GENETIC DETECTION OF SOME TICK-BORNE BACTERIAL AND PROTOZOAN PATHOGENS IN TICKS COLLECTED IN RAYMOND MHLABA LOCAL MUNICIPALITY, EASTERN CAPE PROVINCE, SOUTH AFRICA

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Together in Excellence

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE

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BY

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DECLARATION

I Ayabulela Nqoro hereby declare that the Master's Research Project I have succumbed to the University of Fort Hare for the degree Master of Science in Microbiology in the Faculty of Science and Agriculture is my own work and has not been previously submitted to any other University in South Africa. I played a major role in the preparation and execution of this project, and the data analysis and interpretation are entirely by own work unless where stated. Any contributions from colleagues in the collaboration, such as diagrams or calibrations, are explicitly referenced in the text.

Signature ……. Date ……

DEDICATION

I dedicate this work to the most High God for giving me strength to push hard on this research, secondly, my family who have shown great support throughout the days of my life, their motivaton and courage to believe in myself. Most importantly, I dedicate this work to young scientists like me who might find interest in doing beyond and exploring more of what I have done.

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ABBREVIATIONS

ATBF	African Tick-bite Fever
BB	Binding Buffer
CLD	Cell lysis buffer
CWD	Column Wash Solution
DNA	Deoxynucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
gltA	Citrate-synthase
HGA	Human Granulocytic Anaplasma
IFA	Immuno-Fluorescence Antibody
IFAT	Indirect fluorescent antibody test
IHC	Immunohistohemical assay
IgG	Immunoglobulin
LB	Lyme Borrelios
LD	Lyme disease
MSF	Mediterranean Spotted Fever
NCBI	National Centre of Biotechnology Information
*omp*A	Outer-Membrane Protein A
*omp*B	OuterMembrane Protein B

PCR	Polymerase Chain Reaction
PK	Proteinase K
qPCR	Taq-Polymerase Chain Reaction
RBCs	Red Blood Cells
rRNA	Ribosomal Ribonucleic Acid
SFG	Spotted Fever Group
TG	Thyphus Group
TGR	Transitional Group Rickettsiae

ABSTRACT

Ticks and tick-borne diseases are becoming a major life threatening concern to wildlife, domesticated animals and human health. Besides causing skin damage, ticks infestations have become a growing burden in food security, economic losses and transmitting multides of pathogens. Little data and knowledge is available regarding the occurrence of etiologic agents of tick-borne diseases in the Eastern Cape of South Africa, hence the study was conducted and aimed at screening for genetic material of *Anaplasma, Rickettisia, Ehrlichia, Borrelia, Babesia* and *Theileria* species in ticks collected in Raymond Mhlaba District at Eastern Cape, South Africa.

Ticks were collected from domesticated animals in Raymond Mhlaba Municipality, and were morphologically identified and processed for DNA extraction. Ticks were chopped into bits and DNA was extracted from the samples with commercial DNA extraction kit. The extracted DNA samples was used to molecularly identify the tick as well as assess the presence of tick-borne pathogens belonging to *Rickettsia, Babesia, Borrelia, Anaplasma* and *Erhlichia*, and *Theileria* spp. by PCR using specific primer pairs published in literature. Positive amplicons were sequenced in a commercial sequencing facility. The obtained chromatograms were edited with Geneious bioinformatics software and were subjected to BLASTn and phylogenetic analyses using MEGA7 version for evolutionary relationships with curated reference sequences in GenBank.

Nine hundred and sixty two tick samples were collected from domestic animals. Collected tick samples belonged to three genera, which were the *Amblyomma, Rhipicephalus* and *Haemophalis* in decreasing order of their abundance. Screening of tick DNA samples by PCR did not show presence of *Babesia, Borrelia, Anaplasma* and *Erhlichia*.

