrant bordered by Lang'ata road and Mombasa road (C) and the west quadrant bordered by Lang'ata road and Waiyaki Way (D) (Figure 3.1).

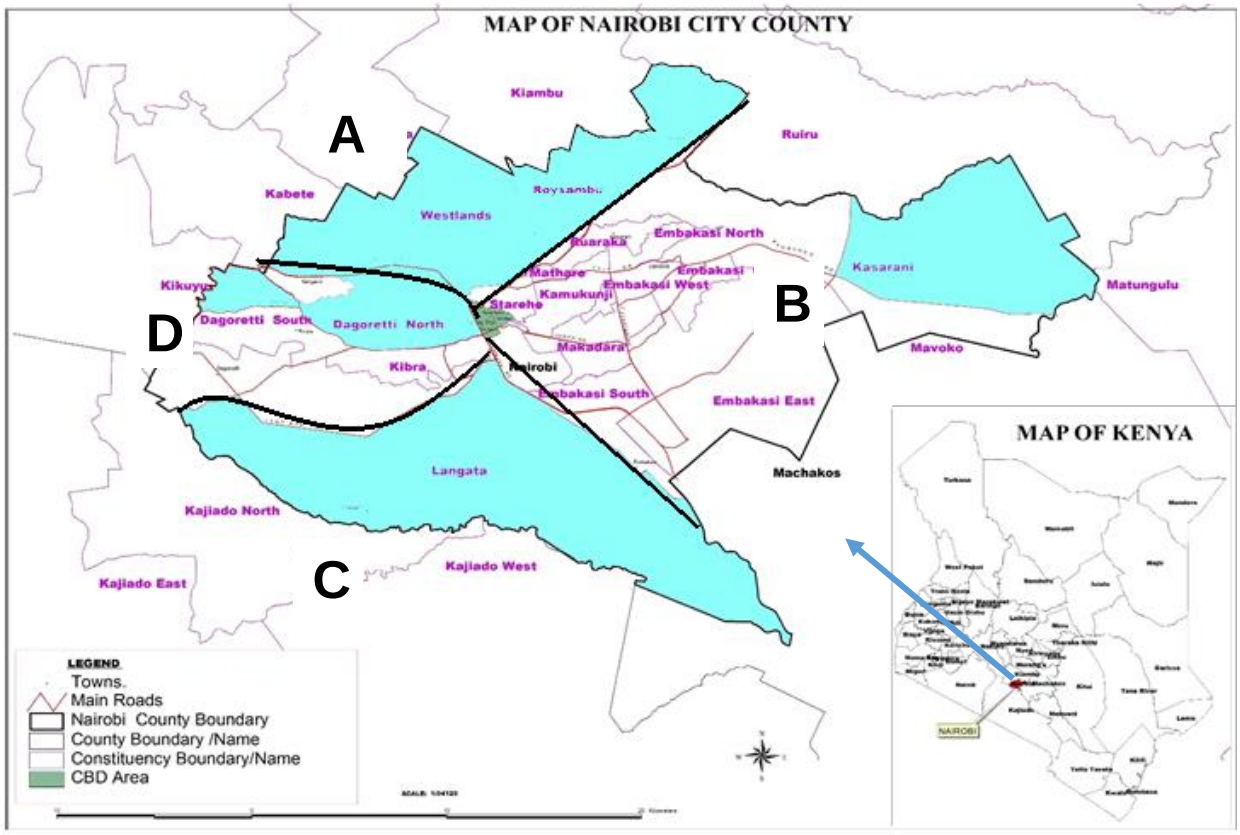

Figure 3.1: Map of Nairobi County highlighting the sub-counties that were sampled. Source: Independent Electoral and Boundaries Commission, Kenya. https://www.iebc.or.ke/uploads/resources/WHXao7x83D.pdf.

3.2 Sample size calculation

The prevalence of *Anaplasma* and *Ehrlichia* species was presumed to be 11.4% from an average of previous studies in Western Uganda by Muhanguzi *et al.* (2010) at 5.2%, Iran at 24% (Soosaraei *et al.,* 2020) and Sudan at 5.1% (Eisawi *et al.,* 2020). Using the Cochran's sample size calculation formula (Bartlett *et al.,* 2001), the sample size was calculated as follows:

Sample size = $(Z_{1-\alpha/2}^2\ p\ (1-p))/\ d^2$ where;

$Z_{1-\alpha/2}^2$ = Standard normal variate (at 5% type 1 error ($p < 0.05$) it is 1.96)

p = Expected proportion of *Anaplasma* and *Ehrlichia* infection in the population based on previous studies

d = absolute error of precision (5% was used)

$(1.96^2\ X\ 0.114\ (1-0.114))/\ 0.05^2 = 156$ cattle

A minimum of 156 cattle were to be included into the study.

A smallholder dairy unit in peri-urban Nairobi has been described to have 1-5 dairy cattle (Alarcon *et al.,* 2017; Nguhiu-Mwangi *et al.,* 2013) with nearly 71% keeping 1-3 cattle (Odero-Waitituh, 2017). Taking the median number of animals per farm to be 3 dairy cattle, a minimum of 52 farms were required. Consequently, the number of cattle recruited from the four sub-counties were as follows: Dagoretti - 105 cattle in 53 farms, Kasarani - 102 cattle in 38 farms, Lang'ata - 60 cattle in 10 farms and Westlands - 47 cattle in 8 farms. These were randomly selected as described below.

3.3 Study design

This study was approved by the Biosecurity, Animal Use and Ethics Committee (BAUEC) at the Faculty of Veterinary Medicine, University of Nairobi, Kenya (FVM BAUEC/2016/122). Blood collection in the cattle was carried out in accordance with the guidelines laid down by BAUEC requiring keen adherence to animal welfare and strict observance of biosecurity measures during handling of infectious materials.

In each of the four study quadrants of Nairobi County, purposive sampling was used to identify one sub-county with the highest cattle population and with a database of dairy farms for inclusion to the study. The sub-counties identified in each quadrant were Westlands (north quadrant), Kasarani (east quadrant), Lang'ata (south quadrant) and Dagorretti (west quadrant). Using the databases kept in each sub-county veterinary office as the sampling frames, random sampling was used to identify farms to be recruited to the study.

Every tenth farm listed was contacted on phone and the study was well described to the farm owner. The farm owner then gave verbal consent if they were willing to participate, otherwise if they declined to give consent, the next dairy unit was recruited. Cattle in the study farm were grouped into three age groups (calves< 12 months, yearlings 12 ≤ 24 months and adults > 24 months). Maximum of two cattle in each age-group were included in the study for each farm. If there were more than two cattle in the specified age-group in one farm, simple random sampling was used to select the two. In this case, each animal was allocated a number and this was inscribed on a piece of paper before folding it, then the farmer was asked to

select two of the papers. The animals indicated on the papers that the farmer picked were to be included in the study.

A pretested questionnaire was administered by the principal investigator to the animal owner or the stockman who spent most time with the cattle to collect farm level management factors (feeding system, source of fodder, cowshed cleaning, tick control, nature of housing, introduction of new animals) as well as animal-level factors (age, sex, breed, and lactation status) that were thought to be associated with occurrence of *Ehrlichia* and *Anaplasma* infections (Appendix 1).

3.4 Sample collection

3.4.1 Blood collection and serum harvesting

The study cattle were identified and physically restrained in a crush. Using the coccygeal vein, a total of 6 milliliter (ml) of blood were collected using a syringe and needle after swabbing the veni-puncture site with 70% alcohol-soaked cotton wool. Approximately 3 ml of whole blood was put into ethylenediaminetetraacetic acid (EDTA)-coated vacutainers (Becton Dickinson (BD) Vacutainer Systems Europe, UK) to be used for molecular analysis while 3 ml was collected in plain vacutainers (Becton Dickinson(BD) Vacutainer Systems Europe, UK) for ELISA assay. These were labelled by date and unique identity of animal sampled and the farm of origin.

A thin blood smear was prepared immediately after collecting the blood while still in the farm, labelled and fixed using absolute ethanol awaiting Giemsa staining later in the laboratory. The blood smears and the blood collected from plain vacutainers were transported to the Hematology and Biochemistry Laboratories in the Department of Clinical Studies, University of Nairobi while the whole blood samples were transported in a cool box with ice packs to the Molecular Laboratory at the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi and stored at -20^{o}C pending subsequent analysis. Blood collected in vacutainers without anticoagulant was allowed to stand for 2 hours and then the serum was decanted into a labeled Eppendorf tube, centrifuged for 5 min at 6000 revolutions per minute (rpm), then decanted into the final eppendorf tube and stored at −20°C awaiting ELISA test (Byaruhanga, 2017).

3.4.2 Tick collection and identification

Each of the sampled cattle, was thoroughly examined in the ears, legs, dew lap, flanks, udder and perineal areas for the presence of ticks. Ticks found attached to the cattle was picked using forceps taking precautions not to break the mouth parts. They were collected and stored in labeled containers containing 70% alcohol before transportation to the Parasitology Laboratory in the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. Morphological identification of ticks was done to species level based on taxonomic keys as described by Walker *et al.* (2014a) using a binocular microscope. The taxonomic keys used were: colour, shape and size of capitulum, eyes, presence or absence of festoons, position of anal groove spur of coxa and presence or absence of adanal shield. Each identified tick was stored in a labelled collection tube containing 70% alcohol.

3.5 Laboratory analysis

3.5.1 Blood smear preparation

A drop of blood was used to prepare one thin smear per sampled animal. The smears were then air dried, and fixed in absolute ethanol for 1 minute. The blood smears were then stained in Giemsa for 15 minutes. This was then rinsed and allowed to air dry and observed under a light microscope on oil immersion (x1000 magnification) (Aktas and Özübek, 2017). The blood smears were screened for the presence of hemoparasites in both white and red blood cells.

3.5.2 Serological screening for *Ehrlichia ruminantium*

Enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of *E. ruminantium* antigens in the harvested serum using a commercial kit (Bovine Ehrlichiosis

ELISA kit-BIOS microwell ELISA diagnostic systems, USA) following manufacturer's instructions (Bell-Sakyi *et al.,* 2003). The microtiter plate wells were coated with monoclonal capture antibodies for *E. ruminantium* derived from mouse hybridoma cells and purified by affinity chromatography. There were two positive and two negative wells to be used to validate the test and calculate cut-off point and one blank well for calibration of the ELISA reader.

Ten microliters of the serum were added to the microtiter plates and incubated for 30 minutes at 37^{o} C before washing in a wash buffer. Fifty microliters of an enzyme labelled detection antibody was then added to each well to attach to a different epitome of the *E. ruminantium* antigens. This then formed antibody-antigen-enzyme-antibody complex. This was then washed to removed non-combinative components. Fifty microliters of the substrate were added and this changed colour to blue through the action of the enzyme. The reaction was terminated by addition of fifty microliters of a stop solution containing sulphuric acid and the colour change (blue to yellow) measured using spectrophotometry by an ELISA reader (Multiskan® FC, ThermoFisher Scientific, China) at a wavelength of 450 nm. For validation of the test, the average positive control wells were ≥ 1.00, while the average negative wells were ≤ 0.10. The critical cut off values were calculated as; average of negative control wells + 0.15. Therefore, samples whose optic density (OD) was equal or higher than the critical cut off were classifies as *E. ruminantium* positive.

3.5.3 DNA extraction from blood

Genomic DNA (gDNA) was extracted from aliquots of 200µl whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instructions (Ringo *et al.,* 2019). Twenty microliters of proteinase K were pipetted into 1.5 ml eppendorf

tubes before adding 200µl of whole blood. Two hundred microliter of lysis buffer (Buffer AL) was then added to the sample and mixed by pulse-vortexing (Vortex-Genie® 2, Scientific industries, USA) for 15 seconds to lyse the cells and release the DNA. The mixture was incubated for 10 minutes at 56^{o} C after which it was briefly centrifuged (Eppendorf Centrifuge 5424R, Germany) to remove any drops from the inside of the lid.

Two hundred microliter of absolute ethanol ((200 proof), Molecular Biology Grade, Fisher BioReagents, USA) was added to the sample and pulse-vortexed for 15 seconds to precipitate the DNA, then briefly centrifuged to remove any drops from the lid. The mixture was transferred into QIAmp Mini spin column in a 2 ml collection tube. This was then centrifuged at 8000 rpm for 1 minute so that the DNA is bound by the silicon column while the filtrate passes on to the collection tube. The QIAmp Mini spin column was transferred to a clean 2ml collection tube. Five hundred microliter of wash buffer (Buffer AW1) was added to the mini spin column and centrifuged at 8000 rpm and the filtrate discarded. Five hundred microliter of second wash buffer (Buffer AW2) was added to the column and centrifuged at full speed (14,000 rpm) for 3 minutes. The wash buffers were used remove possible DNA contaminants. Centrifuging at full speed was repeated with a new 2ml collection tube to completely remove the filtrate and avoid carryover of second wash buffer. The QIAmp mini spin column was placed in a sterile 1.5 ml eppendorf tube and 100 µl elution buffer (Buffer AE) added to elute the DNA. This was incubated for 5 minute at room temperature before being centrifuged at 8000 rpm for 1 minute. The quality of the DNA was verified analyzed using QIAexpert slides in QIAxpert machine (Qiagen, Hilden, Germany) and the integrity verified by running 5µl of the eluted DNA using 1% agarose gel (Sigma, USA). The DNA was stored in -20^{o} C awaiting analysis.

3.5.4 DNA extraction from ticks

Genomic DNA was extracted from ticks using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following manufacturer's instructions (Kanduma *et al.,* 2019). DNA extraction was undertaken only on unengorged ticks to avoid PCR inhibition from excess erythrocytes as recommended by Silaghi *et al.* (2017). The ticks were removed from the alcohol, air dried and rinsed in distilled water before being dried on a filter paper. The tick was then put in 1.5 ml eppendorf tube and 180µl of Buffer ATL added then vortexed thoroughly. A pipette tip was used to draw the tick to the upper rim of the opened tube. The tick was sliced into several pieces using surgical blade (one blade per tick). The tube was then centrifuged so that all the cut pieces are collected into the bottom of the tube. Twenty microliters of proteinase K was added into the tubes and vortexed thoroughly before incubation at 56^{o} C for 1 hour. The samples were occasionally shaken during the incubation time. After the incubation the samples were vortexed thoroughly before adding 200 µl of lysis buffer (Buffer AL) and incubating at 70^{o} C for 10 minutes. Two hundred and thirty microliters of absolute ethanol were added and thoroughly mixed by vortexing. The mixture was then pipetted into DNeasy Mini spin columns. The wash and elution steps are similar to the ones described during DNA extraction in blood. Evaluation the quality and integrity of the eluted DNA was undertaken as described during DNA extraction from whole blood. The DNA was stored in -20^{o} C awaiting analysis.

3.5.5 Primer design

The Primer Blast tool of the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/tools/primer-blast) (Ye *et al.,* 2012) was used to design the primers. The 16S rRNA gene sequences for representative organisms in the *Anaplasma* and *Ehrlichia*

pathogens were used to generate the primers. *Anaplasma phagocytophilum* (accession no. MG519284.1) and *Ehrlichia ruminantium* (accession no. NR_074513.2) sequences were used as reference sequences for *Anaplasma* and *Ehrlichia* species respectively. The sequences were input on the software and the primers generated on default parameters except for the database changed to non-redundant databases (nr) and the organism changed to anaplasmataceae.

The resultant primers generated were: forward primer, ANAF 5'-TAGTGGCAGACGGGTGAGTA-3' and a reverse ANAR 5'-AATTCCGAACAACGCTTGCC-3' targeting an approximately 424 bp for *Anaplasma* species and a forward primer EHRF 5'-AGCTGGTCTGAGAGGACGAT-3' and a reverse primer EHRR 5'-GAGTGCCCAGCATTACCTGT-3' targeting an approximately 838bp of the 16S rRNA for *Ehrlichia* species. The primer sequences were submitted to Macrogen Europe Laboratory, Amsterdam, The Netherlands for synthesis.

3.5.6 Amplification of *Anaplasma* and *Ehrlichia* DNA from blood and ticks

Polymerase Chain Reaction (PCR) amplifications were performed using a thermal cycler (Applied Biosystems Veriti 96 well, ThermoFisher). The *Anaplasma* and *Ehrlichia* 16S rRNA were amplified using a final volume of 20µl reaction each containing 3 µl of genomic DNA, 10 µl Master-mix (Taq PCR 2x mastermix, Qiagen, Germany), 0.2 µl (10 µM final concentration of each primer) and 6.6 µl double distilled water to up the volume topped.

The thermocycling conditions for *Anaplasma* involved a pre-denaturation at 95^{o} C for 5 minutes followed by 40 cycles of denaturation at 95^{o} C for 45 seconds, annealing at 57^{o} C for 45 seconds and extension at 72^{o} C for 45 seconds. A final cycle of extension at 72^{o} C for 7

minutes was performed. The amplification conditions for *Ehrlichia* 16S rRNA involved an initial denaturation cycle at 95^{o} C for 5 minutes followed by 35 cycles of denaturation at 95^{o}C for 45 seconds, annealing at 62^{o} C for 45 seconds and extension at 72^{o} C for 45 seconds. The amplification cycles were followed by a final cycle of extension at 72^{o} C for 7 minutes.

To avoid contamination, the DNA extraction, reaction set up, PCR and electrophoresis were done in separate laboratory working areas. Double distilled water was used as negative control for both assays. The amplified products were electrophoresed using 1.5 % agarose gel in Tris-Borate-EDTA (TBE) buffer, pH 8, stained with Ethidium Bromide and visualized using UV-illuminator (UVP GelMax® 125 Imager, USA). The sizes of the amplicons were determined using molecular ladder (Gelpilot 1kb plus ladder (100), Qiagen, Germany).

3.5.7 Purification and sequencing of *Anaplasma* and *Ehrlichia* DNA from blood and ticks

The resulting PCR amplicons were purified and sequenced at Macrogen Europe Laboratories (Amsterdam, The Netherlands) for further analysis. After enzyme purification of the amplicons, Sanger sequencing reactions were performed in the Eppendorf Master Cycler pro 384 Thermocycler using the ABI Big Dye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) following manufacturer protocols. The single-pass sequencing was performed on each DNA template using the same forward and reverse primers as for the PCR reactions. The fluorescent-labelled fragments were purified from the unincorporated terminators with BigDye XTerminator® Purification Kits (Applied Biosystems). The samples were injected for electrophoresis in an ABI 3730xl DNA Analyzer (Applied Biosystems). The obtained sequences were viewed and manually verified using chromatogram peaks, edited and assembled using CLC Main Workbench 6.8.3 software (CLC bio, Qiagen GmbH, Germany).

3.6 Data analysis

3.6.1 Statistical analysis

Questionnaire data, ELISA and PCR results were input into Excel version 2016 (Redmond, WA, USA) before being exported to Stata 15.0 (StataCorp LLC, USA) for analysis. Descriptive statistics expressed as proportions and frequencies were computed for the farm and animal level factors as well as the seropositivity of *E. ruminantium*, PCR detected and confirmed species of *Anaplasma* and *Ehrlichia*. In the dataset, there was clustering at the individual farms introducing the random effect at the farm level.

Mixed effects logistic regression model was used to assess the association between farm-level and animal level factors (fixed effects) taking the farm as the random effect and the outcome of *Anaplasma* and *Ehrlichia* species. Univariable mixed effects logistic regression was used to test for association between the individual risk factors as explanatory variables and the *E. ruminantium* ELISA positive, *Anaplasma* and *Ehrlichia* PCR positive as separate outcomes in two independent models. Factors with p value of ≤ 10% were further analyzed using a multivariable mixed effects logistic regression model. Possible interaction and confounding factors were assessed in the multivariable model.

Backward elimination of the factors was done so that only factors with $p < 5\%$ and confounders were left in the final model and were identified as statistically associated with *E. ruminantium*, *Anaplasma* and *Ehrlichia* species. Diagnostic accuracy between microscopy and PCR was analyzed using McNemar's chi-square test. The null hypothesis for the test was that: the proportion of microscopy positive samples was similar for both microscopy and

PCR, while the alternative hypothesis was that the proportion of samples positive on microscopy was different from those that were positive on PCR.

3.6.2 Bioinformatics analyses of the sequences

Bioinformatics analysis of the parasites' 16S rRNA sequences was done by using Basic Local Alignment Search Tool nucleotide (BLASTn), multiple sequence alignment and phylogenetic analyses. Genetic identities of the *Anaplasma* and *Ehrlichia* species were confirmed by BLASTn analysis (Schäffer *et al.,* 2001) at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Multiple Sequence alignment was done using Log-Expectation (MUSCLE) v3.8.31 (Edgar, 2004). Sequence similarity was calculated using Clustal Omega to obtain identity matrix (Madeira *et al.,* 2019). A phylogenetic reconstruction was done using MEGA 6.0 (Tamura *et al.,* 2013). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Felsenstein, 1985)

CHAPTER 4: RESULTS

4.1 Description of smallholder dairy farms in peri-urban Nairobi

In this study, of the 109 smallholder dairy farms included, 60.7% (66/109) were headed by female farmers. Majority of the male farmers has secondary education and above 22% (24/109) compared to their female counterparts at 12.8% (14/109). The average duration that the dairy enterprise had been running in the study farms was 18 years. Sixty-four point two percent (70/109) of the farms had employed non-family members aged averagely 31 years to take care of the cattle and majority of them had primary education level or below 60% (42/70).

Of the 109 study farms, 92.7% (101/109) practised livestock and crop farming. Majority of these farms obtained additional income from businesses 49.5% (54/109) with 31.2 (34/109) solely depending on income from the dairy cattle. Cattle were mainly stall-fed 81.7% (89/109) with most fodder being sourced through cut and carry from either own farm 36.7% (40/109) or roadside 28.4% (31/109). Hay feeding was a common practice where 83.5% (91/109) of the dairy farms practised it. Most of the dairy farms reported fencing off their pastureland 82.6% (90/109).

Majority of cowshed floors were cemented 56% (61/109) while others had stones 26.6% (29/109) and some earthen 17.5% (19/109). Floor bedding was present in 50.1% (55/109) of the farms and 95.4% (104/109) reported roofing their cowsheds. In these dairy farms, cowshed cleaning was commonly done daily 75.2% (82/109) with few farms cleaning every fortnightly 15.6% (17/109).

4.2 Microscopic identification of the parasites in dairy cattle from smallholder farms in peri-urban Nairobi

A total of 314 blood samples were collected from apparently healthy cattle in 109 smallholder dairy farms from the four selected regions in Nairobi County. The samples were distributed in the four sub-counties as follows: Dagoretti (n=105 in 53 farms), Kasarani (n=102 in 38 farms), Lang'ata (n=60 in 10 farms), and Westlands (n=47 in 8 farms). On microscopic examination of blood smears, *Ehrlichia*-like inclusion bodies were observed in neutrophils, lymphocytes and monocytes in 79.3% (249/314) (95% confidence interval [CI] 74.4-83.6%) of the samples examined but no parasite was seen in the red blood cells. Farms in Dagoretti Sub-county had the highest proportion of animals with inclusion bodies in their white blood cells, totaling 89.5 % (94/105). Farms in Westlands and Kasarani Sub-counties reported lower [70.2% (33/47) and 70.6% (72/102), respectively] proportions of animals with inclusion bodies among the animals examined (Table 4.1).

Table 4.1: Distribution of numbers and percentages of blood samples, collected from dairy cattle in four sub-counties in Nairobi County, Kenya and those found to have *Ehrlichia*-like inclusion bodies on microscopy

Sub-county	No. of samples collected	No. of blood samples with *Ehrlichia*-like Inclusion bodies present (%)
Dagorreti	105	94 (89.5)
Lang'ata	60	50 (83.3)
Kasarani	102	72 (70.6)
Westlands	47	33 (70.2)
Total	**314**	**249(79.3%)**

Out of the 249 samples that had inclusion bodies on microscopic analysis, slightly more than half of them 58.2% (145/249) were randomly selected and scrutinized further to assess the specific white blood cells that had *Ehrlichia*-like inclusion bodies. Of all the samples that were examined, 49.7% (72/145) had *Ehrlichia*-like inclusion bodies in monocytic cells, while only 4.8% (7/145) were in granulocytic cells. The other 45.5% (66/145) of the samples had inclusion bodies in both granulocytic and monocytic cells. Among the monocytic cells, the lymphocytes were most commonly affected, representing 37.2% of the cells (54/145) followed by monocytes, representing 12.4% (18/145) of the cells. Figure 4.1 shows the *Ehrlichia*-like inclusion bodies as they appeared in different white blood cells.

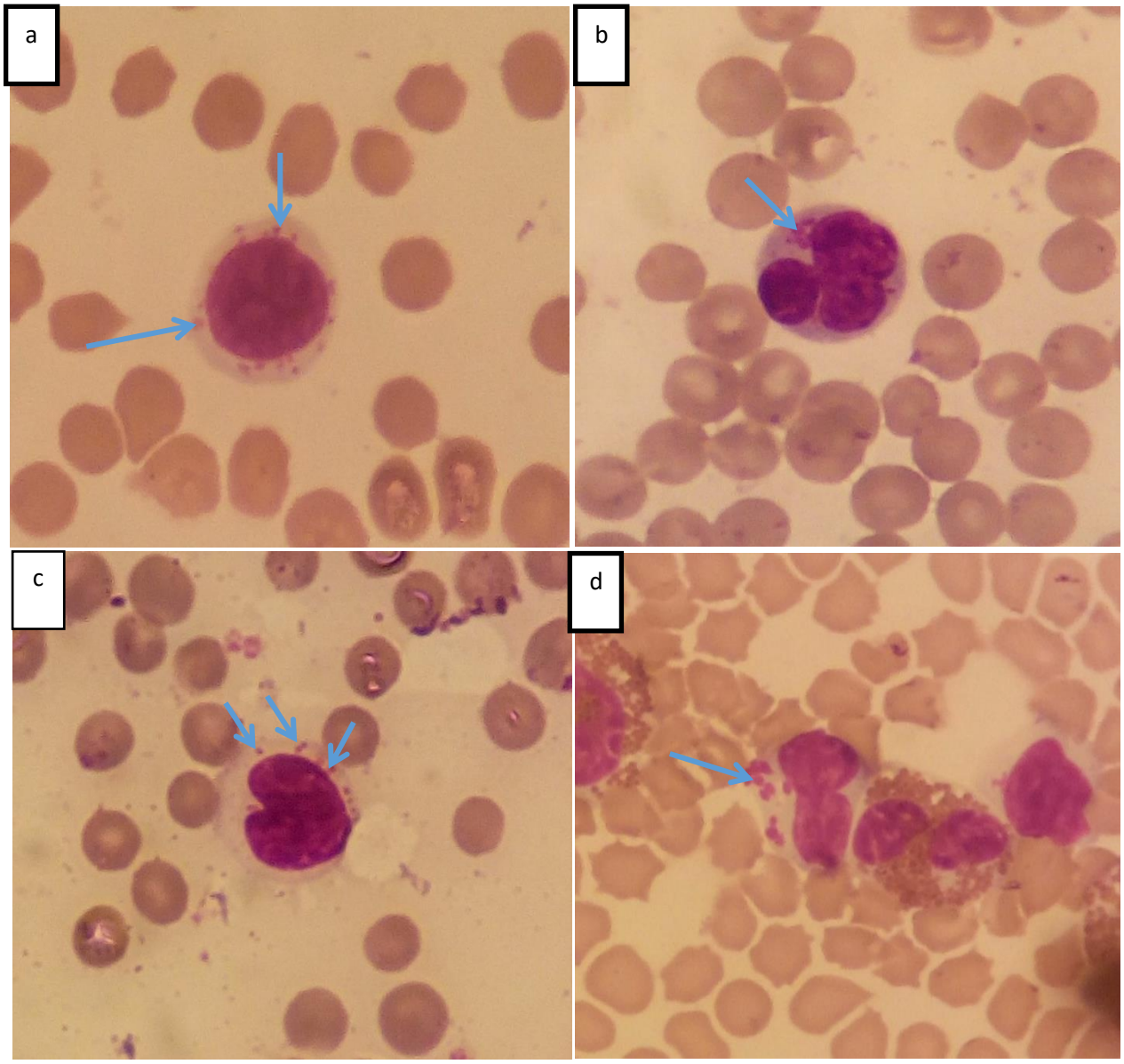

Figure 4.1: *Ehrlichia*-like inclusion bodies (blue arrows) as observed under a light microscope (x1000 magnification) oil immersion in a lymphocyte (a), neutrophil (b) and a monocyte (c and d).

4.3 *Ehrlichia ruminantium* infections in cattle from smallholder dairy farms in peri-urban Nairobi, based on serology

4.3.1 Prevalence of *Ehrlichia ruminantium* infections in the smallholder dairy farms in peri-urban Nairobi.

For serological analysis, eighteen (18) serum samples of the 314 blood samples collected were excluded because of extensive hemolysis of the red blood cells during sample collection since this could potentially affect the optic density (OD) readings.

The samples excluded were distributed across various farms and resulted in exclusion of two dairy farms; one from Dagoretti and the other from Lang'ata Sub-counties. The remaining samples for serological analysis were 296 from 107 farms. Out of the 296 samples analyzed, 18.6% (55/296) (95% CI [14.2-23.0%]) were positive for *E. ruminantium.* The samples positive for *E. ruminantium* on ELISA were from approximately one third 35.5% (38/107) (95% CI [27.0-45.3]) of the 107 farms analysed.

4.3.2 Description of factors associated with *Ehrlichia ruminantium* infections based on serology in the smallholder dairy farms in peri-urban Nairobi.

The distribution of various farm level factors associated with *E. ruminantium* infections in the study farms based on serology are described in Table 4.2. Majority of the farms where animals were found positive for *E. ruminantium* were in Kasarani Sub-county 42.1% (16/38). *Ehrlichia ruminantium* positive samples were more prevalent in farms where the farm head was a female, representing 60% (23/38), compared to male-headed farms representing 39.5% (15/38) of the farms. Farms that had an employee to take care of the cattle had higher infections 63.2% (24/38) than those that did not 36.8% (14/38).

There were more farms with *E. ruminantium* infections in which employees had only attained a primary level of education or lower, representing 44.7% (17/38) of the farms, in comparison to farms in which employees had attained a higher level of educational, representing 18.4% (7/38) of the farms. Farms where the owners had attained secondary level of education and above had higher proportion of *E. ruminantium* 71.1% (27/38) than those where farm owners had attained primary level of education or below 28.9% (11/38). There were more samples positive for *E. ruminantium* in farms where the owners had additional sources of income from businesses, representing 57.8% (22/38) compared to farms in which the owners depended solely on livestock 23.7% (9/38) or their salary 18.4% (7/38).

A higher proportion of farms which practised crop farming and livestock keeping were positive for *E. ruminantium* 84.2%(32/38) compared to those that only practiced livestock keeping 15.8%(6/38). Despite majority of the study farms having fenced off the pastureland 83.1%(89/107), *E. ruminantium* infections was still higher in those farms 84.2%(32/38)

compared to those that had not fenced 15.8%(6/38). Stall feeding was the main method of feeding the dairy cattle, and the majority of the infected farms, representing 76.3% (29/38), were in this category. *Ehrlichia ruminantium* infections were lower in farms in which fodder fed to the animals was sourced from the same farm, representing 26.3% (10/38) of farms, in comparison to farms that sourced some of the fodder from the same farm with additional fodder being purchased 39.5% (15/38) and those where fodder was purchased from outside the farm and some cut from the roadside 34.2% (13/38). Additionally, farms that supplemented the fodder with hay feeding had higher infection rates of 86.8% (33/38), compared to those that did not feed hay 13.2% (5/38).

Ehrlichia ruminantium infections were high in farms in which the cowshed was cleaned on a daily basis, representing 60.5% (23/38) of farms, and the cowshed floor was cemented, representing 50% (19/38) of the farms. Moreover, cowsheds without bedding on the floor reported higher infections 52.6%(20/38) than those that had some bedding 47.4%(18/38). A higher proportion of farms where the cowshed had roofs were *Ehrlichia ruminantium* positive 89.5%(34/38) compared to those that did not have roofs 10.5% (4/38) although very few farms had cowsheds without roofs (n=7).

Tick control was a major practice in majority of the study farms, representing 73.8% (79/107), although *E. ruminantium* infections remained high in those farms, affecting 76.3% (29/38) and especially in farms that used hand spraying 81.6% (31/38). Acaricide application was commonly applied weekly 43.9% (47/107) with few farms applying every three months 18.7%(20/107). *Ehrlichia ruminantium* infections were however, highest in those farms that

applied acaricide monthly 50%(19/38) followed by the weekly application 44.7%(17/38) and the least were those that applied every three months 5.3%(2/38).

Majority of the farms reported not to have introduced a new animal to the herd 71%(76/107), although this group had higher infections 68.4%(26/38) compared to those that reported introduction of a new animal to their herd 31.2%(12/38). Farms in which the cattle owner had never seen a tick attached to their cattle had slightly higher *E. ruminantium* infections 52.2% (12/38) that those who had ever seen ticks attached to their animals 47.8%(11/38).

Table 4.2: Description of farm-level factors in 107 smallholder dairy farms in peri-urban Nairobi, with (%) of farms positive for *E. ruminantium* infections on serology

Parameter	Description (number of farms sampled)	No. (%, total = 38) of farms positive for *Ehrlichia ruminantium*
Sub-county	Dagoretti (n=52)	12 (31.6)
	Kasarani (n=38)	16 (42.1)
	Westlands (n=8)	4 (10.5)
	Lang'ata (n=9)	6 (15.8)
Gender of farm head	Female headed farms (n= 42)	23 (60.0)
	Male headed farms (n= 65)	15 (39.5)
Employee present	Yes (n=68)	24(63.2)
	No (n= 39)	14(36.8)
Employee's highest education level	Primary level or lower(n=40)	17 (44.7)
	Secondary education and above (n=28)	7(18.4)
Farmer's highest education level	Primary and below level (n= 27)	11 (28.9)
	Secondary and above (n=80)	27 (71.1)
Farming system	Livestock only (n=7)	6 (15.8)
	Livestock and crops (n=100)	32 (84.2)
Feeding system	Stall feeding only (n= 86)	29 (76.3)
	Free grazing only (n=3)	2 (5.3)
	Stall feeding and free grazing (n= 18)	7 (18.4)
Source of fodder	Fodder from own farm (n= 38)	10 (26.3)
	Fodder from own farm and purchase (n=32)	15 (39.5)
	Fodder purchase and cut from road side grazing (n=37)	13 (34.2)
Hay feeding	Fed (n=90)	33 (86.8)
	Not fed (n= 17)	5 (13.2)
Cleaning of cowshed	Daily (n=78)	23 (60.5)
	Every other day (n=10)	3 (7.9)
	Fortnightly (n=19)	12 (31.6)
Type of cowshed floor	Earthen floor (n=19)	10 (26.3)
	Cemented floor (n=60)	19 (50.0)
	Stoned floor (n=28)	9 (23.7)
Presence of cowshed floor bedding	Bedding present (n=52)	18 (47.4)
	Bedding absence (n=55)	20 (52.6)
Presence of cowshed roof	Roof present (n=100)	34 (89.5)
	Roof absent (n=7)	4 (10.5)
Tick control on cattle	Practicing tick control (n=79)	29 (76.3)
	Not practicing tick control (n=28)	9 (23.7)
Frequency of tick control	Acaricide applied weekly (n=47)	17 (44.7)
	Acaricide applied monthly (n=40)	19 (50.0)
	Acaricide applied every 3 months (n=20)	2 (5.3)
Method of acaricide application	Hand spray (n=96)	31 (81.6)
	Pour on (n=8)	5 (13.2)
	Other methods (n=3)	2 (5.3)
Presence of new animal in the herd	Introduction of new animals (n=31)	12 (31.2)
	No new animals introduced (n=76)	26 (68.4)

Table 4.3 describes the various animal-level factors associated with *E. ruminantium* infections in the study cattle based on serology. The majority of animals sampled were adults 48.3% (143/296) and the total number of animals found infected with *E. ruminantium* was 55, representing 18.6% (55/296) of the total number of animals sampled. Of the 55 animals infected, 30 were adults, representing 54.5% (30/55) (Table 4.3). The main breed kept in the study farms was Friesian 61.8% (183/296), and being dairy farms, the majority of animals were females 90.5% (268/296). The highest number of those infected were Friesians 49.1% (27/55) followed by indigenous breeds 40%(22/55) and none of the Jersey breed was infected. Ticks were found on only 21.3% (63/296) of the animals sampled, which accounted for 40% (22/55) of those that were found infected with *E. ruminantium*.

Table 4.3: Description of various animal-level factors in 292 dairy cattle sampled in Nairobi County, with number (%) of cattle found positive for *Ehrlichia ruminantium* infections using serology

Parameter	Description (number of animals sampled)	No. (%, total = 55) of animals positive for *Ehrlichia ruminantium*
Age of the animal	Calves (n=80)	13(23.6)
	Yearlings (n=73)	12(21.8)
	Adults (n=143)	30(54.5)
Animal breed	Friesian (n= 183)	27(49.0)
	Guernsey (n=10)	2(3.6)
	Ayrshire (n=55)	4(7.3)
	Indigenous (n=39)	22(40.0)
	Jersey (n=9)	0(0)
Sex of the animal	Female (n=268)	46(83.6)
	Male (n=28)	9(16.4)
Lactational status	Lactating (n=123)	22(40)
	Pregnant (n=10)	3(5.5)
	Calves/heifers/male (n=163)	30(55.5)
Ticks present on examination	Yes (n=63)	22(40.0)
	No (n=233)	33(60.0)

4.2.3 Analysis of risk factors associated with *E. ruminantium* infections among 296 dairy cattle across 107 smallholder dairy farms in peri-urban Nairobi.

On univariate analysis of factors statistically associated with *E. ruminantium* infection, those found to be significant at $p \leq 0.1$, were sub-county of origin, the gender of farm head, farming systems, cleaning of cowshed, type of cowshed floor, presence of cowshed roof, breed of the animal and presence of ticks on examination (Table 4.4).

Multivariable logistic regression analysis revealed that the factors significantly associated with higher *E. ruminantium* sero-positivity at $p \leq 0.05$, were farms from the Lang'ata Sub-county (p=0.009), farms in which the farm head was female (p=0.005), cleaning of the cowshed every fortnight (p=0.008), farming systems that involved livestock and crops (p=0.008) and cattle that had ticks on examination (p=0.007) (Table 4.5). Farms in the Lang'ata Sub-County were 3.6 times more likely to be infected with *E. ruminantium* than farms in the Dagoretti Sub-County, while farms that cleaned their cowsheds fortnightly were 3.3 times more likely to be infected with *E. ruminantium* than those that cleaned their cowsheds on a daily basis. Farms which practiced livestock and crop farming were 3.5 times more likely to be infected than those that practiced livestock keeping only. Animals that were infested by ticks at the time of examination were 2.9 times more likely to be infected with *E. ruminantium* than those that had no ticks. Farms which were female-headed were 2.6 times more likely to have *E. ruminantium* infections than the male-headed farms (Table 4.5).

Table 4.4: Univariate mixed effects logistic regression of factors associated with *E. ruminantium* among 296 dairy cattle across 107 smallholder farms in peri-urban Nairobi

Parameter	Estimate	p-value	95% Confidence Interval	
			Lower	Upper
Sub-County	0.263	**0.099***	-0.049	0.5745
Gender of farm head	-1.356	**0.003***	-2.194	-0.514
Gender of owner	0.117	0.671	0.655	0.421
Employee present	-0.336	0.549	-1.250	0.578
Employee education	-0.740	0.168	-1.792	0.311
Employee age	-0.041	0.232	-0.109	0.026
Farmer's education level	-0.204	0.454	- 0.738	0.330
Farming system	-0.204	**0.005***	-2.948	-0.526
Other sources of income	0.067	0.795	-0.438	0.572
Duration of farming	0.016	0.995	-0.031	0.031
Feeding system	-0.019	0.950	-0.571	0.536
Source of fodder	0.266	0.901	-0.553	0.488
Feeding of hay	-0.057	0.929	-1.314	1.200
Area for fodder growing	-0.133	0.258	-0.363	0.097
Fencing of pastureland	-0.158	0.773	-1.230	0.915
Cleaning of cowshed	0.989	**<0.001***	0.514	1.464
Type of cowshed floor	-0.839	**0.006***	-1.437	-0.241
Presence of cowshed bedding	-0.371	0.389	-1.215	0.473
Presence of cowshed roof	-2.551	**<0.001***	-3.720	-1.382
Tick control on cattle	0.309	0.559	-0.727	1.344
Frequency of tick control	-0.108	0.781	-0.866	0.651
Method of acaricide application	0.643	0.279	-0.521	1.807
New animals in the herd	0.008	0.986	-0.919	0.936
Ticks present	1.177	**0.002***	0.435	1.919
Age of the animal	0.171	0.423	-0.248	0.590
Breed of the animal	0.263	**0.062***	-0.013	0.540
Sex of the animal	-0.668	0.212	-1.717	0.380
Parity	-0.064	0.491	-0.248	0.119

Key: * Factors statistically significant at p≤ 0.1

Table 4.5: Multivariable mixed effects logistic regression analysis of the factors significantly associated with *E. ruminantium* among 296 dairy cattle across 107 smallholder farms in peri-urban Nairobi.

Parameter	Description	Odds Ratio (OR)	95% Confidence Interval		p-value
			Lower	Upper	
Sub-county	Dagorreti	Ref			
	Kasarani	1.0	0.4	2.6	0.950
	Westlands	1.3	0.3	5.5	0.648
	Lang'ata	**3.6**	**1.3**	**9.6**	**0.009***
Farm head	Male headed farms	Ref			
	Female headed farms	**2.6**	**1.3**	**5.4**	**0.005***
Cowshed cleaning	Daily cleaning of cow shed	Ref			
	Every other day cleaning of cow shed	3.4	0.7	14.7	0.216
	Cleaning of cow shed fortnightly	**3.3**	**1.4**	**8.0**	**0.008***
Farming system	Livestock only	Ref			
	Livestock and crops	**3.5**	**1.4**	**8.8**	**0.008***
Ticks present on examination	No	Ref			
	Yes	**2.9**	**1.3**	**6.2**	**0.007***

Key: * Factors statistically significant at p≤ 0.05

4.4 Molecular detection and characterization of *Anaplasma* and *Ehrlichia* pathogens infecting cattle in smallholder dairy farms in peri-Urban Nairobi.

4.4.1 Molecular prevalence of *Anaplasma* and *Ehrlichia* species infecting dairy cattle in smallholder farms in peri-urban Nairobi

Polymerase Chain Reaction (PCR) was used to further characterize the pathogens observed on microscopy. A total of 306 DNA samples were successfully extracted from 314 blood samples previously collected. The eight samples had poor DNA yields that could not be used for analysis, therefore only 306 DNA samples were analyzed. Of the 306 blood DNA samples analyzed, 61 (19.9%) [95% CI 15.6 -24.9 CI] were PCR positive for *Anaplasma* species while 10 (3.3%) [95% CI 1.6 - 5.9]) were positive for *Ehrlichia*. Two percent (6/306) of the cattle were co-infected with *Anaplasma* and *Ehrlichia* species. Therefore, the total prevalence of *Anaplasma* and/or *Ehrlichia* infections was 21.2% [95% CI 16.8-26.3] (65/306).

The *Anaplasma* species yielded a specific band corresponding to 424 bp (Figure. 4.2A) while primers targeting the 16S rRNA gene of *Ehrlichia* species produced a specific band corresponding to the expected size of 838 bp (Figure. 4.2B). For the co-infected cattle, each yielded the specific band for *Anaplasma* and *Ehrlichia* separately since the assay used was simplex. The distribution of the positive samples in different sub-counties is shown in Table 4.6. The highest numbers of both *Anaplasma* 55.7% (34/61) and *Ehrlichia* 70% (7/10) infections were found in Kasarani Sub-County while Lang'ata had the least number of cattle positive for *Anaplasma* 9.8% (6/61) infection. *Ehrlichia* infections were however not detected in cattle in Dagorreti Sub-county.

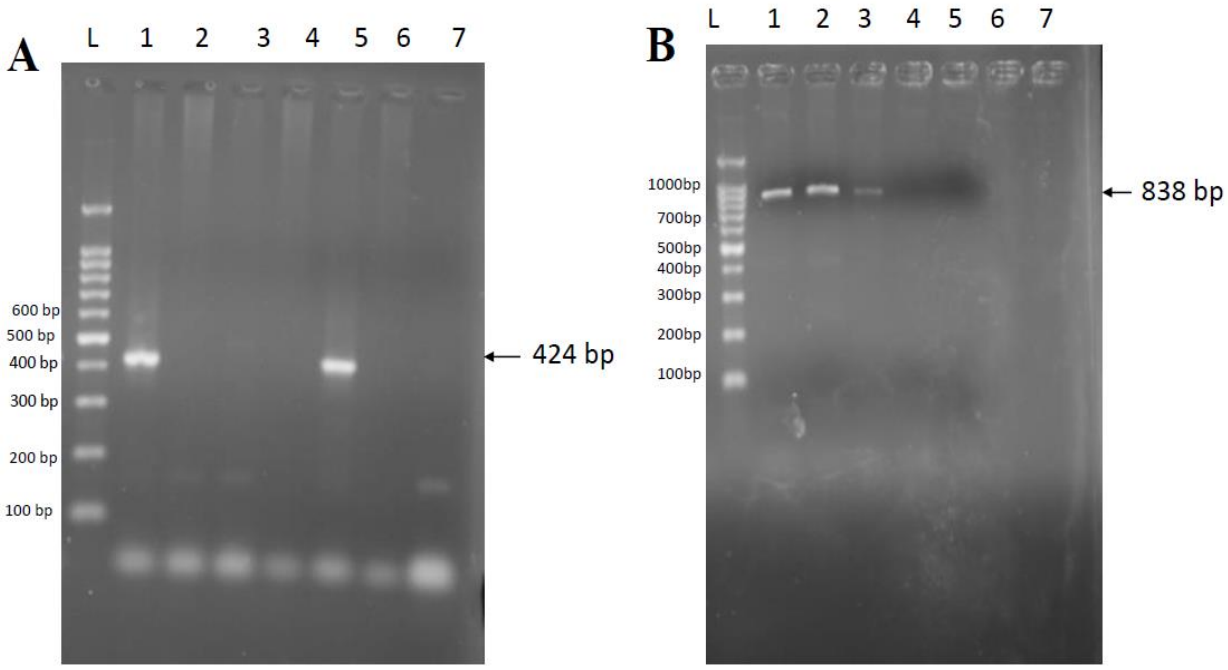

Figure 4.2: Representative PCR amplicons of *Anaplasma* and *Ehrlichia* 16S rRNA A) PCR product of *Anaplasma* species. Lane L: molecular ladder lane lanes 1 and 5: positive samples showing amplicon at approximate 424 bp, lane 2, 3, 4 and 6: no amplicons were observed, 7: negative control. B) PCR product of *Ehrlichia* species. Lane L: molecular ladder lane, lanes 1, 2 and 3: positive samples showing amplicon band at approximate 838 bp, lanes 4, 5 and 6: no amplicons were observed, 7: negative control.

Table 4.6: Distribution of 61 *Anaplasma* species positive and 10 *Ehrlichia* species positive blood DNA samples from cattle in four sub-counties of Nairobi County, based on Polymerase Chain Reaction (PCR)

Sub-County	No. (%) of *Anaplasma* spp. positive blood samples	No. (%) of *Ehrlichia* spp. positive blood samples
Kasarani	34 (55.7)	7(70.0)
Westlands	11 (18.0)	1(10.0)
Lang'ata	6(9.8)	2(20.0)
Dagorreti	10(16.4)	0(0.0)
Total	**61(100)**	**10(100)**

4.4.2 Description of factors associated with *Ehrlichia* and *Anaplasma* infections in the smallholder dairy farms in peri-urban Nairobi.

Of the 109 sampled dairy farms 37.6% (41/109) were positive for either *Anaplasma* or *Ehrlichia* species or both. Among the sub-counties sampled, Kasarani had the highest proportion of *Anaplasma* and *Ehrlichia* positive farms representing slightly over half of the farms 51.2%(21/41) with Lang'ata Sub-county reporting the least 9.8%(4/41) of the infected farms. Female headed farms had higher infections 51.2%(21/41) than the male-headed farms 48.8%(20/41). Farms that had employees accounted for a higher proportion of *Anaplasma* and *Ehrlichia* infections 63.4%(26/41) compared to those that did not have them 36.6%(15/41). Farms where the employee had attained primary level of education and below had higher *Anaplasma* and *Ehrlichia* infections 34.1%(14/41) than those with secondary level of education and above 29.3% (12/41). On the contrary, a higher proportion of farms where the owners had attained secondary level of education and above had higher infections with

Anaplasma and *Ehrlichia* species 68.3% (28/41) than those whose education level was primary and below 31.7%(13/41).

Study farms that practiced crop farming and livestock keeping had higher *Anaplasma* and *Ehrlichia* infections 87.8% (36/41) than those that practicing livestock keeping only 12.2% (5/41). Infections were highest in farms where the owners had additional income sources from business 51.2% (21/41) compared to salary 19.5% (8/41) or no additional income 29.3% (12/41). Farms where cattle were mainly stall fed had higher *Anaplasma* and *Ehrlichia* infections 73.2% (30/41) compared to those which combined stall feeding and free-grazing 22% (9/41) or free grazing alone 4.9% (2/41). The infections were higher in farms where the fodder was cut from the roadside and purchased 36.6% (15/41) than in farms where the fodder was either sourced from own farm 29.3% (12/41) or combined own farm fodder and purchasing 34.1% (14/41). Higher proportion of *Anaplasma* and *Ehrlichia* infections were observed in farms where cattle were fed on hay 78% (32/41) than those not fed and in farms where the pastureland was fenced off 75.6% (31/41) compared to those that had not fenced 24.4% (10/41).

Farms where the cowshed was cleaned daily had higher *Anaplasma* and *Ehrlichia* infections 51.2% (21/41) than those which cleaned fortnightly 24.4% (10/41) or every other day 4.9% (2/41). Higher proportion of infections were reported in farms where the cowshed floors were cemented 43.9% (18/41) compared to the earthen 29.3% (12/41) or those with stones 26.8% (11/41). Additionally, the cowsheds without any bedding reported more *Anaplasma* and *Ehrlichia* infections 61% (25/41) than those which had bedded floors 39% (16/41).