Positive PCR products were observed for *Rickettsia* and *Theileria* spp.. The positive amplicons were purified, sequenced and analysed for speciation of *Theileria* and *Rickettsia*. The presence of *Rickettsia* was detected in 60/994 (6%) from the three genera of ticks. Phylogenetic analyses shows that the sequences obtained are phylogenetically related to members of Spotted fever group *Rickettsiae*. Genetic material of *Theileria* spp. was detected from 10/994 ticks with an overall infection of 1% obtained in *Rhipicephalus* genera. Analyses shows that the sequences obtained are phylogenetically related to *T. orientalis* complex.

The finding from this study therefore expands the knowledge on recent emergence of *Theileria* and *Rickettsia* spp. in Raymond Mhlaba Municipality in Eastern Cape, South Africa.

CHAPTER ONE

1.1 Introduction

An emerging public health concern known as tick-borne zoonotic diseases is becoming a major problem throughout the world, with rapid mortality in wildlife population and domestic animals (Vesco *et* al., 2011; Titcomb *et al*., 2017). The conceivable reason for this observation is the rapid distribution of ticks, which are one of the agents of zoonotic diseases rating the second to mosquitoes. Ticks are known as external obligate parasites capable of transmitting a huge amount of pathogens comprising, bacterial, protozoan and viral agents of zoonotic diseases. They create a growing burden in well-being of human and animal, while surviving and completing their life cycles by sucking blood of vertebrates such as mammals, birds, and amphibians (Paddock, 2011, Estrada-Peña, 2015, Mitchell *et al*., 2016).

The world tick fauna comprises of nine-hundred tick species detected in diverse arrays of hosts and are proficient in infesting both humans and animals worldwide (Lopes *et al.,* 2016). However, in Africa, there are about fifty endemic tick species found to be remarkably diverse and are responsible for economic losses by affecting animal health and efficiency with the highest impact on livestock. A large body of theoretical and empirical studies on animal and human vector-borne infections have played a role in understanding of the significance of tick vectors, their evolution and ecology in the transmission of disease pathogens with the sole aim of designing efficient control strategies (Vesco *et al*., 2011; Anna *et al*., 2012; Reye *et al*., 2012; Rascalou *et al*., 2012).

Apart from damaging skin, a single tick bite can transmit multiple pathogens, thus leading to a typical presentation of tick-borne diseases including lyme diseases, African tick-bite fever and Human Granulocytic fever disease, as reported globally (Tahir *et al*., 2016). Routine application of tools such as PCR to detect the fragments of DNA in ticks for identification and

quantification of disease risks for humans, has led to a remarkably increase in the number of reports on the ecology and epidemiology of tick-borne infection in humans and animals (Rojas, 2011).

In most countries, zoonoses are rarely reported hence making it difficult to evaluate their levels of involvements in human infections (Cripps, 2000). The consequence of unreported zoonotic diseases has lead to underestimation of their global burden. Therefore, the initial attempt of identification of ticks made it possible to detect and understand the basic regulation and prevention of tick-borne diseases (Lv *et al*., 2014). The use of molecular approach has brought forward crucial information on the detection of pathogens and rapid diagnostic assays that results in rapid treatment of tick-borne diseases (Gariepy *et al*., 2012).

Additionally, zoonoses are a major concern in food security as they limit animal and agricultural exports thus causing a high socio-economic impact (Rojas, 2011). They also contribute to human illnesses, more especially to people with low immune system due to diseases like HIV/ Aids (Cripps, 2000). Moreover, in local communities, livestock and farming serve as a source of income and survival in poor households, yet the impact of the zoonotic diseases lead to drastic losses in terms of economic wellbeing on the community (Epstein and Price, 2009).

Despite the broad studies conducted in South Africa on ticks and tick-borne infections, there is insufficient information concerning the prevalence of tick-borne pathogens in the Raymond Mhlaba local Municipality at Eastern Cape Province. The present study was therefore carried out to fill in the research gap and document the incidence of tick-borne bacterial and protozoan pathogens in this region.