Despite tick control being a common practice among the study farms where 74.3 % (81/109) of the farms practised, higher proportion of *Anaplasma* and *Ehrlichia* 87.8% (36/41) was reported in these farms than those that did not practice 12.2% (5/41). Among those that practised tick-control, the proportion of infections was higher in those that controlled ticks monthly 46% (19/41) compared to those that did so weekly 39% (16/41) or every three months 2.4% (1/41). Hand spraying was the most common method of acaricide application 65.1% (71/109) and they accounted for higher proportion of infected farms 90.5% (32/41) than those that used pour on or used other methods such as washing with a cloth each representing 4.7% (2/41). *Anaplasma* and *Ehrlichia* infections were more common in farms where the owners had ever seen ticks attached to the cattle 63.4% (26/41) compared to those that had not seen ticks 36.6% (15/41).

Anaplasma and *Ehrlichia* infections were lower in farms where a new animal had been introduced to the herd 29.3% (32/41) compare to those that had not brought in any new animal 70.7% (29/41). Nearly half of the study farms 46.8% (51/109) owned dogs, although lower infections 46.3% (19/41) were reported in these farms compared to those that did not own a dog 53.7% (22/41).

Description of animal-level factors for *Anaplasma* and *Ehrlichia* infections is shown in Table 4.7. Female cattle 90.5% (277/306) and the Friesian breed 62.4% (191/306) were overrepresented in this study mainly because the study animals were dairy. Consequently, Friesian cattle had higher *Anaplasma* and *Ehrlichia* infections 61.5% (40/65) compared to the other breeds. There were higher infections among the adult cattle 52.3% (34/65) than in the yearling 24.6% (16/65) and calves 23.1% (15/65). *Anaplasma* and *Ehrlichia* infections were lower 36.5% (23/65) among cattle that had ticks attached on examination than those that did not have ticks 63.5% (40/65).

Table 4.7: Descriptive and univariate analysis of animal-level risk factors associated with *Anaplasma* and *Ehrlichia* infections in 306 dairy cattle from smallholder farms in peri-urban Nairobi.

Parameter	Description (Number of animals sampled)	No. (%, total = 65) of animals positive for *Anaplasma* and *Ehrlichia* species	Estimate	p-value	95% Confidence Interval	
					Upper	Lower
Breed of the animal	Friesian (n=191)	40(61.5)	0.118	0.271	-0.092	0.328
	Guernsey (n=10)	0(0)				
	Ayrshire (n=56)	7(10.8)				
	Indigenous (n=40)	17(26.2)				
	Jersey (n=9)	1(1.5)				
Sex of the animal	Female (n=277)	57(87.7)	-0.385	0.382	-1.250	0.479
	Male (29)	8(12.3)				
Lactation status	Lactating (n=128)	32(49.2)	-0.062	0.763	-0.468	0.343
	Pregnant (n=11)	1(1.5)				
	Other (n=167)	32(49.2)				
Age of the animal	Calf <1 year (n=83)	15(23.1)	0.139	0.410	-0.192	0.469
	Yearling 1-2 years (n=74)	16(24.6)	-0.068	0.536	-0.285	0.148
	Adults (n=149)	34 (52.3)				
Ticks present on examination	Yes (n=63)	23(36.5)	1.009	**0.042***	0.036	1.982
	No (n=243)	40(63.5)				

Key: * Factors statistically significant at p≤ 0.10

4.4.3 Analysis of risk factors associated with *Anaplasma* and *Ehrlichia* infections in smallholder dairy farms in peri-urban Nairobi based on PCR

On univariate analysis, factors found to be statistically significant at $p \leq 0.1$ were; employee's education level, sourcing of hay for the animals, cleaning of cowshed, type of cowshed floor, presence of cowshed bedding, presence of roof in the cowshed, practicing of tick-control, owner having seen ticks attached to the cow and ticks being present on animal on examination (Table 4.7 and 4.8).

On further multivariate logistic regression analysis, employees whose education level was secondary and above (p=0.018) and cleaning of the cowshed fortnightly (p=0.034) were found to be significantly associated with the occurrence of *Anaplasma* and *Ehrlichia* infections at $p \leq 0.05$ (Table 4.9). Type of cowshed floor was found to be confounding cowshed cleaning, therefore it remained in the final multivariate logistic regression model. Concrete or stone floors were reported to be cleaned more frequently than earthen floors. Of the cowsheds that were cleaned daily, 90.2% (74/82) of them were either concrete or stone floors while majority of the earthen floored cowsheds were cleaned fortnightly 47.4% (9/19). Farms whose cowshed floors were cleaned fortnightly were 2.3 times more likely to be infected with *Anaplasma* and *Ehrlichia* infections than those cleaned daily. Farms where the employee had attained secondary level of education and above were 2.0 times more likely to be infected than those where the employee had attained primary or lower level of education.

Table 4.8: Univariate analysis of farm-level risk factors associated with *Anaplasma* and *Ehrlichia* infections on PCR in 109 smallholder farms in peri-urban Nairobi

Parameter	Estimate	p-value	95% Confidence Interval	
			Upper	Lower
Sub-county	0.378	0.117	-0.094	0.850
Gender of farm head	-0.863	0.160	-2.066	0.340
Employee present	-0.586	0.356	-1.831	0.657
Farmer's highest education level	-0.077	0.840	-0.829	0.674
Employee's highest education level	1.537	**0.004**	0.492	2.677
Farming system	-1.317	0.205	-3.354	0.721
Other sources of income	-0.079	0.820	-0.758	0.600
Feeding system	0.558	0.159	-0.219	1.337
Source of fodder	-0.136	0.708	-0.847	0.575
Feeding of hay	-1.447	**0.083***	-3.08	0.186
Fencing of pastureland	-0.888	0.177	-2.181	0.402
Cleaning of cowshed	0.627	**0.108***	-0.138	1.393
Type of cowshed floor	-0.752	**0.102***	-1.653	0.149
Presence of cowshed bedding	-1.121	**0.053***	-2.258	0.016
Presence of cowshed roof	-2.086	**0.090***	-4.497	0.324
Tick control on cattle	1.808	**0.029***	0.187	3.429
Frequency of tick control	-0.210	0.665	-1.163	0.742
Method of acaricide application	0.1513	0.829	-1.226	1.528
Ever seen ticks on the cattle	1.198	**0.049***	0.006	2.389
Dog ownership	0.133	0.827	-1.056	1.322
New animals in the herd	0.102	0.876	-1.180	1.384
Age of Employee	0.027	0.483	-0.049	0.104
Duration of farming	-0.023	0.312	-0.067	0.021
Own-land under fodder	-0.010	0.920	-0.223	0.202
Parity	-0.064	0.491	-0.248	0.119

Key: * Factors statistically significant at p≤ 0.10

Table 4.7: Multivariable mixed effects logistic regression analysis of the factors significantly associated with *Anaplasma* and *Ehrlichia infections* among 306 dairy cattle in 109 smallholder farms in peri-urban Nairobi

Parameter	Description	Odds Ratio (OR)	95% Confidence Interval		p-value
			Lower	Upper	
Employee's education level	Primary level or informal education	Ref			
	Secondary level and above	**2.0**	**0.2**	**3.1**	**0.018***
Cleaning of cowshed floor	Daily	Ref			
	Every other day	0.2	-3.3	3.8	0.893
	Fortnightly	**2.3**	**1.1**	**4.4**	**0.034***
Type of cowshed floor	Earthen	Ref			
	Cemented	-0.7	-2.8	1.3	0.471
	Stones	1.02	-1.4	3.4	0.63

Key: * Factors statistically significant at p≤ 0.05

Analysis of the test diagnostic accuracy between microscopy and PCR on detection of *Anaplasma* and *Ehrlichia* species using McNemar's Chi-square test was done using the 306 samples that had microscopy and PCR results (Table 4.10). The test chi-square value was 148 and the p-value <0.001. This suggested a strong evidence against the null hypothesis that was stated: the proportion of samples testing positive on microscopy were equal to those testing positive on PCR. Therefore, the alternative hypothesis was true that the proportion of microscopy positive samples was different from that of PCR positive. This implied a low sensitivity of microscopy in comparison to PCR which is a confirmatory test in the detection of *Anaplasma* and *Ehrlichia* pathogens.

Table 4.8: Comparison of microscopy and PCR in the detection of *Anaplasma* and *Ehrlichia* species in dairy cattle in peri-urban Nairobi.

		PCR *Anaplasma* and *Ehrlichia* species		
		Negative	Positive	**Total**
Anaplasma and *Ehrlichia* on	Negative	49	16	65
microscopy	Positive	192	49	241
Total		241	65	306

4.4.4 Genetic identities of the *Anaplasma* and *Ehrlichia* species detected

Based on strong PCR bands as observed on gel electrophoresis, representative samples; 54.1% (33/61) for *Anaplasma* and 60% (6/10) for *Ehrlichia* were sequenced for confirmation of species of the detected pathogens. The stronger bands imply a higher concentration of the pathogen's DNA therefore better base-calling and subsequently good chromatographs that can be analyzed. Of these, twenty-nine PCR amplicons for *Anaplasma* and four for *Ehrlichia* had good chromatograms that were analyzed further. Table 4.11 and 4.12 indicate the sequence identities of the sequenced amplicons for *Anaplasma* and *Ehrlichia* species respectively following BLASTn search. BLASTn analysis revealed that majority, 44.8% (13/29) of the *Anaplasma* 16S rRNA sequences were similar to *A. platys* with sequence identity of between 98.72% and 100 % to annotated sequences in Genbank (Table 4.11).

Nine (31%) of the sequences were similar to *A. marginale* with a sequence identity of between 99.07% and 100%. Other sequences matched *A. bovis* 13.8% (4/29) with sequence identity of between 99.28% and 100% and unidentified *Anaplasma* species 10.3% (3/29) sequence identity of 97.85% to 100% (Table 4.11).

All the four *Ehrlichia* sequences were similar to those of *E. minasensis* revealing a sequence identity of between 99.42% and 100 % (Table 4.12). Of the sequenced samples, two of them were co-infected; isolate 86 with *A. bovis* and *E. minasensis* GenBank Accession numbers MT160357 and MT163430 respectively while isolate 175 had *A. platys* and *E. minasensis* GenBank Accession numbers MT163388 and MT163431 respectively.

Table 4.9: *Anaplasma* species detected by BLASTn analysis of 16S rRNA gene sequences of the peri-urban Nairobi, Kenya isolates.

Isolate	Accession no. (this study)	Matching sequence	Accession no. of highest match	E-value	% Identity
20	MT163376	*A. platys*	MN630836.1	0.0	100.00
46	MT163377	*A.* platys	MK408655.1	0.0	99.28
79	MT163378	*A. platys*	MN630836.1	0.0	100.00
85	MT163379	*A. platys*	MN630835.1	0.0	99.73
97	MT163380	*A. platys*	MN401150.1	0.0	99.76
100	MT163381	*A. platys*	MK408655.1	0.0	99.77
117	MT163382	*A. platys*	MN630836.1	0.0	100.00
173	MT163387	*A. platys*	MN630836.1	6^{e-154}	98.72
175	MT163388	*A. platys*	MN401150.1	0.0	99.51
268	MT163383	*A. platys*	MN401150.1	0.0	100.00
318	MT163384	*A. platys*	MN159065.1	0.0	100.00
381	MT163385	*A. platys*	MN630836.1	0.0	100.00
425	MT163386	*A. platys*	MN861060.1	0.0	99.76
127	MT163438	*A. marginale*	MK310488.1	0.0	99.76
139	MT163439	*A. marginale*	MK310488.1	0.0	100.00
159	MT163440	*A. marginale*	MK016525.1	0.0	100.00
168	MT163441	*A. marginale*	MK310488.1	0.0	100.00
171	MT163442	*A. marginale*	MK310488.1	0.0	100.00
172	MT163443	*A. marginale*	MK016525.1	0.0	99.07
239	MT163444	*A. marginale*	MK310488.1	0.0	99.04
243	MT163445	*A. marginale*	MK016525.1	0.0	100.00
342	MT163446	*A. marginale*	MK310488.1	0.0	99.77
39	MT160355	*A. bovis*	MT036513.1	0.0	100.00
75	MT160356	*A. bovis*	MK028574.1	0.0	100.00
86	MT160357	*A. bovis*	MT036513.1	0.0	99.28
326	MT160358	*A. bovis*	MK028573.1	0.0	100.00
103	MT163684	Unidentified *Anaplasma* spp.	KY924885.1	0.0	100.00
112	MT163683	Unidentified *Anaplasma* spp.	KY924884.1	0.0	99.18
166	MT163685	Unidentified *Anaplasma* spp.	KY924884.1	0.0	97.85

Table 4.10: *Ehrlichia* species detected by BLASTn analysis of 16S rRNA gene sequences of the peri-urban Nairobi, Kenya isolates

Isolate	Accession number (this study)	Matching sequence	Accession no. of highest match	E-value	% Identity
32E	MT163429	*E. minasensis*	MH500005.1	0.0	100.00
86E	MT163430	*E. minasensis*	MH500005.1	0.0	99.42
175E	MT163431	*E. minasensis*	MH500005.1	0.0	99.71
181E	MT163432	*E. minasensis*	MH500005.1	0.0	100.00

4.4.5 Multiple sequence alignments of the *Anaplasma* and *Ehrlichia* species detected in dairy cattle from peri-urban Nairobi

Multiple sequence alignment was done to assess the genetic similarity of the Kenyan isolates. The nucleotide sequences of three *A.bovis* isolates were conserved while one (MT160357) had three nucleotide polymorphisms at position 267, 268 and 332 (Table 4.13 and Figure 4.3). The *A. platys* sequences MT163377 and MT163388 indicated multiple single nucleotide polymorphism while the other five isolates showed single nucleotide polymorphism (SNP) (Table 4.13). *Anaplasma platys* sequences showed divergence of upto 4% (Table 4.14) with regions of nucleotide polmorphism (Figure 4.4). All the *Anaplasma marginale* sequences from this study were however highly conserved sharing 97.6% to 100 % nucleotide similarity (< 2.5% divergence) (Table 4.15) and (Figure 4.5).

For *E.minasensis*, two isolates had conserved sequences while isolates MT163430 and MT163431 appeared to be genetically different showing multiple SNPs (Table 4.13) and (Figure 4.6). The multiple sequence nucleotide polymorphisms observed in the Kenyan isolates of *A.bovis*, *A.platys* and *E.minasensis* indicate that various strains of the pathogens may exist in the dairy cattle in peri-urban Nairobi.

Table 4.11: Nucleotide polymorphisms among 16S rRNA sequences of *A. platys*, *A. bovis* and *E. minasensis* of the peri-urban Nairobi, Kenya isolates.

		[a]Nucleotide position – *Anaplasma platys*							
		1	30	55	118	257	258	407	408
Isolate	[b]Accession no.								
20	MT163376	A	A	A	T	C	G	T	T
46	MT163377- MSNP	*	G	*	*	T	T	*	*
79	MT163379-SNP	*	*	*	*	*	*	G	*
97	MT163380-SNP	*	G	*	*	*	*	*	*
100	MT163381-SNP	*	G	*	*	*	*	*	*
175	MT163388- MSNP	G	G	G	*	*	*	*	C
381	MT163385-SNP	*	*	*	C	*	*	*	*

		[a]Nucleotide position – *Ehrlichia minasensis*			
		1	130	257	652
Isolate	[b]Accession no.				
32E	MT163429	G	C	A	G
181E	MT163432	*	*	*	*
86E	MT163430- MSNP	*	T	C	T
175E	MT163431-SNP	A	*	*	*

		[a]Nucleotide position – *Anaplasma bovis*		
		267	268	332
Isolate	[b]Accession no.			
39	MT160355	C	G	G
75	MT160356	*	*	*
326	MT160358	*	*	*
86	MT160357-MSNP	T	T	A

Key: [a]Numbers denotes the nucleotide position on the sequence. Conserved nucleotide positions relative to the first sequence are indicated using asterisks while the specific nucleotide is indicated where a substitution occurred. MSNP- Multiple Single Sequence Polymorphism, SNP-Single nucleotide polymorphism. Nucleotides: T-thymine, C-cytosine, G-guanine, A-adenine. [b]Genbank Accession numbers

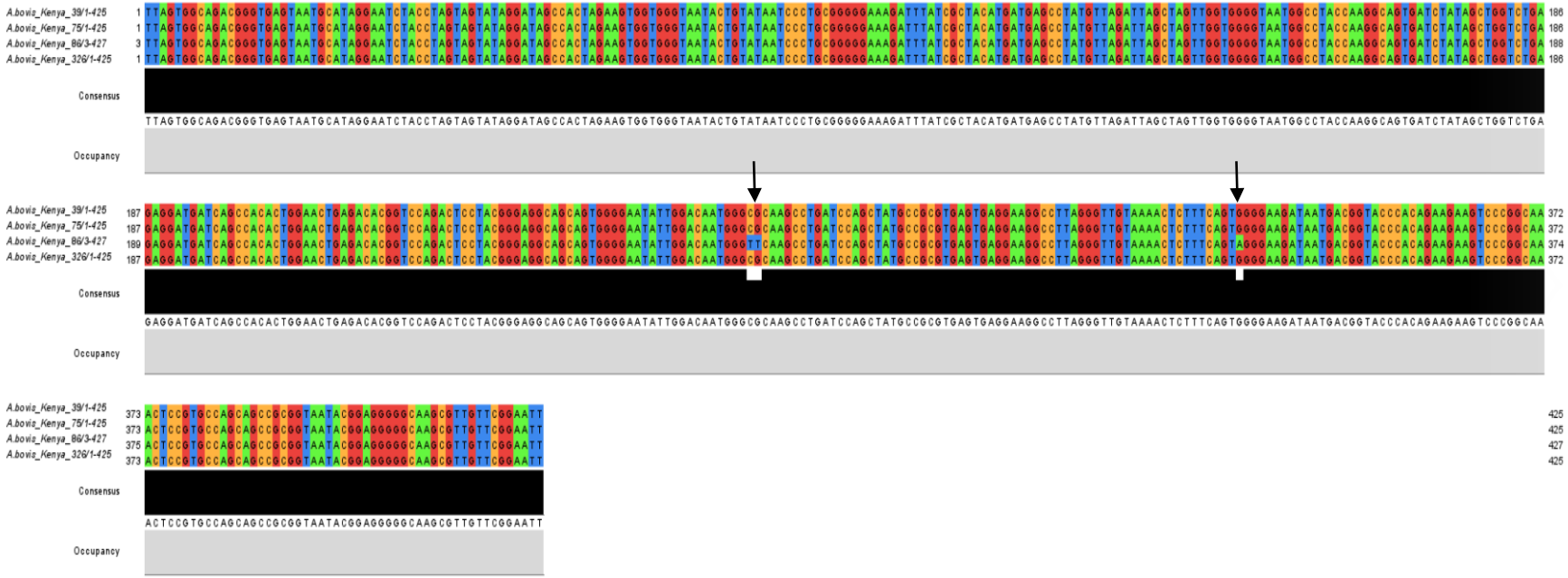

Figure 4.3: Multiple sequence alignment of *A. bovis* 16S rRNA, indicating areas of sequence polymorphism (black arrows). Numbers at the ends of each sequence indicate nucleotide lengths while the isolate names are indicated on the far left end of the nucleotide

Table 4.12: Pairwise percent identity matches of 16S rRNA sequences of *A. platys* isolated from cattle in Nairobi, Kenya.

Isolates	ApN173	ApN46	ApN268	ApN20	ApN117	ApN381	ApN425	ApN100	ApN85	ApN97	ApN175	ApN79	ApN318
ApN173	100.0	97.8	96.2	96.2	96.8	98.1	98.4	98.4	98.4	98.4	95.9	98.1	98.4
ApN46	97.8	100.0	97.6	97.6	98.1	99.3	99.5	99.5	99.5	98.6	96.7	98.1	98.3
ApN268	96.2	97.6	100.0	99.8	99.8	98.4	98.1	98.1	97.9	97.2	98.1	97.2	99.0
ApN20	96.2	97.6	99.8	100.0	99.8	98.4	98.1	98.1	97.9	97.2	98.1	97.2	99.0
ApN117	96.8	98.1	99.8	99.8	100.0	98.8	98.6	98.6	98.4	97.6	98.6	97.6	99.0
ApN381	98.1	99.3	98.4	98.4	98.8	100.0	99.8	99.8	99.7	98.8	97.4	98.8	99.0
ApN425	98.4	99.5	98.1	98.1	98.6	99.8	100.0	100.0	100.0	99.1	97.2	98.6	98.8
ApN100	98.4	99.5	98.1	98.1	98.6	99.8	100.0	100.0	100.0	99.1	97.2	98.6	98.8
ApN85	98.4	99.5	97.9	97.9	98.4	99.7	100.0	100.0	100.0	100.0	97.6	99.7	99.7
ApN97	98.4	98.6	97.2	97.2	97.6	98.8	99.1	99.1	100.0	100.0	97.6	99.5	99.8
ApN175	95.9	96.7	98.1	98.1	98.6	97.4	97.2	97.2	97.6	97.6	100.0	97.6	99.0
ApN79	98.1	98.1	97.2	97.2	97.6	98.8	98.6	98.6	99.7	99.5	97.6	100.0	99.8
ApN318	98.4	98.3	99.0	99.0	99.0	99.0	98.8	98.8	99.7	99.8	99.0	99.8	100.0

Key: Abbreviation ApN-*Anaplasma platys* Nairobi, followed by the isolate number. The numbers denote the nucleotide identity rates found between the sequences.

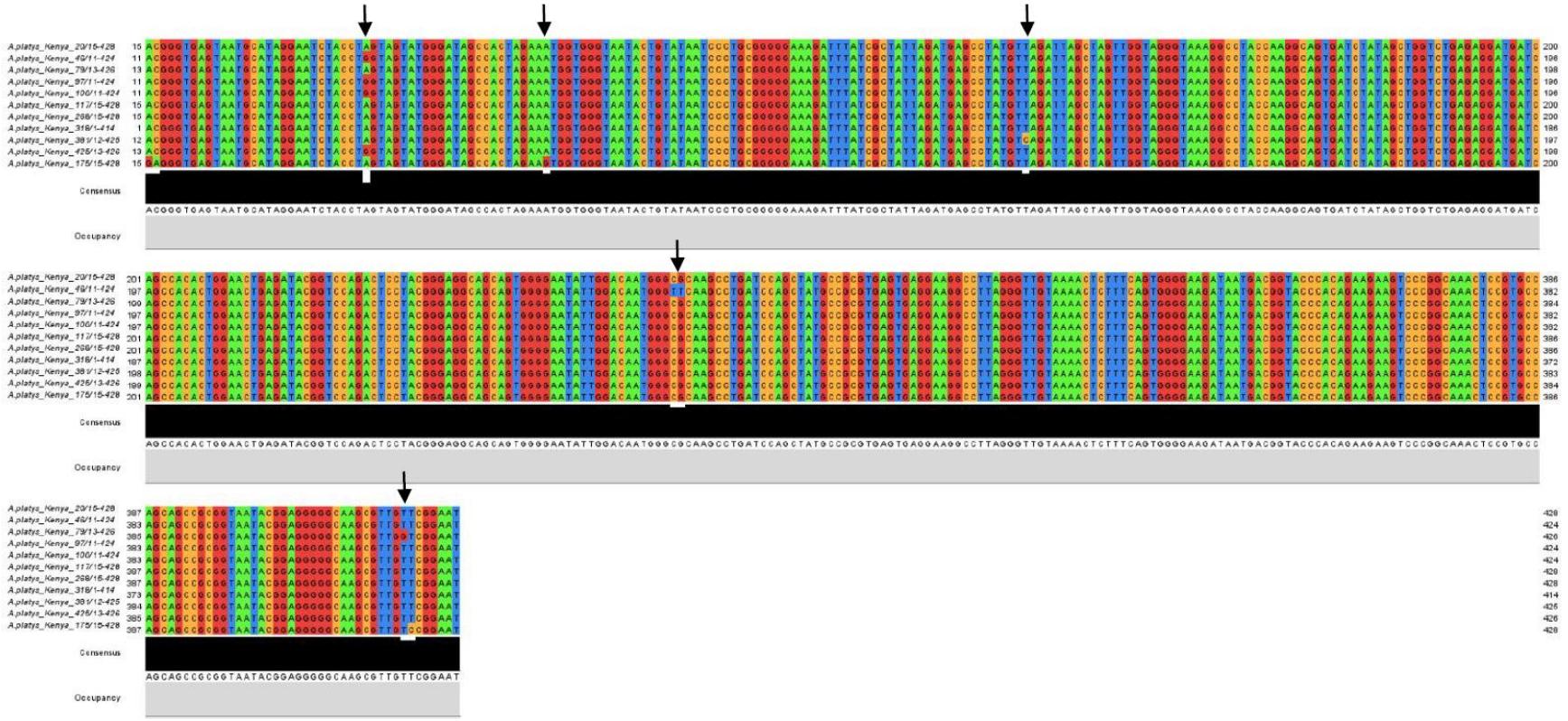

Figure 4.4: Multiple sequence alignment of *A. platys* 16S rRNA, indicating areas of sequence polymorphism (black arrows). Numbers at the ends of each sequence indicate nucleotide lengths while the isolate names are indicated on the far left end of the nucleotide sequences.

Table 4.13: Pairwise percent identity matches of 16S rRNA sequences of *A. marginale* isolated from cattle in Nairobi, Kenya.

Isolates	AMN239	AMN172	AMN168	AMN139	AMN159	AMN171	AMN243	AMN127	AMN342
AMN239	100.0	99.1	97.6	97.6	97.6	97.6	97.6	98.1	98.3
AMN172	99.1	100.0	98.4	97.6	97.6	97.6	97.6	98.8	99.1
AMN168	97.6	98.4	100.0	97.6	97.6	97.6	97.6	99.1	99.1
AMN139	97.6	97.6	97.6	100.0	100.0	100.0	100.0	98.1	98.3
AMN159	97.6	97.6	97.6	100.0	100.0	100.0	100.0	98.1	98.3
AMN171	97.6	97.6	97.6	100.0	100.0	100.0	100.0	98.1	98.3
AMN243	97.6	97.6	97.6	100.0	100.0	100.0	100.0	98.1	98.3
AMN127	98.1	98.8	99.1	98.1	98.1	98.1	98.1	100.0	100.0
AMN342	98.3	99.1	99.1	98.3	98.3	98.3	98.3	100.0	100.0

Key: The numbers denote the nucleotide identity rates found between the sequences. Abbreviation AMN-*Anaplasma marginale* Nairobi, followed by the isolate number.

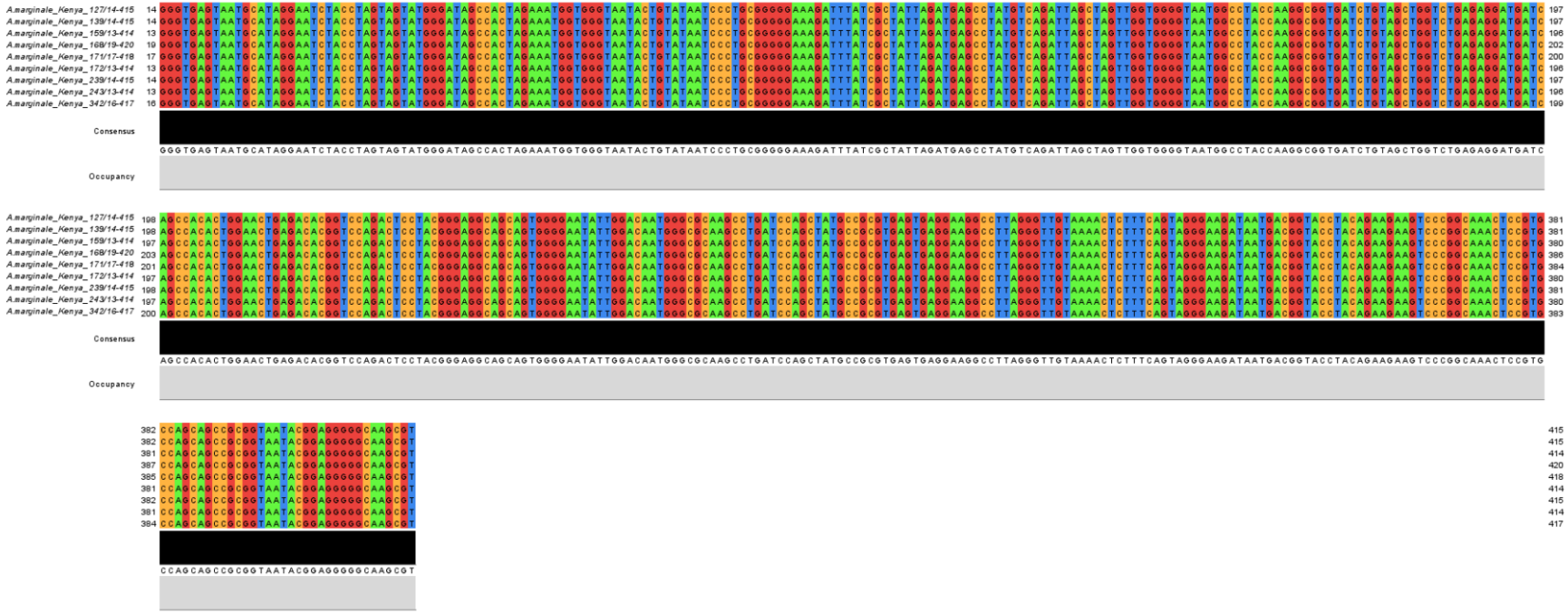

Figure 4.5: Multiple sequence alignment of *A. marginale* 16S rRNA, highly conserved sequences. Numbers at the ends of each sequence indicate nucleotide lengths while the isolate names are indicated on the far left end of the nucleotide sequences.

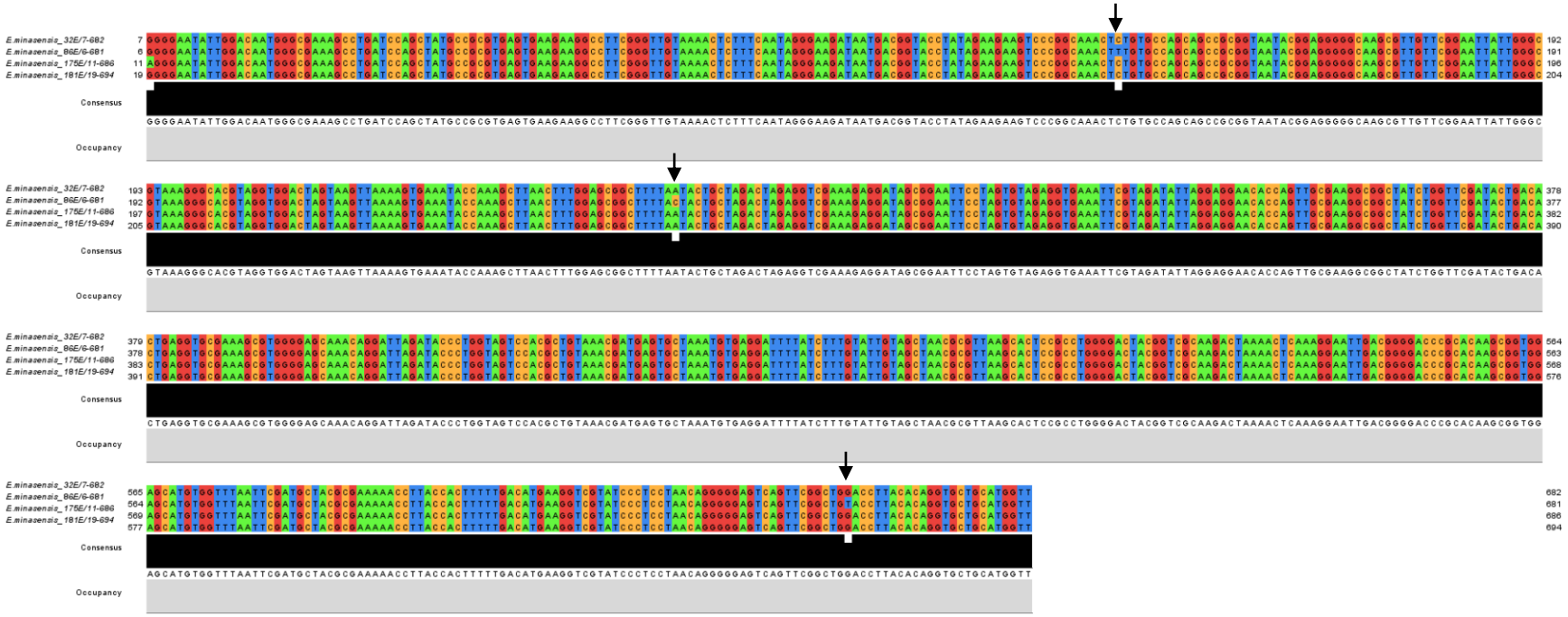

Figure 4.6: Multiple sequence alignment of *E. minasensis* 16S rRNA, indicating areas of sequence polymorphism (black arrows). Numbers at the ends of each sequence indicate nucleotide lengths while the isolate names are indicated on the far left end of the nucleotide sequences.

4.4.6 Phylogenetic positioning of the *Anaplasma* and *Ehrlichia* species detected in dairy cattle from peri-urban Nairobi

Phylogenetic analysis was done to understand genetic relatedness of the peri-urban Nairobi, Kenya isolates in the two genera with those of annotated sequences in the GenBank database (Figure 4.7 and 4.8). The Nairobi, Kenya isolates of *A. platys* clustered in the same clade as those of *A. platys* isolated from South Africa, Nigeria and Iran. They were however distinct from an isolate from India accession number MG711856.1 (Figure 4.7-Clade 1). The Nairobi, Kenya isolates of *A. marginale* were closely related to those from Uganda, USA, Australia and Iran (Figure 4.7-Clade 3). *Anaplasma bovis* isolates from Nairobi, Kenya were closely related to those from China but distantly related to those from South Korea and Japan (Figure 4.7-Clade 2). The unidentified *Anaplasma* species from this study clustered in their own clade separate from *A. platys*, *A. marginale* and *A. bovis* (Figure 4.7-Clade 4). For the *Ehrlichia* species, phylogeny was done to compare the detected *E. minasensis* genetic relatedness to other characterized species such as *E. canis*, the dog pathogen and the more common ruminant pathogen, *E. ruminantium.* The *E. minasensis* isolated in this study grouped in one clade with other isolates from USA, Australia and Brazil. These isolates were however closely related to *E. canis* than *E. ruminantium* (Figure 4.8).

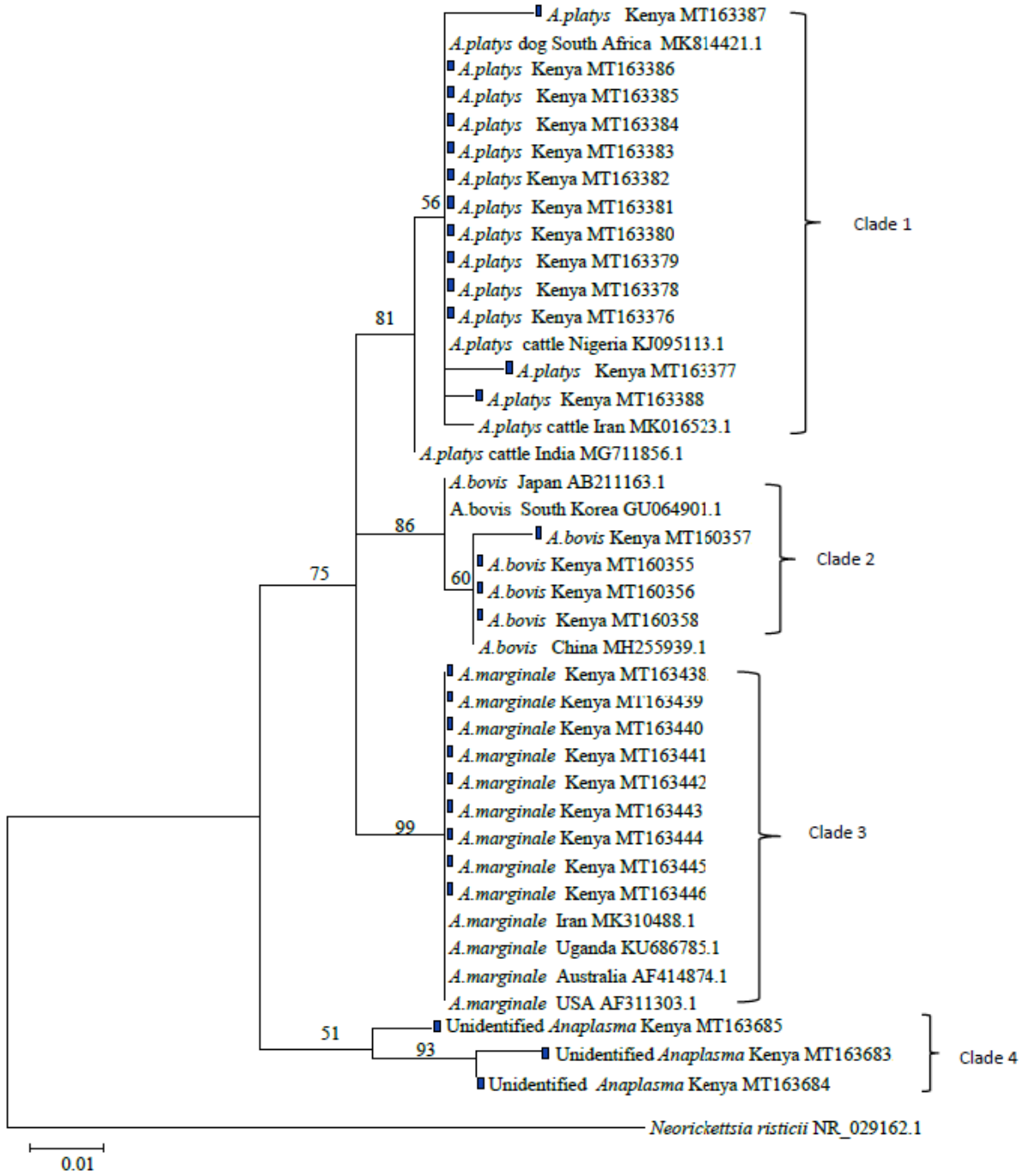

Figure 4.7: Maximum Likelihood tree of *Anaplasma spp.* constructed using partial sequences of 16S rRNA gene. The tree is drawn to scale with branch lengths measured in the number of substitutions per site. The analysis involved 29 nucleotide sequences from this study and 12 others obtained from Genbank. The tree shows the phylogenetic relatedness of *Anaplasma* isolates obtained from cattle blood in Nairobi, Kenya marked with dark box and sequences from other countries. *Neorickettsia risticii* was used as the outgroup. Sequence accession numbers are given at the end of each isolate.

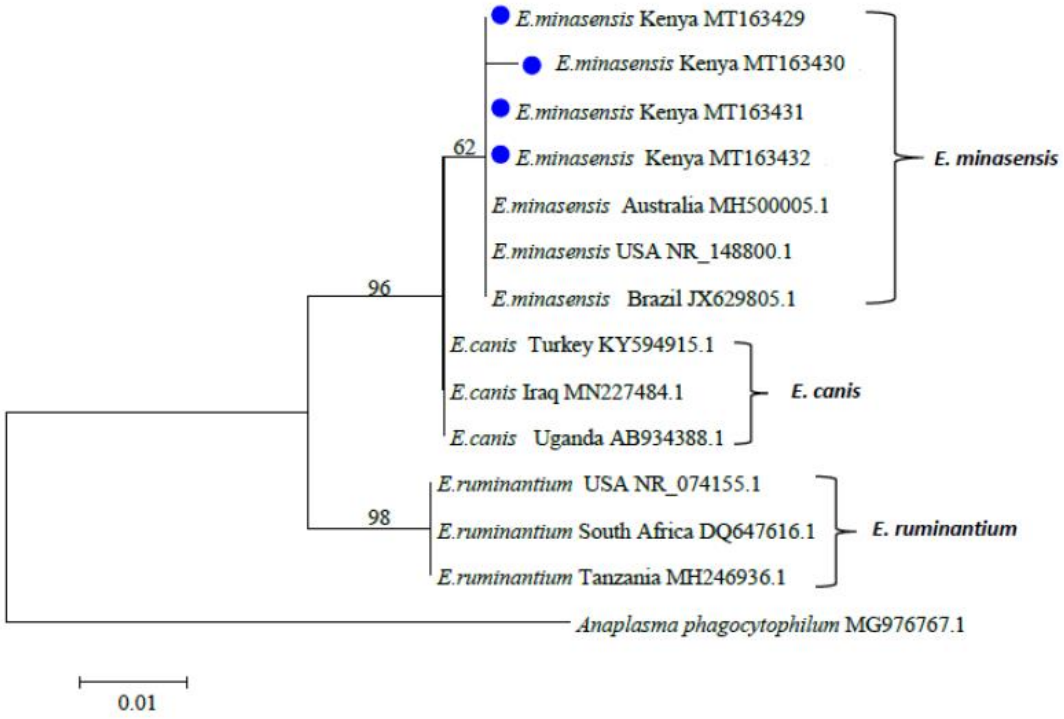

Figure 4.8: Maximum Likelihood tree of *Ehrlichia* spp. constructed using partial sequences of 16S rRNA gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 4 nucleotide sequences from this study and 10 others obtained from Genbank. The phylogeny shows the relatedness of *E. minasensis* isolated from this study marked with blue dot with other isolates from USA, Brazil and Australia and its relation to *E. canis* and *E. ruminantium*. *Anaplasma phagocytophilum* was used as the outgroup.

4.4.7 Nucleotide sequence accession numbers for the pathogens detected in dairy cattle in peri-urban Nairobi

The partial 16S rRNA gene sequences obtained in this study were deposited in the GenBank under the following accession numbers; MT163376 to MT163388 for *A. platys*, MT160355 to MT160358 for *A. bovis*, MT163438 to MT163446 for *A. marginale*, MT163683 to MT163685 for unidentified *Anaplasma* species and MT163429 to MT163432 for *E. minasensis*.

4.5 Ticks infesting dairy cattle in smallholder dairy farms in peri-urban Nairobi and the pathogens they harbor

4.5.1 Morphologically identified ticks infesting dairy cattle on smallholder dairy farms in peri-urban Nairobi

Out of the 314 animals examined, 21.0% (66/314) were found to be infested with one or more ticks. A total of 94 adult ticks were found attached to the cattle at the time of sampling. Among them, twelve tick species in the three genera *Rhipicephalus*, *Amblyomma* and *Hyalloma* were identified (Table 4.16 and Appendix 3). The majority 67% (63/94) of the ticks were *Rhipicephalus* species while *Hyalloma* species were the least abundant 13.8%(13/94). In the *Rhipicephalus* genera, *R(b). decoloratus* was the most prevalent tick species and only one tick was identified as *R. praetextatus*. *Amblomma variegatum* and *Hyalloma rufipes* were the most prevalent in their respective genera. All the tick species were found in cattle from Kasarani Sub-county.

Table 4.14: Distribution of the tick species identified from dairy cattle in peri-urban Nairobi, Kenya

Tick species	No. collected (%, n=94)	Sub-county
	***Rhipicephalus* (*R.*) species**	
R (boophilus) decoloratus	23(24.5)	Kasarani
R. evertsi evertsi	11 (11.7)	Kasarani
R. pulchellus	11(11.7)	Kasarani
R.(boophilus) microplus	6(6.4)	Kasarani
R. sanguineous	5(5.3)	Kasarani
R. simus	3(3.2)	Kasarani
R. appendiculatus	3(3.2)	Kasarani
R. praetextatus	1(1.2)	Kasarani
	***Ambylomma* (*A.*) species**	
A.variegatum	15(15.6)	Kasarani
A.gemma	3(3.2)	Kasarani
	***Hyalloma* (*H.*) species**	
H. rufipes	8(8.5)	Kasarani
H. truncatum	5(5.3)	Kasarani
Total	**94(100)**	

4.5.2 Pathogens detected from ticks infesting dairy cattle in smallholder farms in peri-urban Nairobi

The tick DNA was analyzed for the presence of *Anaplasma* and *Ehrlichia* pathogens using similar primers as used for whole blood analysis. Out of 94 ticks that were collected, 25.6% (24/94) of the samples yielded PCR bands at approximately 424 bp (Figure 4.9A). Of these, 9 representative amplicons which had strong bands were selected for sequencing. Eight of them had good chromatograms that were analyzed and on BLASTn analysis, they were all *Rickettsia* species except one *Anaplasma ovis* (Table 4.17).

Anaplasma ovis was detected in *R. evertsi evertsi* tick while all the three tick genera were found to harbour *rickettsia* species. *Rickettsia aeschlimanii* was detected in *R(boophilus) microplus* and *Hyalloma rufipes* ticks. On the other hand, *R. conorii* was detected in *Ambylomma gemma* and *A. variegatum* (Table 4.17). *Rickettsia conorii* was detected in 62.5%(5/8) of the sequenced amplicons indicating sequence similarity of between 98.30% and 99.38%. *Rickettsia aeschlimanii* was confirmed in two of the sequenced amplicons 25%(2/8) with a sequence similarity of between 99.30% and 99.76% (Table 4.17).

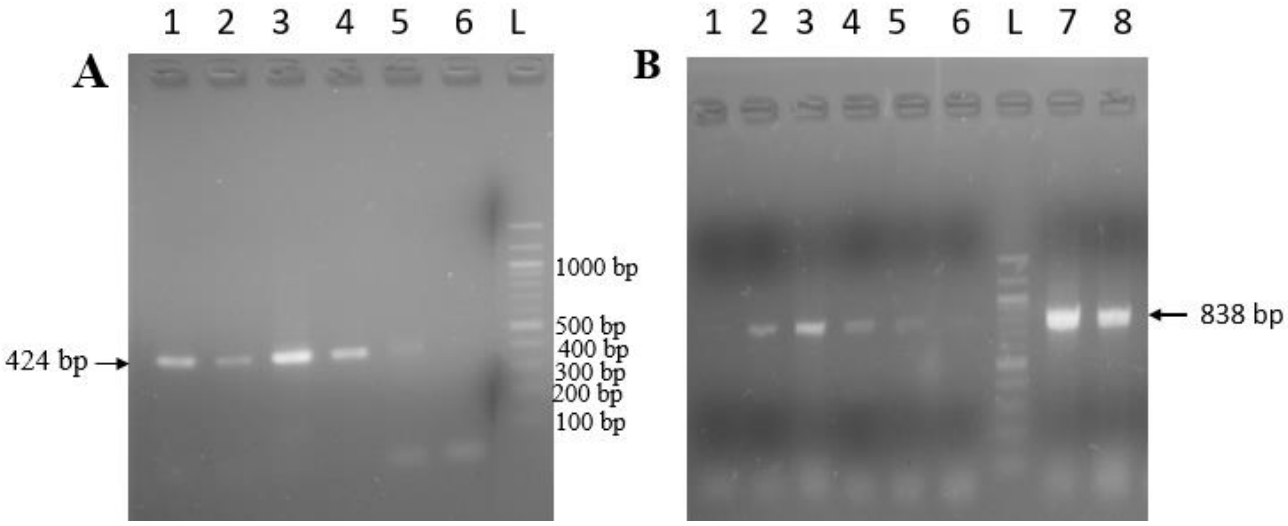

Figure 4.9: PCR amplification of 16S rRNA gene of *Rickettsia* and *Ehrlichia spp.* detected from ticks infesting cattle. A: The black arrow indicates amplicon band at approximately 424 bp for *Rickettsia* and *Anaplasma* spp. L-Molecular ladder (100 kbp plus Ladder (100)), lanes 1- 5 positive samples, lane 6-negative control B: The black arrow indicates an amplicon band at approximately 838 bp for *Ehrlichia* spp. L-Molecular ladder (100 kbp plus Ladder (100)), Lanes 2-6, 7 and 8-positive sample, lane 1: negative control.

Table 4.15: Pathogens detected from ticks collected from cattle in peri-urban Nairobi, Kenya

Isolate	Accession no. (this study)	Tick species	Accession no. of highest BLASTn Match	Pathogen detected	% identity
522A	MT366207	*R. evertsi evertsi*	MG869525.1	*Anaplasma ovis*	99.77
281C	MT366164	*R.(boophilus) microplus*	HM050274.1	*R. aeschlimannii*	99.76
290B	MT366165	*H. rufipes*	HM050274.1	*R. aeschlimannii*	99.30
286A	MT366066	*A. gemma*	MG564259.1	*R. conorii*	98.38
524A	MT366070	*A. variegatum*	MG564259.1	*R. conorii*	99.30
519A	MT366069	*A. variegatum*	MG564259.1	*R. conorii*	98.30
501A	MT366067	*A. variegatum*	MG564259.1	*R. conorii*	99.30
508B	MT366068	*A. variegatum*	MG564259.1	*R. conorii*	98.38

For *Ehrlichia* species, out of 94 ticks that were collected, 8.5%(8/94) were positive yielding PCR bands at approximately 838 bp (Figure 4.9B). The eight samples were sequenced, but five had good quality chromatograms that could be analyzed. Three of the sequences; one from *R.(boophilus) decoloratus* and two from *A. variegatum* were similar to *Ehrlichia canis* with a sequence identity (99.76 -100%). One isolate from *A. variegatum* was 100% similar to *E. ruminantium* while the other was an unidentified *Ehrlichia* species from *R. sanguineous*. Table 4.18 shows the tick species from which the *Ehrlichia* species were detected. Two *Ambylomma variegatum* ticks were observed to be co-infected with two pathogens; one with *R. conorii* and *E. canis* (isolate 508B) and the other with *R. conorii* and *E. ruminantium* (isolate 524A).

Table 4.16: *Ehrlichia species* detected from different tick species collected from cattle in peri-urban Nairobi, Kenya

Isolate	Accession no. (this study)	Tick species	Accession no. of highest BLASTn match	*Ehrlichia* spp. detected	% identity
396B	MT734401	*R.(boophilus) decoloratus*	KY594915.1	*E. canis*	100.0
508B	MT734402	*A. variegatum*	KX987326.1	*E. canis*	99.76
524A	MT734403	*A. variegatum*	KX180945.1	*E. canis*	100.0
524AR	MT738235	*A. variegatum*	NR_074155.1	*E. ruminantium*	100.0
277C	MT738242	*R. sanguineous*	KX987325.1	*Unidentified Ehrlichia spp.*	100.0

4.5.3 Multiple sequence alignments of the *Rickettsia* and *Ehrlichia* species isolated from ticks infesting dairy cattle from peri-urban Nairobi

Multiple sequence alignment was done for the *R. conorii* and *E. canis* Kenyan isolates. The *Rickettsia conorii* nucleotide sequences appeared genetically diverse with multiple nucleotide sequence polymorphisms (SNPs) (Figure 4.10) and nucleotide diversity of up to 4% (Table 4.19). On the contrast, the nucleotide sequences for *Ehrlichia canis* were highly conserved (Figure 4.11).