1.2 Problem statement

Tick-borne diseases (TBDs) have arisen as a threat in social and economic life, thus hindering the production of livestock and increasing human diseases on a global portion. The scientific evidence for rapid climate change is convincing and certainly resulting in global distribution of tick species.The emergence of these arthropods in new geographic regions have radically increased the epidemics of tick-borne pathogens (de la Fuente *et al.,* 2016).

South Africa is a known agro-exporting country that is mostly reliant on livestock production for survival according to the Department of Agriculture, Forestry and Fisheries in the year 2016. This country also boasts of many game reserves, which could be regarded as safe havens for ticks, with tourists and locals more probable to fall as targets of tick bites (Fujisawa *et al*., 2012; Snijders, 2012). In developing countries where there are limited resources, tick infestation and diseases are more severe (De Castro, 1997).

However, Eastern Cape of South Africa is one of the places where tick infestation poses a major challenge especially for small-scale farmers (Masika *et al*., 1997). Yet, there are limited reports and studies on the surveillance of tick-borne bacterial and protozoan pathogens in the Province of Eastern Cape in South Africa. This Province is agrarian and has excessive number of domestic animals that are kept in close vicinity to homes, and this could lead to spontaneous spread of diseases from one animal to another and ultimately to humans, hence this study was conducted.

Therefore, this study is significant, as it will ascertain the distribution of the tick-borne bacterial and protozoan pathogens in ticks collected from various livestock in selected communities in Raymond Mhlaba District Municipality, Eastern Cape, South Africa.

1.3 Hypothesis

This study was conducted under the null hypothesis that ticks collected in Raymond Mhlaba District in Eastern Cape, South Africa do not harbour tick-borne pathogens

1.4 Aim

This study aimed at screening for the genetic materials of *Anaplasma*, *Rickettisia*, *Ehrlichia Borrelia, Theileria* and *Babesia* species in ticks collected in Raymond Mhlaba District at Eastern Cape South-Africa.

1.5 Objectives

1. To collect ticks from domestic animals in Raymond Mhlaba District of the Eastern Cape, South Africa, identify them morphologically and molecularly in order to determine the tick types in the study area.

2. To determine the prevalence of tick-borne pathogens causing anaplasmosis, ehrlichiosis, babesiasosis, borreliosis, rickettisiosis in ticks colleted in the study area and to genetically characterize these pathogens.

3. To determine the phylogeny of the detected pathogens using bioinformatics tools.

4. To conduct research uptake within the communities where samples will be collected in order to intimate them with the findings of the study and the implications thereof.

CHAPTER TWO

2.1 Literature Review

Ticks are recognized as small arthropods, blood feeders found almost in every corner of the world (Narasimhan and Fikrig, 2015). Besides causing direct damage to the host, the key concern of ticks lies on transmitting pathogens to humans and animals, that is accomplished by biting mammals, birds, reptiles and amphibians during feeding. This form of survival applies to both sexes and developmental stages of all ticks (Mediannikov and Fenollar, 2014; Narasimhan and Fikrig, 2015).

2.1.1Taxonomy of ticks

Taxonomically, ticks have been classified into the phylum Arthropoda of animal kingdom as part of insects, in subphylum Chelicerata in class Arachnida (Table 1). To date, there are about 900 identified tick species of veterinary and medical importance, globally (Lopes *et al*., 2016). Most of which belong to one of the three main families, the *Ixodidae* (hard ticks), and *Argasidae* (soft ticks) and the *Nuttalliellidae* respectively (Figure 1.1). The two main families of ticks (hard ticks and soft ticks) have numerous morphological and physiological features that clearly delineate them from each other (Sonenshine, 1991; Estrada-Pena, 2015). A third family, *Nuttalliellidae* exhibits features that are related with both hard and soft ticks, it is found only in Southern Africa, and Tanzania and classification in regards to its relationship to the other tick families based on the origin, morphology and biology are consequently quite problematic (Latif *et al.*, 2012).

Table 1. The classification of ticks (e.g *Rhipicephilus*)

Kingdom	Animalia
Classification	e.g. *Rhipicephilus microplus*
Phylum	Arthropoda
Class	Arachnida
Sub-class	Acaria
Order	Anactinotrichidea
Sub-order	Ixodida
Family	Ixodidae
Genus	*Rhipicephilus*
Species	*Rhipicephilus microplus*

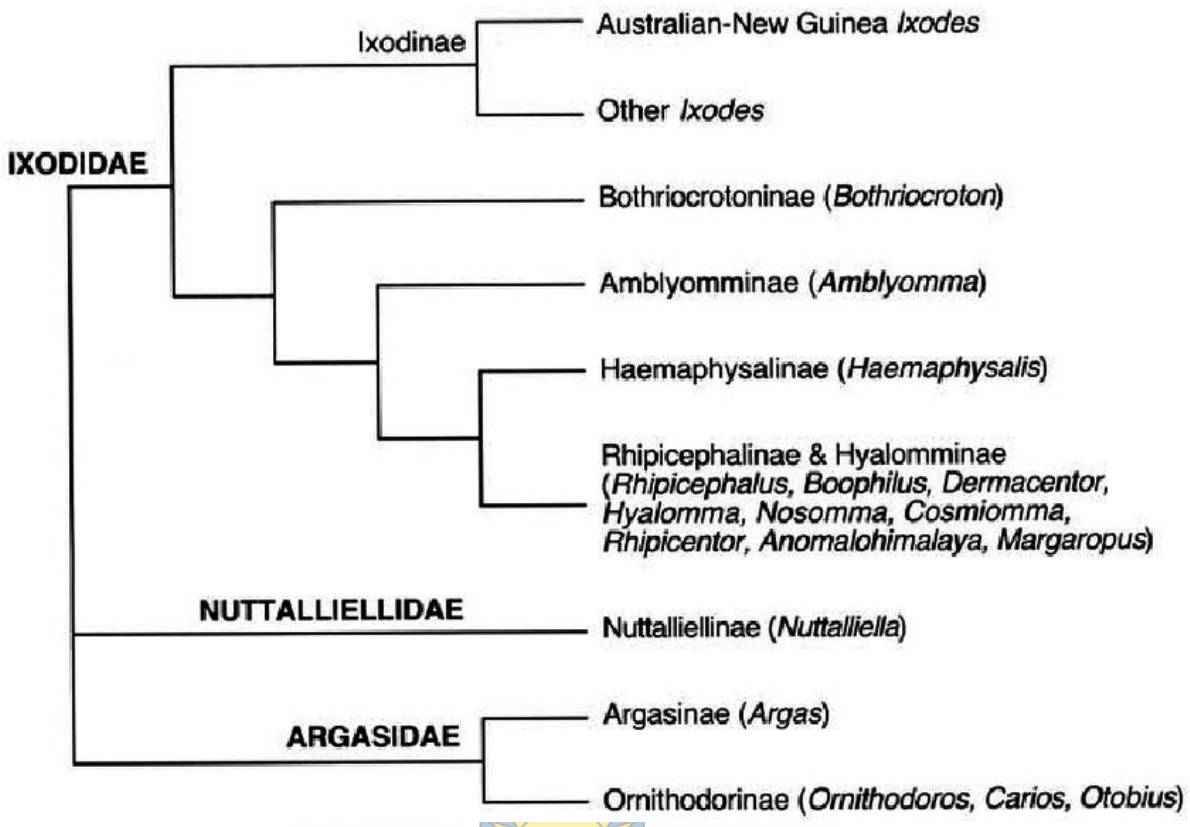

Figure 1. 1 Classification of the three families of ticks (Parola and Raoult, 2001).