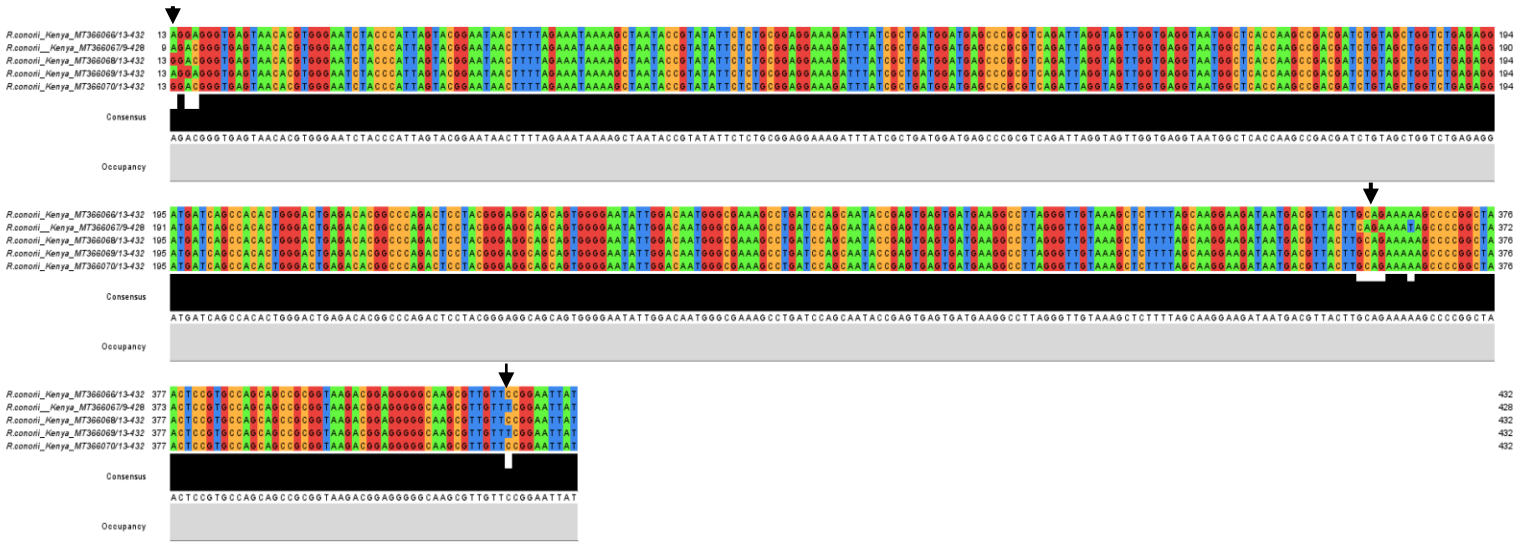

Figure 4.10: Multiple sequence alignment for *R. conorii* nucleotide sequences obtained from ticks infesting cattle in Nairobi, Kenya. The black arrows show regions of multiple nucleotide sequence polymorphism (SNPs).

Table 4.17: Pairwise percent identity matches of 16S rRNA sequences of *R. conorii* isolated from ticks infesting dairy cattle in Nairobi Kenya. The numbers denote the nucleotide identity rates found between the sequences.

Isolate	501A	286A	519A	508B	524A
501A	100.0	96.0	96.1	98.1	97.7
286A	96.0	100.0	99.8	97.0	96.8
519A	96.1	99.8	100.0	97.2	96.2
508B	98.1	97.0	97.2	100.0	99.8
524A	97.7	96.8	96.2	99.8	100.0

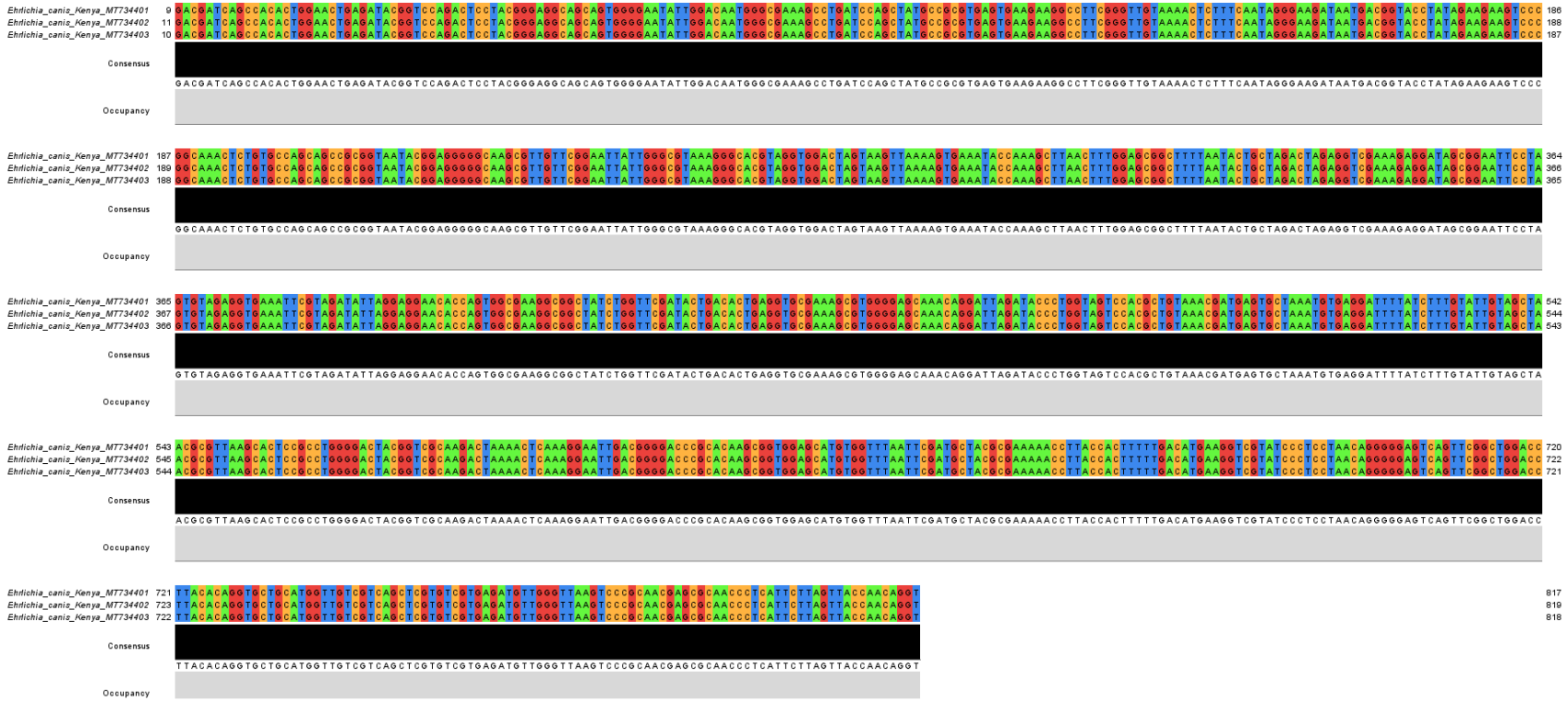

Figure 4.11: Multiple nucleotide sequence alignment for *Ehrlichia canis* detected from ticks in cattle in Nairobi, Kenya indicating highly conserved sequences.

4.5.4 Phylogenetic positioning of the *Rickettsia* and *Ehrlichia* species detected in ticks infesting dairy cattle from peri-urban Nairobi

Phylogenetic analysis was done to confirm the species of *Rickettsia* detected and identify the genetic relatedness to other isolates worldwide (Figure 4.12). The *R. conorii* Kenyan isolates were closely related to those from USA, China, Nigeria and Egypt but differed from those from Zambia and Uganda. *Rickettsia aeschlimanii* Kenyan isolates clustered together with an isolate from Senegal (KY229715.1) and Lebanon (HM050274.1).

Phylogenetic reconstruction of *Ehrlichia* species was done together with the *Ehrlichia minasensis* identified in this study and others annotated in the Genbank (Figure 4.13). *Ehrlichia canis* isolates from the Kenyan ticks were closely related to dog isolates from Iraq and Turkey and a tick isolate from Uganda. The *E. ruminantium* isolated from *A. variegatum* in this study clustered together with a tick isolate from Tanzania and other isolates from cattle in South Africa and USA. The unidentified *Ehrlichia* species was closely related to *E. canis* and *E. minasensis* but distantly from *E. ruminantium*.

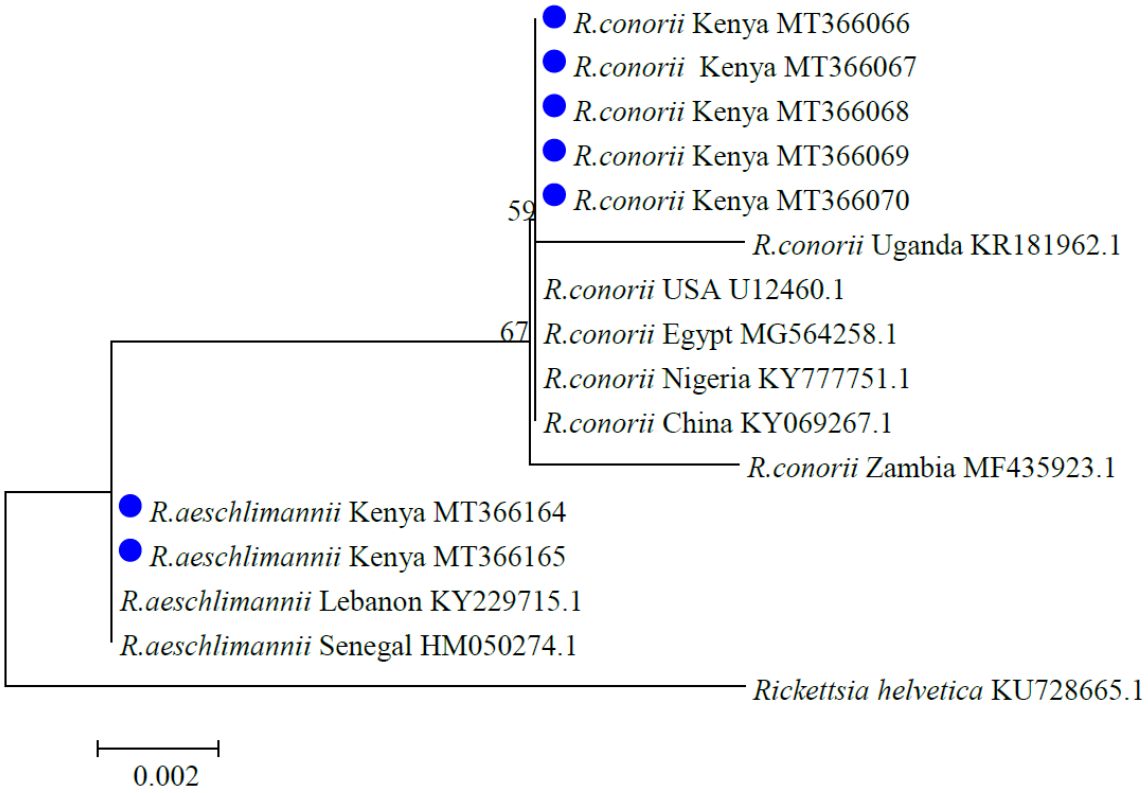

Figure 4.12: Maximum Likelihood tree of *Rickettsia* spp. reconstructed based on partial sequences of 16S rRNA gene with 1000 bootstrap replicates. The analysis involved 16 nucleotide sequences with seven from this study and nine others obtained from the Genbank. The tree indicates the phylogenetic relatedness of *Rickettsia* isolates obtained from ticks infesting cattle in Kenya marked with blue dot and sequences from other countries. *Rickettsia helvetica* was used as outgroup. Sequence accession numbers are given at the end of each isolate.

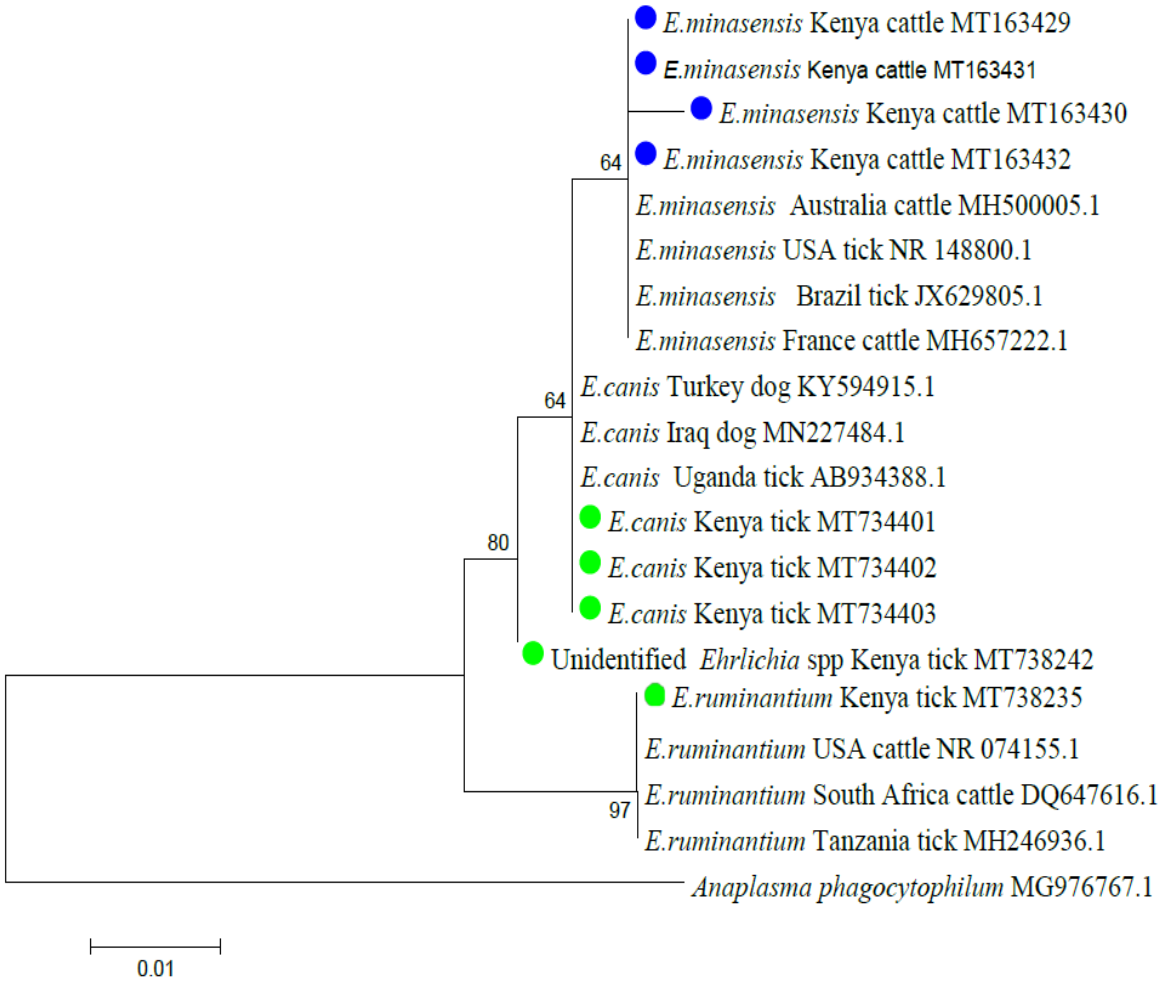

Figure 4.13: Maximum Likelihood tree of *Ehrlichia* spp. reconstructed based on partial sequences of 16S rRNA gene with 1000 bootstrap replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 20 nucleotide sequences with five tick isolates (green dots) and four cattle isolates (blue dots) from this study and the others eleven obtained from the Genbank. The tree indicates the phylogenetic relatedness of *Ehrlichia* isolates from ticks infesting cattle in Kenya marked with green dots and sequences from other countries. *Anaplasma phagocytophilum* was used as outgroup. Sequence accession numbers are given at the end of each isolate.

4.5.5 Nucleotide sequence accession numbers for the pathogens detected in the identified Kenyan ticks

The partial 16S rRNA sequences obtained from tick isolates in this study were deposited in the GenBank under the following accession numbers; MT366066 to MT366070 for *R. conorii*, MT366164 to MT366165 for *R. aeschlimanii*, MT366207 for *Anaplasma ovis*, MT734401 to MT734403 for *E. canis*, MT738235 for *E. ruminantium* and MT738242 for unidentified *Ehrlichia* species.

CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of *E. ruminantium* in dairy cattle from smallholder farms in peri-urban Nairobi, Kenya.

In this study *Ehrlichia*-like inclusion bodies were observed in the cytoplasm of various white blood cells. Microscopic examination of endothelial cells from the brain is the common method of diagnosing *E. ruminantium*, however, the inclusion bodies of the parasite can still be observed on stained blood smears in the neutrophils (The Center for Food Security and Public Health, 2013: Kasari *et al*., 2010).

In this study, inclusion bodies were observed in the neutrophils as well as other white cells such as the lymphocytes and the monocytes. The clinical-pathogenic changes as observed by Dumler and Raoult (2010) and Kasari *et al.* (2010) in *E. ruminantium* infections involving fluctuation in white blood cell counts may suggest that in addition to the neutrophils, other white blood cells such as lymphocytes and monocytes can also be affected. Some of the samples that had the inclusion bodies at microscopy were negative for *E. ruminantium* on ELISA an indication of possible presence of other pathogens possibly other *Ehrlichia* or *Anaplasma* species in the examined samples, which manifested as inclusion bodies. All species in *Ehrlichia* genera and some species in the *Anaplasma* genera are known to infect white blood cells (Eremeeva and Dasch, 2011; Rar and Golovljova, 2011; Gajadhar *et al.*, 2010).

Despite microscopy being cheap and easily available, the low sensitivity and at times misdiagnosis due to the requirement of expertise and presence of artifacts in the blood smears greatly limits its use (Silaghi *et al.*, 2017; Teshale *et al*., 2015). These limitations may explain the high proportion of animals that were observed to have *Ehrlichia*-like inclusion bodies in the white blood cells but negative on analysis using ELISA.

On Ag-ELISA, *Ehrlichia ruminantium* was detected in apparently healthy cattle, a similar observation to that of Matos *et al.* (2019) and Allsopp *et al.* (2007) but contrary to Kelly *et al.,* (2011) who notes that *E. ruminantium* always presents with clinical disease. However due to the antigenic diversity of *E. ruminantium*, low virulent strains may have been in circulation in the study animals or the cattle could have been carriers thereby posing a threat to susceptible animals (Allsopp, 2015; Allsopp *et al.*, 2007).

The overall prevalence of *E. ruminantium* in cattle reported in this study based on serology, was 18.6% which is close to 15% reported in Mozambique (Matos *et al.,* 2019), although lower than 50% by Swai *et al.* (2008) in Tanzania and 33% in Zimbabwe (Semu *et al.,* 2001) but higher than 4.5% in Uganda (Muhanguzi *et al.,* 2010) and 4.1% in Ethiopia (Teshale *et al.,* 2015). This wide variability may suggest differences in cattle management practices, that expose the cattle to infection in the various areas. The prevalence recorded in the present study is however sufficiently high to warrant implementation of appropriate control strategies since clinical disease would be a risk if susceptible animals are present (Swai *et al.,* 2008). The herd prevalence was 35.5%, nearly twice the animal prevalence possibly indicating that *E. ruminantium* infection in this area is a herd health concern more than an individual animal's problem (Swai *et al.,* 2008).

5.1.2 Risk factors associated with *E. ruminantium* on serology, *Anaplasma* and other *Ehrlichia* spp. on PCR in dairy cattle from smallholder farms in peri-urban Nairobi, Kenya.

Farms that cleaned the cowshed fortnightly had increased odds of infection with *Anaplasma*, *E. ruminantium* and other *Ehrlichia* species than those that cleaned daily. Infrequent removal of slurry from cowshed has been shown to compromise animal welfare in small holder dairy units especially because the housing designs are usually poor forcing the animals to lie on the slurry for long hours (Richards, 2017; Nguhiu-Mwangi *et al.*, 2013; Aleri *et al.*, 2012). Poor animal welfare on the other hand increases stress of the affected animals, greatly compromising their immunity and predisposing them to other diseases (Staley *et al.,* 2018; Bonizzi and Roncada, 2007). The decreased immunity in the cattle living in sheds cleaned less frequently explains the increased odds of infection of animals in this group.

Farms located in Lang'ata Sub-county were more likely to have animals infected with *E. ruminantium* compared to Dagorreti Sub-county, possibly because this area borders the Nairobi National Park and the wild animals have been known to be reservoirs for tick-borne pathogens including *E. ruminantium* and their presence has previously been reported as a risk factor by Saito and Walker (2016), Adjou Moumouni *et al.* (2015) and Peter *et al.* (2002). Moreover, Lang'ata Sub-county also borders Kajiado West Sub-county where the pastoral cattle which are commonly heavily infested with ticks, freely graze (Mugambi *et al.*, 2012).

In this study presence of ticks attached to the cattle was found to be a risk factor for *E. ruminantium* infection which is in agreement with a report by Belkahia *et al.* (2015). Transmission by ticks is the major route for *E. ruminantium* infections in susceptible cattle

(Cangi *et al.*, 2017; Allsopp, 2010). This has been documented to occur during blood meal acquisition by the ticks (de la Fuente *et al.*, 2017). On the contrary, Byaruhanga *et al.* (2016) did not find an association between attachment of ticks to cattle and *Ehrlichia* infection, which may have been because their study involved zebu cattle which have been documented to have higher resistance to tick-borne diseases (Mattioli *et al.*, 2000).

Effective tick control using acaricides has been advocated to control tick-borne diseases including *E. ruminantium* infections in cattle (Dinkisa, 2018; Wikel, 2018; Sungirai *et al.*, 2016). However, in this study, tick control, frequency of acaricide application as well as the method used for the application of acaricides were not found to be statistically significantly associated with *Anaplasma, E. ruminantium* or other *Ehrlichia* infections. This finding is similar to that of Swai *et al.* (2005) who did not find any association between method and frequency of acaricide application and tick-borne pathogens in smallholder dairy farms in Tanzania. Perry and Young (1995) and Mugambi *et al.* (2012) note that although use of acaricides is effective in tick control, acaricide mishandling such as wrong dilution and wrong application procedures which are common in the dairy farms in peri-urban Nairobi (Mugambi *et al.*, 2012), negate its effectiveness on tick control. Moreover, these practices have contributed to the challenge of acaricide resistance (Sungirai *et al.*, 2016; Jonsson, 2006) necessitating the need for alternative methods for tick control such as genetic selection (Mapholi *et al.*, 2014).

Additionally, a common practice by most of the farms in smallholder dairy production units in urban and peri-urban areas in Kenya is the cut and carry fodder from open fields or roadside to feed their cattle since they own small land sizes, hence inadequate fodder for the animals as

observed in this study and reported previously by Wesonga *et al.* (2017) and Nguhiu-Mwangi *et al.* (2013). This has been shown to propagate tick-borne diseases in zero-grazed animals (Swai *et al.*, 2005) since ticks are commonly found on long grasses and vegetation therefore are carried together with the fodder (McFee, 2018; Walker *et al.*, 2014a).

Animals from farms that practiced both livestock and crop farming were more likely to be infected with *E. ruminantium* than those that practiced livestock keeping only. Where livestock keeping is the only enterprise practised by the farmers, there may be more attention to disease control especially because it is usually the main source of income to that household (Okuthe and Buyu, 2006).

Cattle reared on farms where the employees had attained secondary education level and above were more likely to be infected with *Anaplasma* and *Ehrlichia* species than those who had primary education or lower. Employees with higher education level may perceive looking after the cattle as inferior work therefore putting less effort while for their counterparts they may perceive it as appropriate for them therefore working hard in the routine activities that are important for disease control.

Farms where the farm head was female were more likely to have animals infected with *E. ruminantium* than those farms headed by males. These findings are similar to those of Sungirai *et al.* (2016) who noted that women may be less educated on management of dairy enterprises hence increased possibility of diseases on farms where they are in charge. Moreover, Tola *et al.* (2016) also noted that women undertake majority of the labor in dairy enterprises therefore with minimum time for decision making such as disease management.

5.1.3 Prevalence and species of *Anaplasma* and *Ehrlichia* identified in the dairy cattle from smallholder farms in peri-urban Nairobi, Kenya.

The prevalence of *Anaplasma* spp. reported in this study was 19.9%. This was higher than 5.3% reported by Muhanguzi *et al.* (2010) in Uganda and 5.1% by Eisawi *et al.* (2020) in Sudan but lower than 24% by Soosaraei *et al.* (2020) in Iran. Additionally, the prevalence of *Ehrlichia* spp. reported in this study was 3.3% and this was lower than previous reports by Muhanguzi *et al.* (2010) at 5.1% and Qui *et al.* (2016) at 3.6%. Eisawi *et al.* (2020) did not report any *Ehrlichia* species in the same study that has reported *Anaplasma* species. The variability in the prevalences of these pathogens may be associated with differences in the grazing systems, climatic conditions or husbandry practices in the various systems (Ben Said *et al.,* 2018). Importantly though, is the need to put in place appropriate control measures to prevent infection with *Anaplasma* and *Ehrlichia* species since they remain economically significant in their impact on livestock production worldwide (Soosaraei *et al.,* 2020; Allsopp 2015).

Overall, a higher proportion of *Anaplasma* than *Ehrlichia* infections were observed in the study cattle population, similar to previous studies in Kenya (Njiiri *et al.,* 2015), Sudan (Eisawi *et al.,* 2020) and Ethiopia (Teshale *et al.,* 2018). The spatial occurrence of tick-borne pathogens has been associated with the presence of their tick vectors (Wikel, 2018; Doudier *et al.,* 2010). In this study, ticks in the *Rhipicephalus* genus which are the known vectors for *Anaplasma* spp. were more prevalent than the *Ambylomma* genus which transmit *Ehrlichia* species possibly explaining the differences in the prevalence in the pathogens in the two genera.

The distribution of the infections varied across sample sub-counties. A higher proportion of cattle were infected with *Anaplasma* and *Ehrlichia* spp. in Kasarani compared to the other three study areas of Dagoretti, Westlands and Lang'ata. A possible explanation of this is that dairy farmers in Kasarani practice mixed production system involving free and zero grazing (Mureithi and Mukiria, 2015) unlike the other areas where farmers practised exclusive zero-grazing. Various studies have shown that free grazing cattle have higher risk of tick-borne infections than zero grazed cattle because of high exposure to tick vectors (Adjou Moumouni *et al.,* 2015; Gachohi *et al.,* 2010; Swai *et al.,* 2005). Additionally, from this study, only cattle in Kasarani Sub-county had ticks which have been the documented vectors for *Anaplasma* and *Ehrlichia* species (Baneth, 2014; Shyma, *et al.,* 2013).

Anaplasma platys pathogens were detected in the study cattle. *Anaplasma platys* has been considered an emerging *Anaplasma* species whose clinical disease is yet to be described (Dahmani *et al.,* 2019; Zobba *et al.,* 2014). Previous studies in Algeria (Dahmani *et al.,* 2015), Senegal (Dahmani *et al.,* 2019) and Tunisia (Ben Said *et al.,* 2017) similarly reported this pathogen in cattle. Machado *et al.* (2016) also detected *A. platys* in buffaloes in Mozambique. Yang *et al.* (2018) suggested a possibility of domestic ruminants acting as alternative hosts or reservoirs for *A. platys* which is typically a canine pathogen (Ybañez *et al.,* 2016). Therefore, the detection of this pathogen in cattle raises questions of host specificity as earlier speculated (Bastos *et al.,* 2015).

Zobba *et al.* (2014) noted that several domestic ruminants can harbor a number of strains of *A. platys* although these strains have different cell tropism compared to those infecting dogs. The ruminant strains infect neutrophils and are thought to be the ancestral pathogens that evolved

to adopt to the canine platelets instead (Ybañez and Inokuma, 2016; Zobba *et al.,* 2014). The investigator speculates that infection with *A. platys* may be associated with co-existence of dogs and cattle in the same farms, a common practice observed in this study. *Rhipicephalus sanguineus* which is typically a dog tick and the documented vector of *A. platys* (Lorusso *et al.,* 2016; Inokuma *et al.,* 2000) was reported infesting the study cattle possibly playing a role in the transmission of this pathogen. Screening of dogs for this pathogen can reveal if they are acting as maintenance hosts of the parasite.

Previous studies have documented the zoonotic potential of *A. platys* causing human disease characterized by headaches, intermittent edema and muscle pains (Arraga-Alvarado *et al.,* 2014; Breitschwerdt *et al.,* 2014; Maggi *et al.,* 2013). In this regard, detection of *A. platys* pathogens in this study would indicate a possible zoonotic health risk to cattle owners who are in constant contact with their cattle during routine farm activities such as milking, feeding and cleaning of the cowsheds (Chien *et al.,* 2019).

To date, bovine anaplasmosis in Kenya is mainly known to be caused by *A. marginale* and to some extent *A. bovis* (Adjou Moumouni *et al.,* 2015; Njiiri *et al.,* 2015; Gakuya and Mulei 2005). However, the current study detected *A. platys* pathogens for the first time in cattle in Kenya possibly contributing equally to the disease burden in dairy cattle. Further studies to investigate the extent of infections with this pathogen using more specific genes such as membrane surface proteins (Msps) (de la Fuente *et al.,* 2005) are needed.

In this study, cattle were also found to be infected with *A. marginale*, a common pathogen of cattle that has been reported in Eastern Africa (Ringo *et al.,* 2018; Byaruhanga *et al.,* 2016; Adjou Moumouni *et al.,* 2015), Southern Africa (Fernandes *et al.,* 2019; Hove *et al.,* 2018; Tembo *et al.,* 2018), North Africa (Dahmani *et al.,* 2019; El-Ashker *et al.,* 2015) and West Africa (Lorusso *et al.,* 2016; M'Ghirbi *et al.,* 2016). It is not surprising to detect this pathogen in cattle in Kenya since *Rhipicephalus (boophilus)* tick species which are the documented vectors of *A. marginale* are widespread in Kenya (Omondi *et al.,* 2017; Mutai *et al.,* 2013; Kariuki *et al.,* 2012; Mugambi *et al.,* 2012; Maingi and Njoroge, 2010) and were even observed in the study cattle. *Anaplasma marginale* causes a mild to severe anemia depending on the susceptibility of the cattle (Aktas and Özübek, 2017). However, cattle in this study were apparently healthy suggesting a possible endemic situation or the animals might have been persistently infected (PI) which is a common occurrence in *A. marginale* infections (Kocan *et al.,* 2010a). Thus, infected animals can appear apparently healthy despite harboring the pathogen.

Anaplasma bovis which is a monocytic pathogen of ruminants was also detected in this study. Different tick species in the genera *Amblyomma* and *Rhipicephalus* have been documented to transmit this pathogen (Omondi *et al.,* 2017) and these were also recovered from cattle in the study area. Similar studies have detected this pathogen in Kenya (Njiiri *et al.,* 2015), China (Yang *et al.,* 2015), South Korea (Park *et al.,* 2018) Tunisia (Belkahia *et al.,* 2015) and Algeria (Rjeibi *et al.,* 2018). Despite it causing a mild disease in cattle, some infected animals have been shown to manifest with decreased weight gain, fever and lymphadenopathy (Noaman and Shayan, 2010). Other studies have documented sub-clinical infection with this

parasite where animals do not show clinical signs of the disease despite the infection (Aktas, *et al.,* 2011) and this may have been the case in this study.

An emerging pathogen *E. minasensis* in the *Ehrlichia* genera was also detected in this study. This novel pathogen was initially reported in cattle from Canada (Gajadhar *et al.,* 2010) and Brazil (Aguiar *et al.,* 2014) but has since been isolated in Ethiopia (Hailemariam *et al.,* 2017), South Africa (Iweriebor *et al.,* 2017), Pakistan (Rehman *et al.,* 2019) and China (Li *et al.,* 2019). The clinical disease due to *E. minasensis* is variable with some reports of severe disease (Aguiar *et al.,* 2019) and at times sub-clinical disease being documented (André *et al.,* 2020; Hailemariam *et al.,* 2017) as observed this study.

Coinfection was also observed in the study cattle. Six cattle were found to be co-infected with more than one tick-borne pathogen. Co-infection of cattle with more than one pathogen are common due to the multiple pathogens vectored by the same tick species (Adjou Moumouni *et al.,* 2015; Njiiri *et al.,* 2015). The coinfections observed in the two sequensed samples were with *E. minasensis* and either *A. bovis* or *A. platys*. Antagonistic interaction between *E. minasensis* and *A. marginale* or *A. platys* in Brazilian cattle have recently been reported where anaemia was not observed in co-infected cattle (André *et al.,* 2020). This implies that *E. minasensis* co-infected cattle may present with mild or sub-clinical disease and this may partially explain the sub-clinical presentation of the study animals. It would be interesting to investigate the role of this newly detected *E. minasensis* in coinfections in the study cattle. Additionally co-infections could potentially complicate microscopic diagnosis especially where the pathogens infect similar blood cells (Zhou *et al.,* 2017).

Although the specific ticks that transmit *E. minasensis* have not been well studied (Cabezas-Cruz *et al.,* 2019), its detection and transstadial transmission by *Rhipicephalus (boophilus) microplus* ticks has been documented (Cabezas-Cruz *et al.,* 2016). Recently, Kanduma *et al.* (2020) detected the presence of this tick in Kwale County of Kenya after nearly a decade since it was last reported by Zulu *et al.* (1998). Additionally, this tick was morphologically identified infesting cattle in this study. Other tick species may still transmit the pathogen in areas where *R. microplus* is absent (Hailemariam *et al.,* 2017). Indeed, Iweriebor *et al.* (2017) detected this pathogen from *R. appendiculatus, R. evertsi evertsi, R. sanguineus* and *Amblyomma hebraeum* ticks. Some of these tick species were observed to be infesting cattle in this study. It is possible that these other ticks as well as the potential vector *R.(boophilus) microplus* may be involved in the transmission of this pathogen.

Phylogenetic analysis based on 16S rRNA has been used elsewhere to infer genetic diversity of *Anaplasma* (Iqbal *et al.,* 2019; Belkahia *et al.,* 2015) and *Ehrlichia* species (Siarkou *et al.,* 2007; Dumler *et al.,* 2001). In this study, *A. marginale* isolates were found to be highly conserved indicating sequence divergence of less than 2.5% and clustered together with those from USA, Uganda, Iran and Australia. These finding is different from that of Rjeibi *et al.* (2018) who detected highly diverse *A. marginale* isolates in Algerian cattle. *Anaplasma bovis*, *A. platys* pathogens and *E. minasensis* strains detected in this study indicated certain levels of nucleotide polymorphism suggesting various strains of the pathogens may exist in the study cattle. This may be related to the increased cattle movement from other regions of the country for slaughter at the country's major export abbatoirs located in Nairobi County. Extensive animal movement has been associated with development of new strains and introduction of the tick-borne pathogens to new geographic areas (Belkahia *et al.,* 2015; Ogden *et al.,* 2013).

In agreement with previous studies, phylogenetic analysis also indicated that *E. minasensis* is closely related to *E. canis* but distantly related to *E. ruminantium* despite infecting similar hosts (Aguiar *et al.,* 2019; Cabezas-Cruz *et al.,* 2019). Indeed, the clinical presentation of *E. minasensis* in cattle has been observed to be similar to the acute form of disease by *E. canis* in dogs (Aguiar *et al.,* 2014; Kitaa, 2014). Cabezas-Cruz *et al.* (2014) links the close relatedness of the two pathogens to possible evolution of *E. minasensis* from highly variable strains of *E. canis* to adapt and infect cattle. The detection of this novel *Ehrlichia* species suggests that it could be circulating in cattle in Kenya and its pathogenicity in the affected animals needs to be determined.

Despite the animals in this study not presenting with the clinical signs of the diseases caused by the pathogens they harbor, poor animal husbandry practices which are common in smallholder dairy farms in peri-urban areas of Nairobi (Nguhiu-Mwangi *et al.*, 2013), causes stress to the cattle consequently lowering their immunity and predisposing them to possible clinical disease and mortalities (Hughes *et al.,* 2014; Garry 2008). This may have been the situation in the previous clinical cases presented to the University of Nairobi Veterinary Hospital. The detection of these pathogen therefore highlights the importance of continued investigation into tick-borne diseases that emerge for effective diagnosis and prevention.

There was a low test accuracy between microscopy and PCR resulting in a high prevalence of *Ehrlichia*-like inclusion bodies (79.3%) being reported on microscopy compared to the overall prevalence of *Anaplasma* and *Ehrlichia* species (21.2%) detected on PCR. This low sensitivity associated with microscopy may be attributed partially to artifacts being confused

for pathogens in blood cells on the blood smear. Additionally, majority of the pathogens in the Family Anaplasmataceae present with intracytoplasmic moruli in white blood cells as reported in infections with *E. ruminantium* (Kasari *et al.,* 2010), *A. bovis* (Liu et al. 2012; Priyanka *et al.,* 2017), *Anaplasma phagocytophilum* (Dahmani *et al.,* 2015; Iqbal *et al.,* 2019), *A. platys* (Zobba *et al.,* 2014) and *E. minasensis* (Aguiar *et al.,* 2019). Therefore, more sensitive techniques which detect pathogen DNA are is imperative in making an accurate diagnosis of causative agents of anaplasmosis and ehrlichiosis in the study area.

A discrepancy in the detection on *E. ruminantium* in cattle blood using sandwich ELISA and not on PCR was observed in this study. This may be explained by the low sensitivity and specificity of serological tests because of cross reactivity with other members of *Anaplasma* and *Ehrlichia* species which is a common finding (OIE, 2018b; Peter *et al.,* 2001) hence has been discouraged in endemic areas (Allsopp, 2010). Therefore, the plates used in this study may have been coated with unspecific monoclonal antibodies targeting crude antigens as earlier discussed (Bell-Sakyi *et al.,* 2003) resulting in low specificity (Sumption *et al.,* 2003) hence the high prevalence reported. Additionally, PCR bias may occur in cases of mixed pathogens where amplification may occur for the most abundant pathogen especially where highly conserved gene such as 16S rRNA is used (Koh *et al.,* 2018). A more robust gene with better resolution for detecting strains of *E. ruminantium* such as major antigen protein 1 (MAP1) (Raliniaina *et al.,* 2010) would be useful to confirm the presence of the pathogen in the study cattle. Despite this, *E. ruminantium* infection cannot be ruled out in the study cattle since the pathogen was detected in the vector tick *A. variegatum* found infecting the cattle.

5.1.4 Ticks infesting dairy cattle from smallholder farms in peri-urban Nairobi and the pathogens they harbor.

Tick infestation was relatively low in the study cattle with all the ticks collected being from in Kasarani Sub-county. The problem of tick-infestation and consequently tick-borne diseases has been well documented as a challenge in smallholder dairy farming in peri-urban Nairobi (Gitau *et al.,* 2010; Gakuya and Mulei, 2005) and especially in Kasarani area (Mureithi and Mukiria, 2015). The increased use of acaricide, which is common in smallholder dairy farms in Kenya (Chenyambuga *et al.,* 2010; Maingi and Njoroge, 2010) and the dry season at the time of sampling (Walker *et al.,* 2014) may explain the low tick infestation reported in this study. Despite the acaricide use, the cattle have been infected with tick-borne diseases as observed in this study and in previous reports (Swai *et al.,* 2005; Maloo *et al.,* 2001) mainly because of acaricide mishandling and tick-resistance (Mutavi *et al.,* 2018; Vudriko *et al.,* 2016).

The high tick infestation in Kasarani Sub-county has been associated with open grazing lands that attract pastoral livestock from the neighboring Kajiado County, which are often heavily infested with ticks (Mugambi *et al.,* 2012). Despite the heavy infestation, there is usually minimal impact to the health of pastoral cattle since they exhibit a certain level of resistance to tick-borne diseases (Chenyambuga *et al.,* 2010; Mattioli *et al.,* 2000). However, they contaminate the grazing pastures with the ticks they carry. Since cut and carry of fodder from the roadsides is the common method of feeding livestock in this area (Alarcon *et al.,* 2017; Mureithi and Mukiria 2015), the ticks are simultaneously carried to the zero-grazed cattle.

Ticks in the genera *Rhipicephalus* including sub-genera *boophilus*, *Ambylomma* and *Hyalloma* were identified in this study. Ticks in these three genera have been previously reported in Kenya (Kariuki *et al.,* 2012; Wesonga *et al.,* 2010), Tanzania (Swai *et al.,* 2008), Uganda (Byaruhanga *et al.,* 2015) and Ethiopia (Mideksa *et al.,* 2017). The wide range of species identified in this study may have been influenced by the conducive climatic conditions that support tick vectors (Keesing *et al.,* 2018; Wikel, 2018). Ticks in these genera consists of important species that transmit diseases of great economic impact to livestock production as well as human health (Walker *et al.,* 2014a).

The African blue tick (*Rhipicephalus (boophilus) decoloratus*) was the most prevalent tick species identified in the study cattle. This tick is the most widespread one host tick in Eastern, Central and Southern Africa (Byaruhanga *et al.,* 2017; Mutai *et al.,* 2013; Odongo *et al.,* 2007) and is known to transmit *Babesia bigemina* (Bock *et al.,* 2004) and *Anaplasma marginale* (Kocan *et al.,* 2004).

The Asian blue tick (*R.* (*boophilus*) *microplus)* was also identified in the study cattle. This tick was first reported in the coastal areas of Kenya by Hoogstraal and Walker (1974) and slightly over a decade the tick was once again identified in the same area (Zulu *et al.,* 1998). Just recently Kanduma *et al.* (2020) characterized these ticks in the coastal strip of Kenya using molecular markers. These tick species are highly invasive and have been reported to replace *R. (boophilus) decoloratus* in areas where both tick species exist (Muhanguzi *et al.,* 2020; Silatsa *et al.,* 2019). They are the most economically important in the subgenera *boophilus* due to their role in transmission of fatal *Babesia bovis* infection (Oliveira *et al.,* 2008; Oliveira-Sequeira *et al.,* 2005) and the novel *E. minasensis* (Carvalho *et al.,* 2016). The

identification of this tick species in other parts of the country implies possible emergence of infections in areas that they were not previously reported (Adjou Moumouni *et al.,* 2015). Adjou Moumouni *et al.* (2015) reported the presence of *B. bovis* in a farm in Ngong' area of peri-urban Nairobi possibly suggesting the possible presence of the vector tick *R. boophilus microplus* as reported in this study. Uncontrolled animal movement in Kenya (Keesing *et al.,* 2018) as well as continued climate change have been suggested to be the key drivers in the spread of different tick species (Vorou *et al.,* 2007; Nyangiwe *et al.,* 2013).

In this study, the brown dog tick (*Rhipicephalus sanguineus*) which typically infests dogs was found attached to the cattle. Majority of the farms where the cattle were sampled were observed to keep dogs, possibly explaining the presence of these ticks. This may result in infection of cattle with dog-related pathogens such as *Anaplasma platys* as observed in this study and reported elsewhere (Hsi *et al.,* 2019; Iweriebor *et al.,* 2017). *Rhipicephalus sanguineus* was also found to harbor unidentified *Ehrlichia* species which clustered in a different clade from *E. canis* which is the pathogen that is known to vector (Bessas *et al.,* 2016; Moraes-Filho *et al.,* 2015; Bremer *et al.,* 2005). Whole genome sequencing of this unknown *Ehrlichia* pathogen would shed light to its identification and studies on its pathogenicity on cattle as well as the role of *R. sanguineus* in its transmission are needed.

Anaplasma ovis, the causative agent for ovine anaplasmosis was detected in one *Rhipicephalus evertsi evertsi* attached to cattle similar to report by Berggoetz *et al.* (2014). Although this pathogen is commonly isolated in small ruminants (Dahmani *et al.,* 2017b), the sharing of hosts by the different tick species may enable ticks to acquire multiple pathogens from different blood meals (Reye *et al.,* 2012). Detection of these pathogens in the ticks do

not always imply vector competence (Sadeddine *et al.,* 2020; Doudier *et al.,* 2010) but mechanical transmission may occur when susceptible hosts such as cattle come in contact with infected ticks (Reye *et al.,* 2012).

Ambylomma variegatum and *A. gemma* were identified in this study. *Ambylomma* species are among the most important tick species in Africa causing devastating animal diseases such as Heart water (Silatsa *et al.,* 2019; Lutomiah *et al.,* 2014). These tick species have been previously identified and reported in Kenya (Oswe *et al.,* 2018; Kariuki *et al.,* 2012; Maingi and Njoroge, 2010; Wesonga *et al.,* 2010). *Rickettsia conorii* was detected in *A. variegatum* ticks similar to a previous study by Mutai *et al.* (2013) in Kenya. Although *R. conorii* has been detected frequently in *R. sanguineous* which is the documented vector tick (Ionita *et al.,* 2016; Liu, 2014), it can also be detected in other tick species (Mediannikov *et al.,* 2010). *Ambylomma* species have been known as the major reservoirs for rickettsial DNA in Africa (Parola *et al.,* 2013), hence not surprising to detect this pathogen in these ticks. Their high attraction of this tick to humans (Parola and Raoult, 2001) increases the risk of human infection by these pathogens.

In this study, *Ambylomma variegatum* was also found to harbor *E. canis* and *E. ruminantium* and in two cases co-infected with *Rickettsia* pathogens. It was not surprising to detect *E. ruminantium* in *A. variegatum* ticks since they are the documented competent vectors for this pathogen (Teshale *et al.,* 2015; Faburay *et al.,* 2007b) and have been previously detected in Kenya (Omondi *et al.,* 2017). Since *A. variegatum* has been strongly implicated in the transmission of *Rickettsia* species (Cicculli *et al.,* 2019; Lorusso *et al.,* 2013), detection of both *Rickettsia* species and *E. ruminantium* is possible (Robinson *et al.,* 2009). The aggressive

feeding habit by this tick species on various hosts (Oswe *et al.,* 2018) may explain the detection of *E. canis* which is commonly a dog pathogen and the co-infection reported in this study.

Hyalloma rufipes and *Hyalloma truncatum* were also identified in this study. These tick species have been reported previously in various parts of Kenya (Lutomiah *et al.,* 2014; Omondi *et al.,* 2017; Kariuki *et al.,* 2012). *Rickettsia aeschlimanii* was detected in *Hyalloma rufipes* ticks in this study. *Hyalloma* species are the documented vectors for *R. aeschlimanii* (Parola *et al.,* 2013). This pathogen was first detected and characterized in *Hyalloma marginatum* in Morocco (Beati *et al.,* 1997) and since then has been isolated in different species of *Hyalloma* ticks in Kenya (Koka *et al.,* 2017; Omondi *et al.,* 2017), Ethiopia and Chad (Mura *et al.,* 2008), Senegal (Mediannikov *et al.,* 2010) and Europe (Chitimia-Dobler *et al.,* 2019; Duscher *et al.,* 2018). Additionally, *R. aeschlimanii* was also detected in *R. microplus* tick. The detection of this pathogen in *R. (boophilus)* microplus is not surprising since Mutai *et al.* (2013) and Reye *et al.* (2012) detected this pathogen in its closely related species of *R.(boophilus) annulatus* attached to cattle from Kenya and Nigeria respectively. Moreover, Pretorius and Birtles (2002) also identified *R. aeschlimanii* in *R. appendiculatus* tick from South Africa. This indicates that *R. aeschlimanii* can be found in many other tick species although the vector competence for these tick species needs to be evaluated. The *Rickettsia* pathogens were only detected in ticks but not in the study cattle. Similar findings have been reported in Kenya (Mutai *et al.,* 2013) and Nigeria (Reye *et al.,* 2012). This may be explained by the low numbers of ticks found on the cattle at the time of sampling.

Although vector competence for some of the tick species found carrying pathogens identified in this study is yet to be established, their recognition in these ticks expands the knowledge on pathogens harbored by ticks infesting dairy cattle in peri-urban Nairobi. Surveillance of pathogens in ticks has been viewed as a good start to pre-empt pathogens that could potentially infect animals and people (Qiu *et al.,* 2014). Additionally, identification of tick species and detection of the pathogens they harbor in various areas of a country has been viewed as a pre-requisite for developing appropriate tick control programs during targeted tick-control (Rajput *et al.,* 2006).

In this study, *R. conorii* and *R. aeschlimanii* which are zoonotic pathogens were detected in ticks infesting cattle. *Rickettsia conorii* has been associated with febrile disease causing severe morbidity (Yoshikawa *et al.,* 2005) and even fatality in tourists returning from Kenya (Rutherford *et al.,* 2004) while *R. aeschlimanii* which is a closely related pathogen manifests with a mild disease (Raoult *et al.,* 2002). Infection of humans with rickettsiosis occurs from contact with domestic animals or pets who act as reservoir host from which tick bites occur (Reye *et al.,* 2012). Indeed, Thiga *et al.* (2015) reported a higher seroprevalence and titers of Spotted Fever Group (SFG) rickettsiosis among pastoralists keeping large numbers of livestock than other communities in Kenya. The febrile disease from rickettsiosis has been commonly confused for Malaria and typhoid especially in Africa including Kenya where these diseases are endemic (Maina *et al.,* 2016; Crump *et al.,* 2013; Richards *et al.,* 2010). In Kenya, there is evidence that rickettsiosis contribute to a great percentage of febrile cases reported in hospitals (Maina *et al.,* 2012).

Since ticks remain infected with rickettsiosis for life while transmitting transtadially and transovarially (Liu, 2014), there is a public health risk posed by their detection in ticks (Reye *et al.,* 2012; Raoult *et al.,* 2002). Additionally, since dogs and ruminants are the implicated domestic reservoirs for these rickettsiosis (Parola and Raoult, 2006), a country wide surveillance to understand the status of the domestic reservoirs would be informative. Additionally, further characterization of the *Rickettsia* species and strains found in ticks as well as cattle using more specific primers targeting Outer Membrane Protein (Omp) A and B, Citrate synthase and 17-kDa proteins (Walker, 2007; Parola *et al.,* 2005) is necessary.

The 16S rRNA gene was used to infer phylogenetics of pathogens detected in the ticks from this study. *Rickettsia species* have a highly conserved genome and their mitochondrial 16S rRNA has been previously used to detect tick microbiome (Buysse and Duron, 2020; Dergousoff *et al.,* 2020; Kim *et al.,* 2020). *Rickettsia conorii* detected in this study showed high diversity and were closely related to an isolate from Nigeria, USA and China but differed from those from Uganda and Zambia. This indicates that different variants of the *Rickettsia* may exists in the study area with some that may be unique to Kenya. The *E. canis* isolates were highly conserved and similar to isolated from Turkey, Iraq and neighboring Uganda, a suggestion that the strains may be similar to those isolated worldwide and their introduction may be associated to international pet movement which has been associated with pathogen spread (Livanova *et al.,* 2018).

The *Rickettsia* and *Ehrlichia* species detected in these ticks were not detected in the cattle on which these ticks were attached. This may be attributed to complexities in transmission dynamics in the tick vector. In addition to pathogens, tick carry a complex microbiota which

includes bacteria and symbionts (Moutailler *et al.*, 2016) which have been shown to influence pathogen transmission, whereby pathogens can be transmitted selectively to the host (Pollet *et al.*, 2020; Narasimhan and Fikrig, 2017). Pathogens have also been shown to mount specific adaptive mechanisms to survive the tick's immunity and be available in the salivary glands to facilitate their transmission (Qiu *et al.*, 2014), therefore allowing transmission of one pathogen and not the other. Moreover, some of the tick transmission mechanisms are unclear and remain to be studied (Perlman *et al.*, 2006).

On the other hand, *Anaplasma* and *Ehrlichia* species detected in the blood of the study animals were not simultaneously detected in the ticks. Since engorged ticks were excluded from molecular analysis, it is possible that the analyzed ticks were not the ones that transmitted the infection. Moreover, PCR bias; where the most abundant pathogen DNA is amplified, has been documented to occur in cases of mixed pathogens when using highly conserved genes such as 16S rRNA (Koh *et al.*, 2018). Hence it is possible that the *Rickettsia* and the *Ehrlichia* pathogens detected in the ticks had more abundant DNA than that of the pathogens detected in the blood of cattle. Overall, the presence of *Anaplasma* and *Ehrlichia* species detected in the cattle blood cannot be ruled out in the ticks. Therefore, utilization of Next Generation Sequencing (NGS) and network analysis have been proposed to resolve the complex association of the tick microbiota including their transmission dynamic (Cabezas-Cruz *et al.*, 2019).

5.2 Conclusions

1. *Anaplasma* and *Ehrlichia* species are prevalent in dairy cattle in peri-urban Nairobi. On microscopy, *Ehrlichia*-like inclusion bodies were observed in 79.3% of the various white blood cells. The serological prevalence of *Ehrlichia ruminantium* was 18.6% and the molecular prevalence of *Anaplasma* and *Ehrlichia* species was 19.9% and 3.3% respectively. Indicating the need for accurate diagnosis and effective tick control so as to reduce infections of cattle with these pathogens.
2. Animal welfare related factors especially cleaning of cowshed frequently and presence of ticks were significantly associated with the occurrence of *Ehrlichia* and *Anaplasma* species, suggesting the importance of managing animal welfare issues on the farms, to reduce the risks of tick-borne diseases.
3. The dairy cattle in peri-urban Nairobi were infected with *E. minasensis* and *Anaplasma* species including the dog-associated *A. platys* and unidentified *Anaplasma* species. This study provides the first reports of *E. minasensis* and *A. platys* infections in dairy cattle in Kenya. Therefore, there is need for continued investigation into emerging tick-borne pathogens to guide in development of appropriate control measures.
4. Dairy cattle in peri-urban Nairobi were infested with different tick species in the genera *Rhipicephalus*, *Ambylomma* and *Hyalloma.* The ticks harbored various *Ehrlichia* species including the dog-associated *Ehrlichia canis* and zoonotic *Rickettsia* pathogens, highlighting the public health concern posed by the infestation of these ticks on the dairy cattle.

5.3 Recommendations

1. Whole genome sequencing is necessary to characterize the unidentified *Anaplasma* and *Ehrlichia* species as well as other unrelated pathogens observed on microscopy in this study. Further studies are needed to comprehensively understand the range of pathogens infecting the dairy cattle in peri-urban Nairobi, especially linking up the presenting clinical signs specific pathogens.
2. The role of cattle as alternative hosts for the dog-associated pathogens such as *A. platys* and *E. canis* detected in blood and ticks infesting cattle respectively needs to be investigated, especially when cattle are in close proximity to the dogs.
3. Despite the study cattle that were infested with the *Anaplasma* and *Ehrlichia* species not presenting with the clinical disease, there is urgent need to put in place effective control measures since the compromising animal welfare practices in peri-urban Nairobi predisposes cattle to possible flaring up of these infections.
4. There is need for molecular characterization of the identified tick species using specific genes such as Interspacer (ITS) and CO1 genes to confirm the presence of the re-emerging invasive *R. microplus* ticks in various counties in Kenya.
5. Investigation of zoonotic pathogens such as *A. platys* and rickettsiosis detected in the cattle and the ticks among the cattle owners and handlers will be important to understand the prevalence of these infections and enable early diagnosis and treatment.

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APPENDICES

Appendix 1: Pre-tested questionnaire used for risk factor analysis

A SURVEY ON THE PREVALENCE AND RISK FACTORS ASSOCIATED WITH *ANAPLASMA* AND *EHRLICHIA* PATHOGENS INFECTING DAIRY CATTLE IN NAIROBI AND ITS' PERI-URBAN AREAS

Farmers Name: …… Farm ID: ……

Sub-county: …….. Ward: ……

Phone number: ……...

A: Demographics:

1. What is the gender of the respondent Male ☐
   Female ☐
2. Who is the dairy farm head Male ☐ Female ☐
3. What is the age of the owner_____?
4. What is the current family size _____
5. What is the age of the employee (if any) _____
   If present, what is the highest level of education for the employee? a) ☐
   Primary/informal/none
   b) Secondary and above ☐
6. What is the highest level of education for the principal farmer? a) Primary/ informal/none ☐
   c) Secondary and above ☐

B: Farming systems

7. Which is your farming system: i) Livestock only ☐ ii) Livestock and crops ☐
8. What are your other source of income i) Business ☐ ii) Salary ☐
   iii) none ☐
9. How long have you been keeping cattle? _____
10. Which types of livestock do you keep?
    a) Dairy cattle ☐ b) Beef cattle ☐ c) Sheep ☐
    d) Goats ☐ e) Pigs ☐ f) Layers ☐

g) Broilers ☐ h) Others (specify) ☐

11. How do you always feed your cattle?

i. Stall feeding ☐
ii. Free grazing ☐
iii. Tethering ☐
iv. Stall feeding and free grazing ☐

12. Where do you always obtain fodder from?

a) Cut and carry from road side ☐
b) Cut and carry from own farm ☐
c) Grazing along the road only ☐
d) Purchase from neighbors ☐
e) Others (Specify)_____

If you purchase, do you consider the source of fodder? a) Yes ☐ b) No ☐

13. Do you use hay in your farm? a) Yes ☐ b)No ☐

If yes (i) purchased (Specify area of origin) ☐ _____

(ii) Own ☐

14. What is the total area used for fodder in acres? _____

15. Is the pasture land fenced? a) Yes b) No

C: Management practices

16. How frequently do you clean the cow shed?

a) Daily ☐ b) Every other day ☐ c) Fortnightly ☐

17. What is the nature of the cow pen floor? a) Earthen ☐ b) cemented ☐

c) stones ☐

18. Is there bedding in the sleeping area? Yes ☐ No ☐

If yes, which ones? _____

19. Is the roof present at the sleeping area? Yes ☐ No ☐

20. Is the walk alley adequate? Yes ☐ No ☐

21. Is the cubicle adequate? Yes ☐ No ☐

22. Do you control ticks in your livestock? Yes ☐ No ☐

23. How frequently do you apply the acaricide?

a) Weekly ☐ b) Monthly

c) Every 3 Months ☐

24. Which method of application of the acaricide do you use?

a) Hand spray ☐ b) Pour on ☐ c) Other

Specify_____

25. Do you own a dog? Yes ☐ No ☐

D: Livestock Health

26. Has any of your cattle died in the last 12 months? Yes ☐ No ☐

If yes, what was the cause of death? _____

27. Have you bought new cattle in the last 2 months? Yes ☐ No ☐

28. Have you seen ticks on your cattle? Yes ☐ No ☐

ANIMALS SAMPLED

Animal ID	**Age**	**Breed**	**Lactational status Adults: (dry, lactating, pregnant)**	**Parity**				**Ticks present**	**Blood collected**	
					Rectal Temp	**Lymp nodes**	**Mucous membranes**		**EDTA**	**PLAIN**

Appendix 2: Some of the partial sequences deposited in the GenBank database

Anaplasma platys

LOCUS MT163376 425 bp DNA linear ENV 10-MAR-2020
DEFINITION Anaplasma platys isolate 20 16S ribosomal RNA gene, partial sequence.
ACCESSION MT163376
VERSION MT163376
KEYWORDS ENV.
SOURCE Anaplasma platys
ORGANISM Anaplasma platys
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Anaplasma.
REFERENCE 1 (bases 1 to 425)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Kanduma,E.G., Gakuya,D.W., Maingi,N. and Mulei,C.M.
TITLE Direct Submission
JOURNAL Submitted (10-MAR-2020) Faculty of Veterinary Medicine, Clinical Studies Department, University of Nairobi, Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..425
/organism="Anaplasma platys"
/mol_type="genomic DNA"
/isolate="20"
/isolation_source="blood"
/host="cattle"
/db_xref="taxon:949"
/environmental_sample
/country="Kenya"
/note="amplified with species-specific primers"
rRNA <1..>425
/product="16S ribosomal RNA"
ORIGIN

1 ttagtggcat gacgggtgag taatgcatag gaatctacct agtagtatgg gatagccact
61 agaaatggtg ggtaatactg tataatccct gcgggggaaa gatttatcgc tattagatga
121 gcctatgtta gattagctag ttggtagggt aaaggcctac caaggcagtg atctatagct
181 ggtctgagag gatgatcagc cacactggaa ctgagatacg gtccagactc ctacgggagg
241 cagcagtggg gaatattgga caatgggcgc aagcctgatc cagctatgcc gcgtgagtga
301 ggaaggcctt agggttgtaa aactctttca gtggggaaga taatgacggt acccacagaa
361 gaagtcccgg caaactccgt gccagcagcc gcggtaatac ggaggggggca agcgttgttc
421 ggaat
//

Anaplasma bovis

LOCUS MT160355 425 bp DNA linear ENV 09-MAR-2020
DEFINITION Anaplasma bovis isolate 39 16S ribosomal RNA gene, partial
sequence.
ACCESSION MT160355
VERSION MT160355
KEYWORDS ENV.
SOURCE Anaplasma bovis
ORGANISM Anaplasma bovis
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales;
Anaplasmataceae; Anaplasma.
REFERENCE 1 (bases 1 to 425)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Kanduma,E.G., Gakuya,D.W.,
Maingi,N. and Mulei,C.M.
TITLE Direct Submission
JOURNAL Submitted (09-MAR-2020) Clinical Studies, University of Nairobi,
Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..425
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/mol_type="genomic DNA"
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/isolation_source="blood"
/host="cattle"

/db_xref="taxon:186733"
/environmental_sample
/country="Kenya"
/note="amplified with species-specific primers"
rRNA <1..>425
/product="16S ribosomal RNA"
ORIGIN
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61 gaagtggtgg gtaatactgt ataatccctg cgggggaaag atttatcgct acatgatgag
121 cctatgttag attagctagt tggtggggta atggcctacc aaggcagtga tctatagctg
181 gtctgagagg atgatcagcc acactggaac tgagacacgg tccagactcc tacgggaggc
241 agcagtgggg aatattggac aatgggcgca agcctgatcc agctatgccg cgtgagtgag
301 gaaggcctta gggttgtaaa actctttcag tggggaagat aatgacggta cccacagaag
361 aagtcccggc aaactccgtg ccagcagccg cggtaatacg gagggggcaa gcgttgttcg
421 gaatt
//

Ehrlichia minasensis

LOCUS MT163429 695 bp DNA linear ENV 10-MAR-2020
DEFINITION Ehrlichia minasensis isolate 32E 16S ribosomal RNA gene, partial sequence.
ACCESSION MT163429
VERSION MT163429
KEYWORDS ENV.
SOURCE Ehrlichia minasensis
ORGANISM Ehrlichia minasensis
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Ehrlichia.
REFERENCE 1 (bases 1 to 695)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Kanduma,E.G., Gakuya,D.W., Maingi,N. and Mulei,C.M.
TITLE Direct Submission
JOURNAL Submitted (10-MAR-2020) Faculty of Veterinary Medicine, Clinical Studies Department, University of Nairobi, Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..695

/organism="Ehrlichia minasensis"

/mol_type="genomic DNA"

/isolate="32E"

/isolation_source="blood"

/host="cattle"

/db_xref="taxon:1242993"

/environmental_sample

/country="Kenya"

/note="amplified with species-specific primers"

rRNA <1..>695

/product="16S ribosomal RNA"

ORIGIN

1 ggggaatatt ggacaatggg cgaaagcctg atccagctat gccgcgtgag tgaagaaggc

61 cttcgggttg taaaactctt tcaataggga agataatgac ggtacctata gaagaagtcc

121 cggcaaactc tgtgccagca gccgcggtaa tacggagggg gcaagcgttg ttcggaatta

181 ttgggcgtaa agggcacgta ggtggactag taagttaaaa gtgaaatacc aaagcttaac

241 tttggagcgg cttttaatac tgctagacta gaggtcgaaa gaggatagcg gaattcctag

301 tgtagaggtg aaattcgtag atattaggag gaacaccagt tgcgaaggcg gctatctggt

361 tcgatactga cactgaggtg cgaaagcgtg gggagcaaac aggattagat accctggtag

421 tccacgctgt aaacgatgag tgctaaatgt gaggatttta tctttgtatt gtagctaacg

481 cgttaagcac tccgcctggg gactacggtc gcaagactaa aactcaaagg aattgacggg

541 gacccgcaca agcggtggag catgtggttt aattcgatgc tacgcgaaaa accttaccac

601 tttttgacat gaaggtcgta tccctcctaa caggggggagt cagttcggct ggaccttaca

661 caggtgctgc atggttgtcg tcagctcgtg tcgtg

//

Rickettsia conorii

LOCUS MT366066 433 bp DNA linear BCT 22-APR-2020

DEFINITION Rickettsia conorii isolate 286A 16S ribosomal RNA gene, partial sequence.

ACCESSION MT366066

VERSION MT366066

KEYWORDS .

SOURCE Rickettsia conorii

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  ORGANISM  Rickettsia conorii
            Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales;
            Rickettsiaceae; Rickettsieae; Rickettsia; spotted fever group.
REFERENCE   1  (bases 1 to 433)
  AUTHORS   Peter,S.G., Aboge,G.O., Kariuki,H.W., Gakuya,D.W., Maingi,N.,
            Mulei,C.M. and Mainga,A.O.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-APR-2020) Faculty of Veterinary Medicine, Clinical
            Studies Department, University of Nairobi, Loresho Ridge, Nairobi,
            Nairobi 00100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /isolate="286A"
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                     /db_xref="taxon:781"
                     /country="Kenya"
                     /note="amplified with species-specific primers"
     rRNA            <1..>433
                     /product="16S ribosomal RNA"
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      121 tgagcccgcg tcagattagg tagttggtga ggtaatggct caccaagccg acgatctgta
      181 gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga ctcctacggg
      241 aggcagcagt ggggaatatt ggacaatggg cgaaagcctg atccagcaat accgagtgag
      301 tgatgaaggc cttagggttg taaagctctt ttagcaagga agataatgac gttacttgca
      361 gaaaaagccc cggctaactc cgtgccagca gccgcggtaa gacggagggg gcaagcgtgt
      421 tccggaatta taa
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Rickettsia aeschlimanii

LOCUS MT366164 426 bp DNA linear BCT 22-APR-2020
DEFINITION Rickettsia aeschlimannii isolate 281C 16S ribosomal RNA gene, partial sequence.
ACCESSION MT366164
VERSION MT366164
KEYWORDS .
SOURCE Rickettsia aeschlimannii
ORGANISM Rickettsia aeschlimannii
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; Rickettsieae; Rickettsia; spotted fever group.
REFERENCE 1 (bases 1 to 426)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Gakuya,D.W., Maingi,N. and Mulei,C.M.
TITLE Direct Submission
JOURNAL Submitted (22-APR-2020) Faculty of Veterinary Medicine, Clinical Studies Department, University of Nairobi, Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..426
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/isolation_source="R.(Boophilus) annulatus"
/host="cattle"
/db_xref="taxon:45262"
/country="Kenya"
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rRNA <1..>426
/product="16S ribosomal RNA"
ORIGIN
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121 ccgcgtcaga ttaggtagtt ggtgaggtaa tggctcacca agccgacgat ctgtagctgg
181 tctgagagga tgatcagcca cactgggact gagacacggc ccagactcct acgggaggca
241 gcagtgggga atattggaca atgggcgaaa gcctgatcca gcaataccga gtgagtgatg
301 aaggccttag ggttgtaaag ctcttttagc aaggaagata atgacgttac ttgcagaaaa
361 agccccggct aactccgtgc cagcagccgc ggtaagacgg agggggcaag cgttgttcgg
421 aattat
//

Ehrlichia ruminantium

LOCUS MT738235 825 bp DNA linear ENV 09-JUL-2020
DEFINITION Ehrlichia ruminantium isolate 524AR 16S ribosomal RNA gene, partial sequence.
ACCESSION MT738235
VERSION MT738235
KEYWORDS ENV.
SOURCE Ehrlichia ruminantium (heartwater rickettsia)
ORGANISM Ehrlichia ruminantium
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales;
Anaplasmataceae; Ehrlichia.
REFERENCE 1 (bases 1 to 825)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Gakuya,D.W., Maingi,N.E., Mulei,C.M. and Mainga,A.O.
TITLE Direct Submission
JOURNAL Submitted (09-JUL-2020) Faculty of Veterinary Medicine, Clinical Studies Department, University of Nairobi, Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..825
/organism="Ehrlichia ruminantium"
/mol_type="genomic DNA"
/isolate="524AR"
/isolation_source="Amblyomma variegatum"
/host="cattle"
/db_xref="taxon:779"

/environmental_sample
/country="Kenya"
/note="amplified with species-specific primers"
rRNA <1..>825
/product="16S ribosomal RNA"
ORIGIN
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121 aaggccttcg ggttgtaaaa ctctttcaat agggaagata atgacggtac ctatagaaga
181 agtcccggca aactctgtgc cagcagccgc ggtaatacgg agggggcaag cgttgttcgg
241 aattattggg cgtaaagggc acgtaggtgg actagtaagt taaaagtgaa ataccaaagc
301 ttaactttgg agcggctttt aatactgcta gactagaggt cgaaagagga tagcggaatt
361 cctagtgtag aggtgaaatt cgtagatatt aggaggaaca ccagtggcga aggcggctat
421 ctggttcgat actgacactg aggtgcgaaa gcgtggggag caaacaggat tagataccct
481 ggtagtccac gctgtaaacg atgagtgcta aatgtgagga ttttatcttt gtattgtagc
541 taacgcgtta agcactccgc ctggggacta cggtcgcaag actaaaactc aaaggaattg
601 acggggaccc gcacaagcgg tggagcatgt ggtttaattc gatgctacgc gaaaaacctt
661 accacttttt gacatgaagg tcgtatccct cctaacaggg ggagtcagtt cggctggacc
721 ttacacaggt gctgcatggt tgtcgtcagc tcgtgtcgtg agatgttggg ttaagtcccg
781 caacgagcgc aaccctcatt cttagttacc aacaggtaat gcttg
//

Ehrlichia canis

LOCUS MT734401 825 bp DNA linear ENV 09-JUL-2020
DEFINITION Ehrlichia canis isolate 396B 16S ribosomal RNA gene, partial sequence.
ACCESSION MT734401
VERSION MT734401
KEYWORDS ENV.
SOURCE Ehrlichia canis
ORGANISM Ehrlichia canis
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Ehrlichia.
REFERENCE 1 (bases 1 to 825)
AUTHORS Peter,S.G., Aboge,G.O., Kariuki,H.W., Gakuya,D.W., Maingi,N.E., Mulei, C.M. and Mainga,A.O.
TITLE Direct Submission

JOURNAL Submitted (09-JUL-2020) Faculty of Veterinary Medicine, Clinical Studies Department, University of Nairobi, Loresho Ridge, Nairobi, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
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/mol_type="genomic DNA"
/isolate="396B"
/isolation_source="R.(Boophilus) decoloratus"
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/db_xref="taxon:944"
/environmental_sample
/country="Kenya"
/note="amplified with species-specific primers"
rRNA <1..>825
/product="16S ribosomal RNA"
ORIGIN
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61 gcagtgggga atattggaca atgggcgaaa gcctgatcca gctatgccgc gtgagtgaag
121 aaggccttcg ggttgtaaaa ctctttcaat agggaagata atgacggtac ctatagaaga
181 agtcccggca aactctgtgc cagcagccgc ggtaatacgg agggggcaag cgttgttcgg
241 aattattggg cgtaaagggc acgtaggtgg actagtaagt taaaagtgaa ataccaaagc
301 ttaactttgg agcggctttt aatactgcta gactagaggt cgaaagagga tagcggaatt
361 cctagtgtag aggtgaaatt cgtagatatt aggaggaaca ccagtggcga aggcggctat
421 ctggttcgat actgacactg aggtgcgaaa gcgtggggag caaacaggat tagataccct
481 ggtagtccac gctgtaaacg atgagtgcta aatgtgagga ttttatcttt gtattgtagc
541 taacgcgtta agcactccgc ctggggacta cggtcgcaag actaaaactc aaaggaattg
601 acggggaccc gcacaagcgg tggagcatgt ggtttaattc gatgctacgc gaaaaacctt
661 accacttttt gacatgaagg tcgtatccct cctaacaggg ggagtcagtt cggctggacc
721 ttacacaggt gctgcatggt tgtcgtcagc tcgtgtcgtg agatgttggg ttaagtcccg
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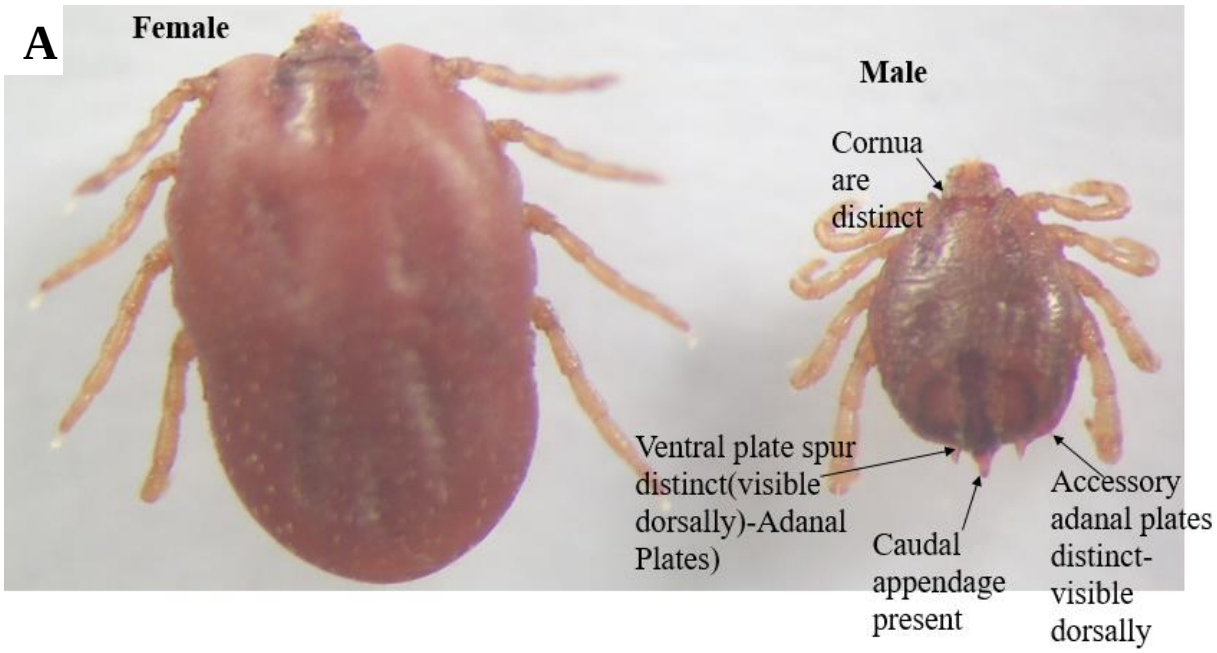
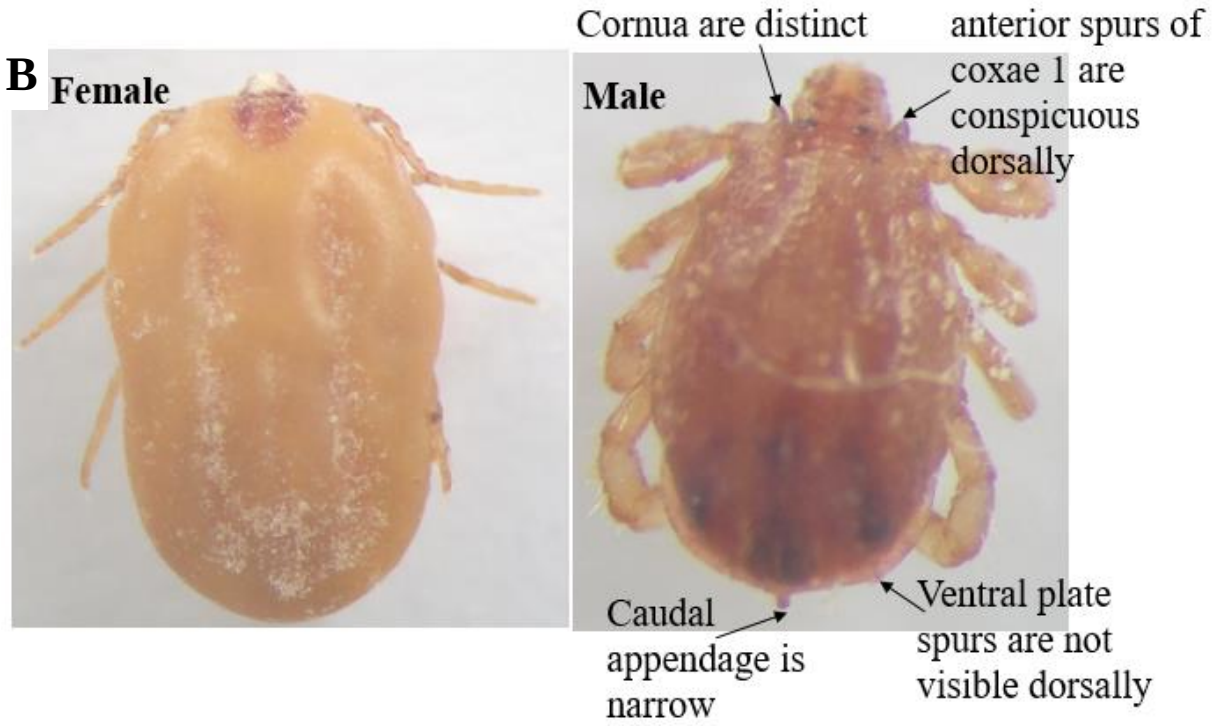
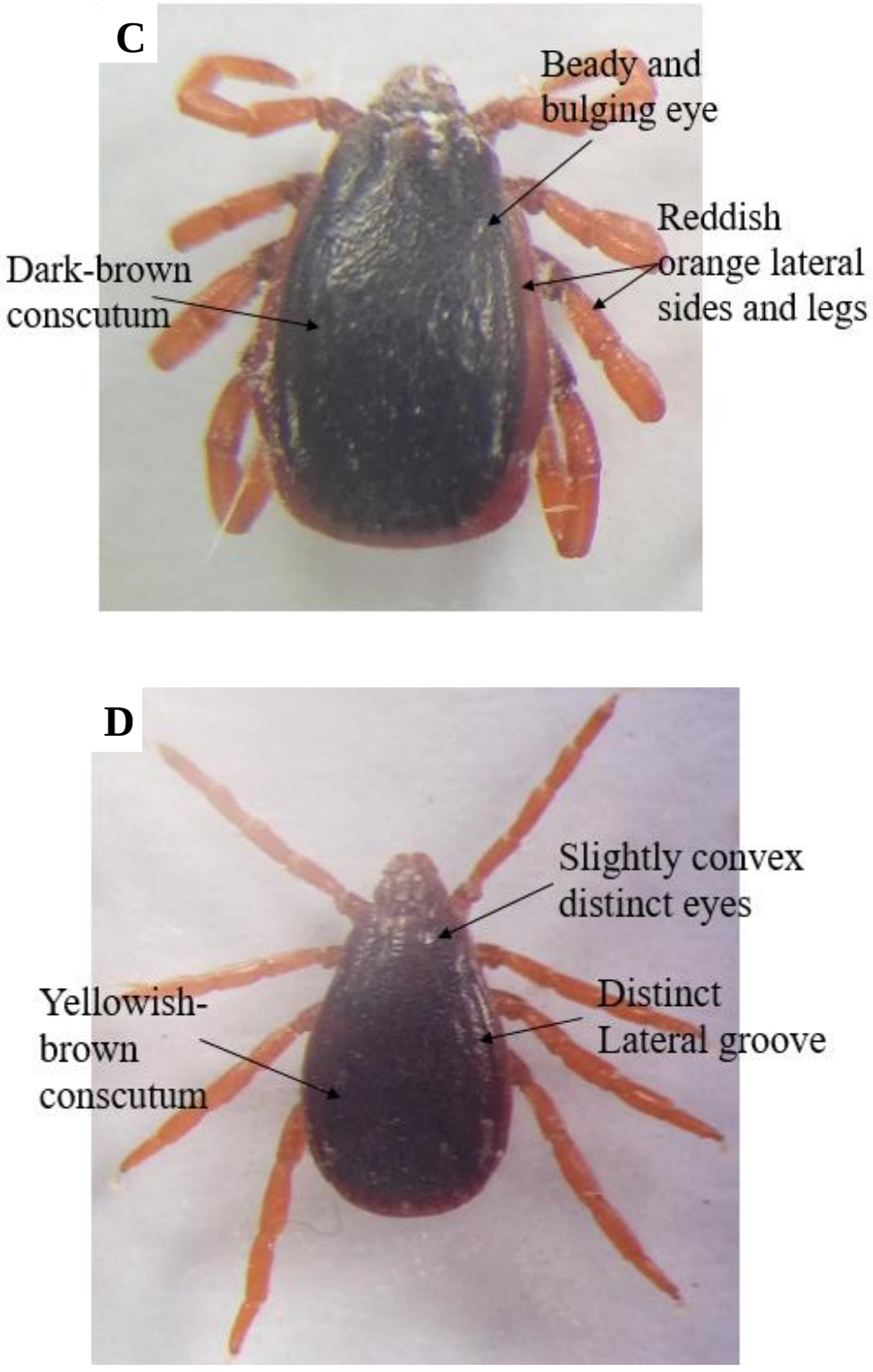
Appendix 3: Morphologically identified tick species infesting dairy cattle in peri-urban Nairobi.

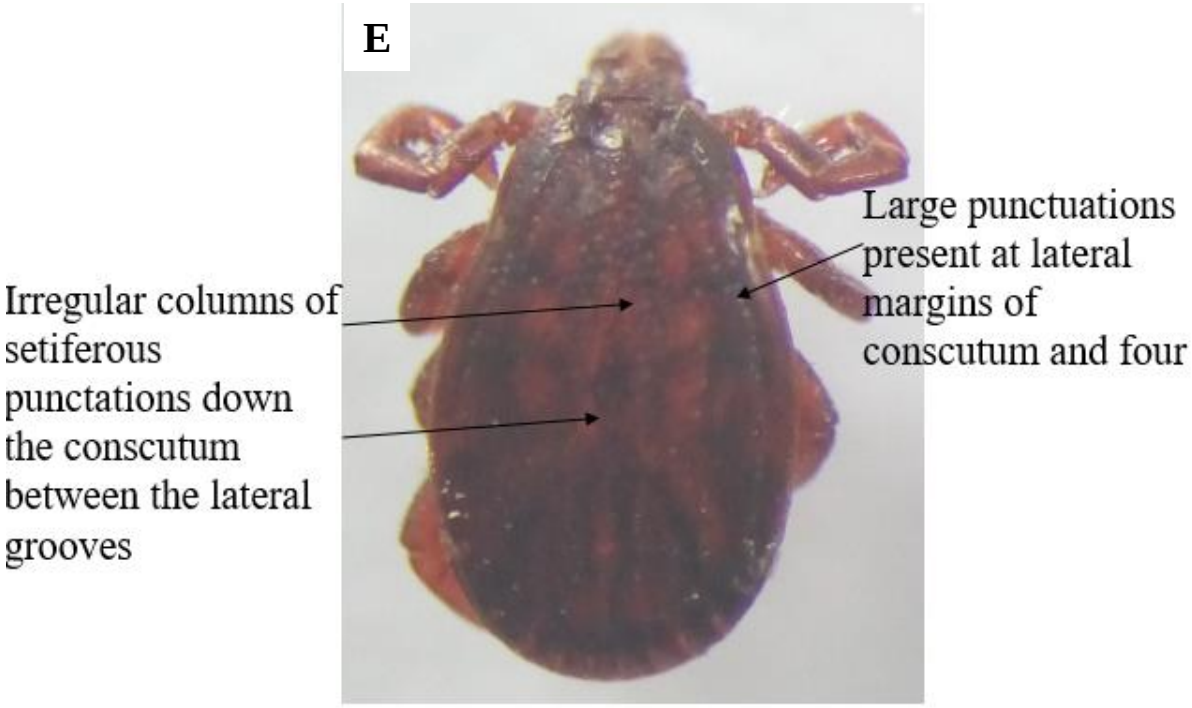


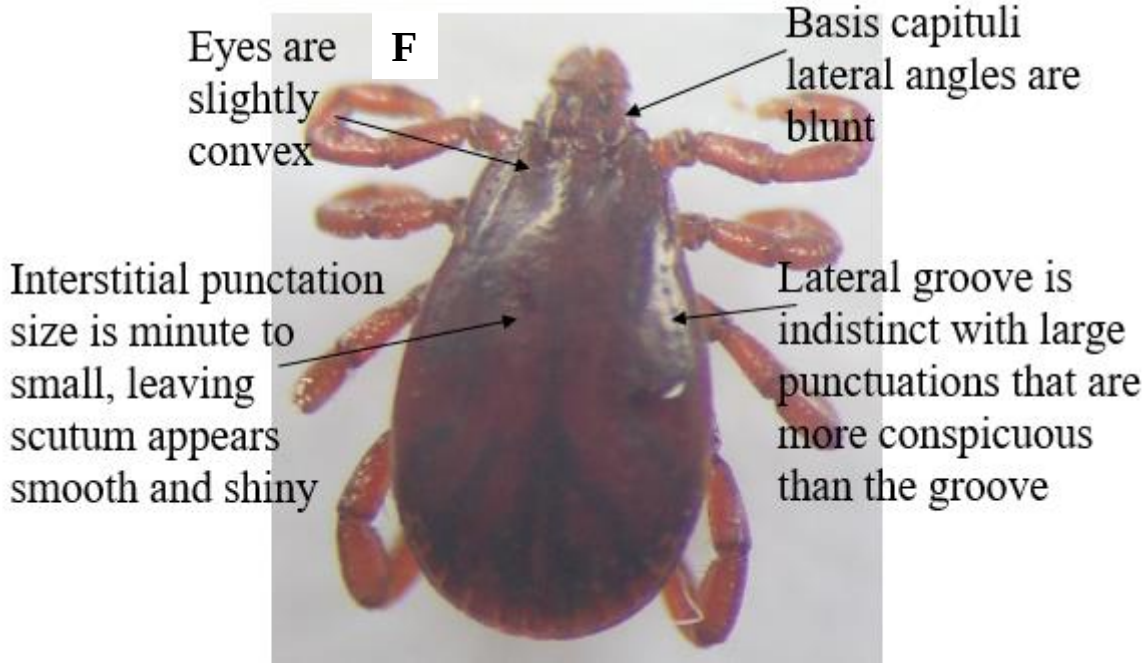


A: ***Rhipicephalus (boophilus) decoloratus*** **B:** ***R. (boophilus) microplus***

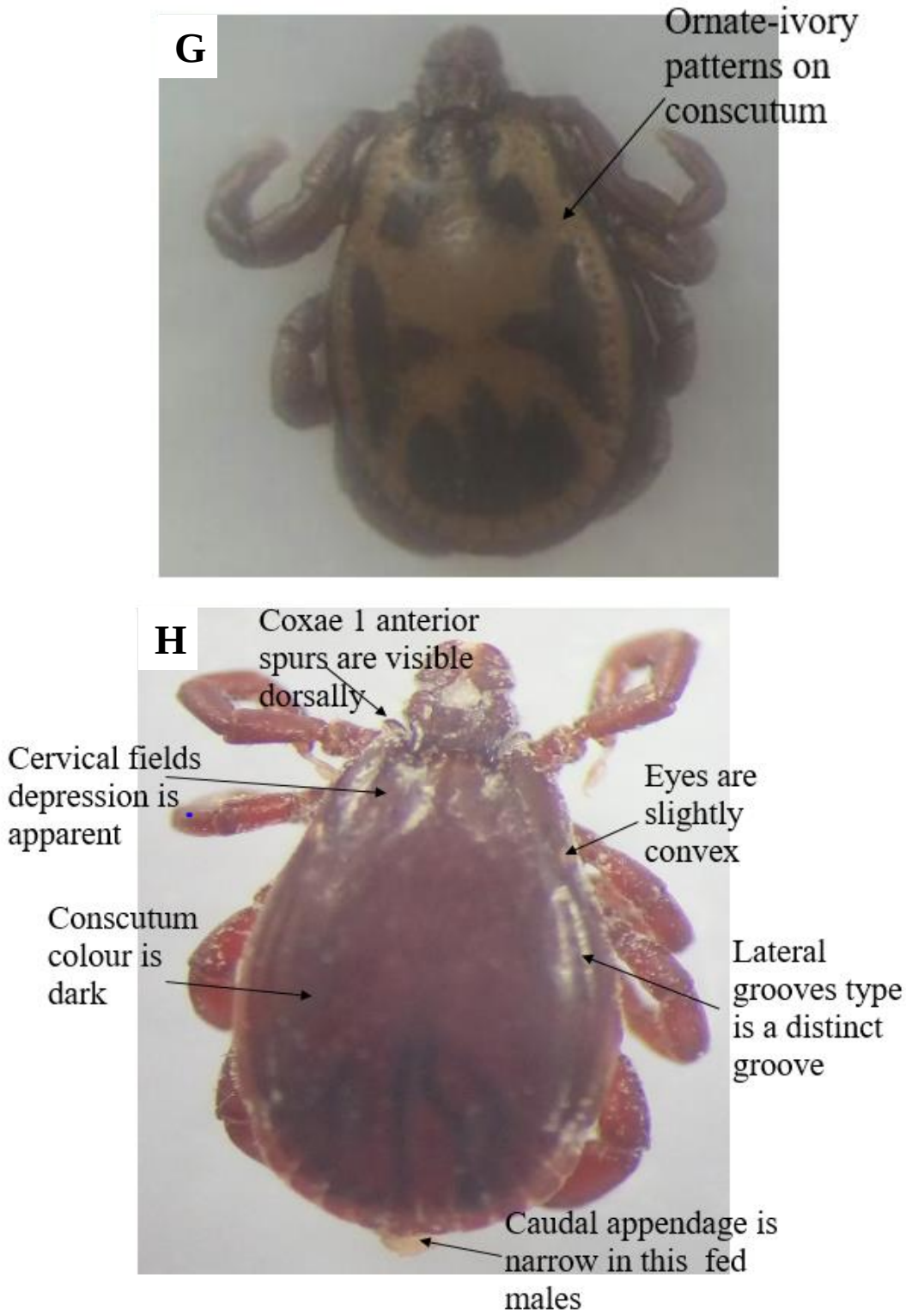


C: ***Rhipicephalus evertsi evertsi*** **D:** ***Rhipicephalus. sanguineous***



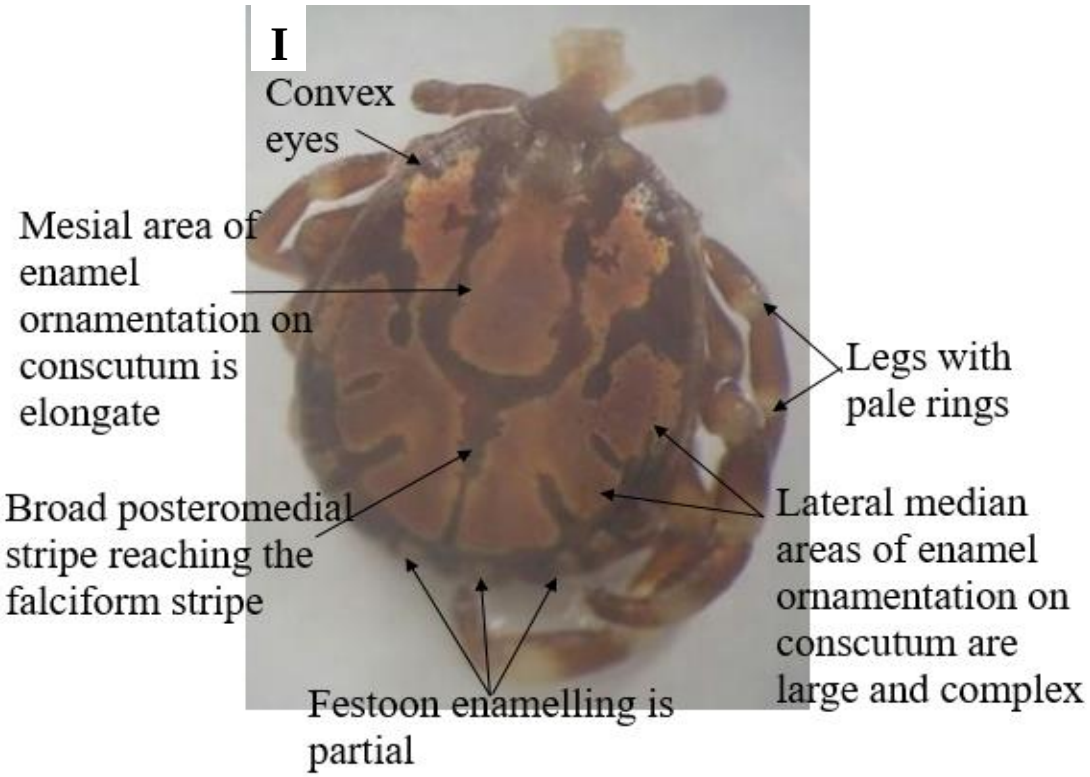


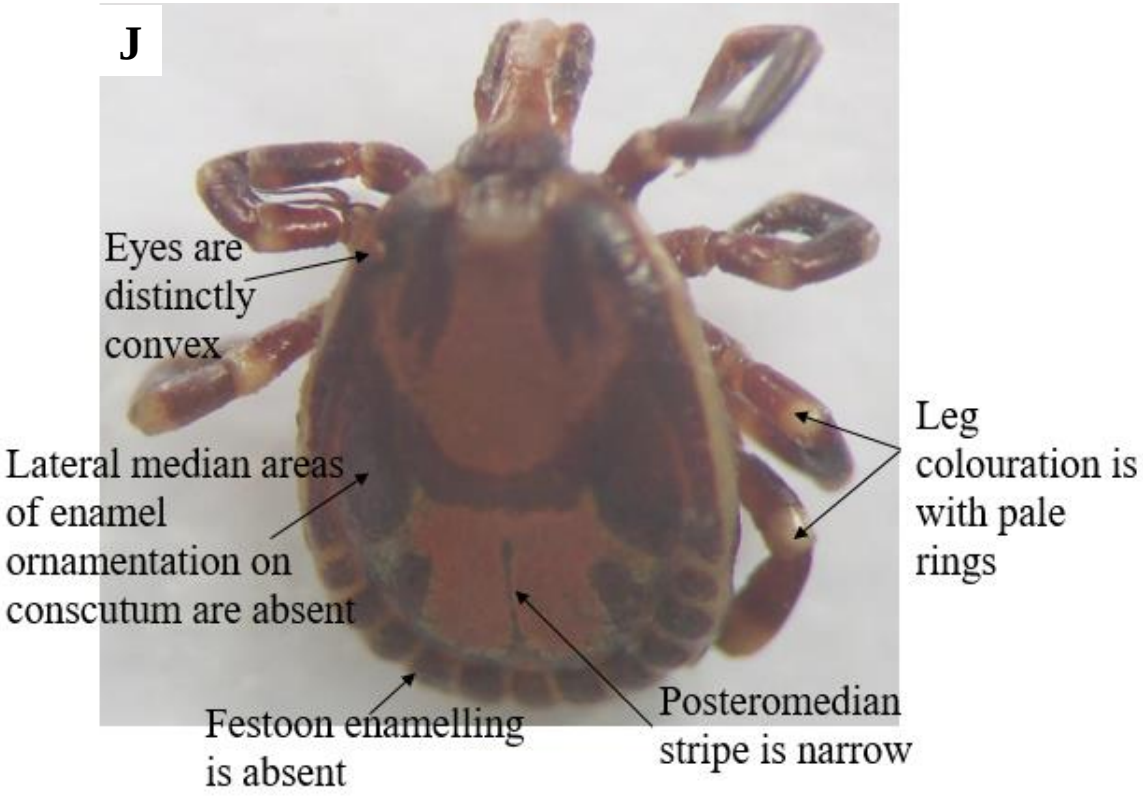
E: ***Rhipicephalus simus*** **F:** ***Rhipicephalus praetextatus***



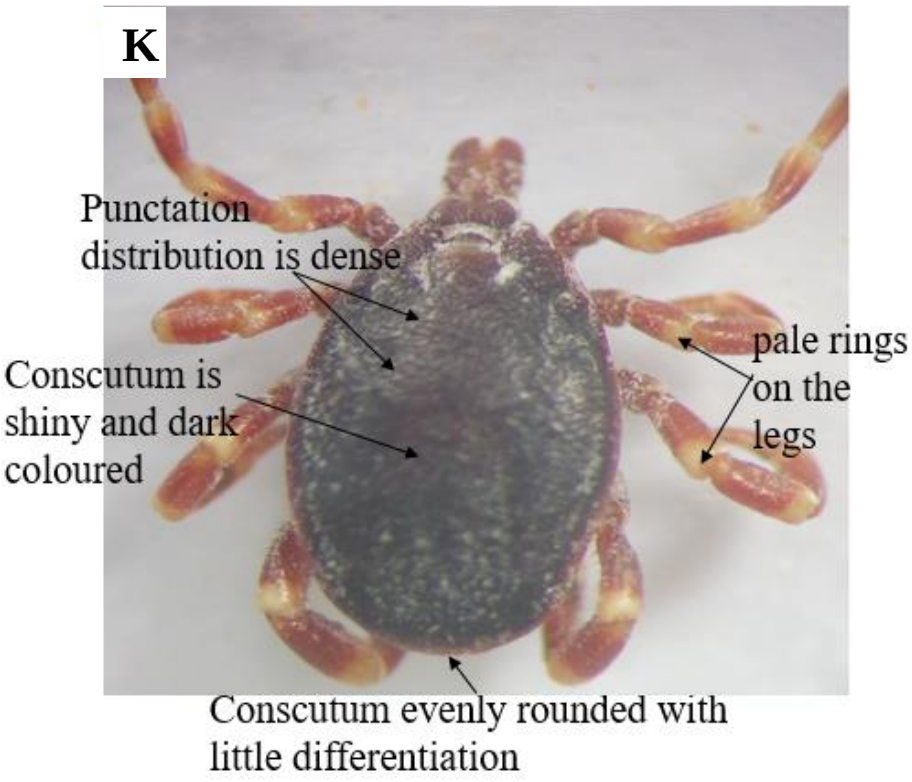
G: ***Rhipicephalus pulchellus***

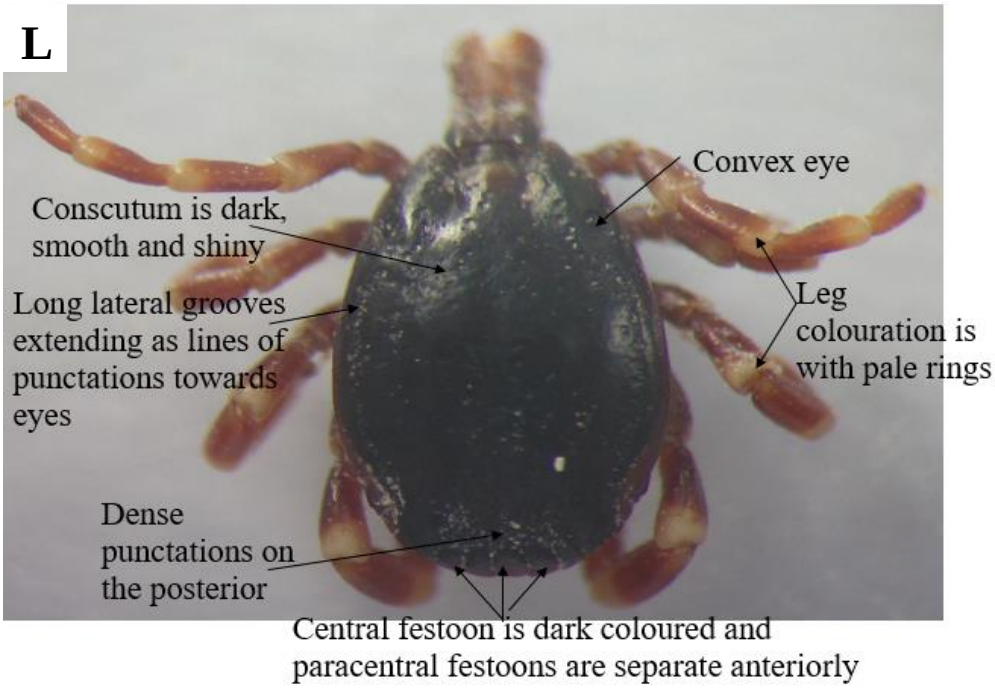
H: ***Rhipicephalus appendiculatus***





I: ***Amblyomma gemma*** **J:** ***Ambylomma variegatum***





K: ***Hyalloma rufipes*** **L:** ***Hyalloma truncatum***

Appendix 4: Publications from this research work

1. Prevalence and risk factors associated with *Ehrlichia* infections in smallholder dairy cattle in Nairobi City County, Kenya, *Veterinary World*, 12(10): 1599-1607. doi: www.doi.org/10.14202/vetworld.2019.1599-1607.
2. Molecular prevalence of emerging *Anaplasma* and *Ehrlichia* pathogens in apparently healthy dairy cattle in peri-urban Nairobi, Kenya. *BMC Veterinary Research*, 16(1): 364. doi:https://doi.org/10.1186/s12917-020-02584-0.