2.1.2 Morphology

2.1.2.1 Ixodidae (hard ticks)

Hard ticks takes up a largest part of tick fauna, with about 702 identified species (Guglielmone *et al*., 2010). A major morphological difference distinguishing the families of ticks is the presence of scutum in hards ticks which covers completely the dorsal body, although it is limited in nymphs and larvae stages. The presencne of scutum plays a crucial role in body expansion during feeding (Figure 1.3). The size in hard ticks is relatively large (2-20 mm) (Brites-Neto *et al*., 2015) with legs varying between the three stages of hard ticks (Figure 1.2) (Parola and Raoult, 2001). The capitulum functions as re-curved teeth that assist in anchoring the tick to the skin of the host during feeding (Figure 1.3) (Estrada-Peña, 2015).

These ectoparasites possess a number of peripheral organs for sensing, using sensory organs such as hair-like structures on their body surfaces,mouthparts and legs (Figure 1.2) and sensory complex which has olfactory in clusters and gustatory receptors. However, these organs have been proven to be crucial for sense of communication with other ticks and contribute in finding its host (Parola and Raoult, 2001).

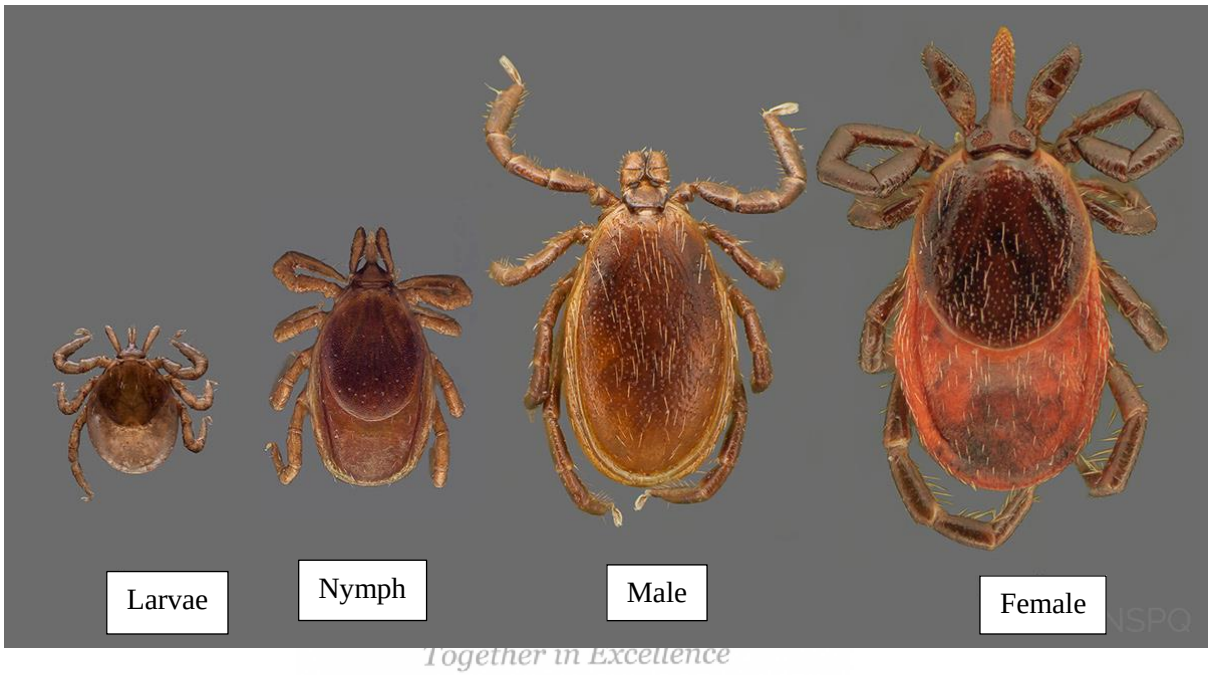

Figure 1. 2. The basic four stages of an *Ixodes scapularis*. From Left to right: larval, nymphal, adult female and male Deer tick (www.inspq.qc.ca).

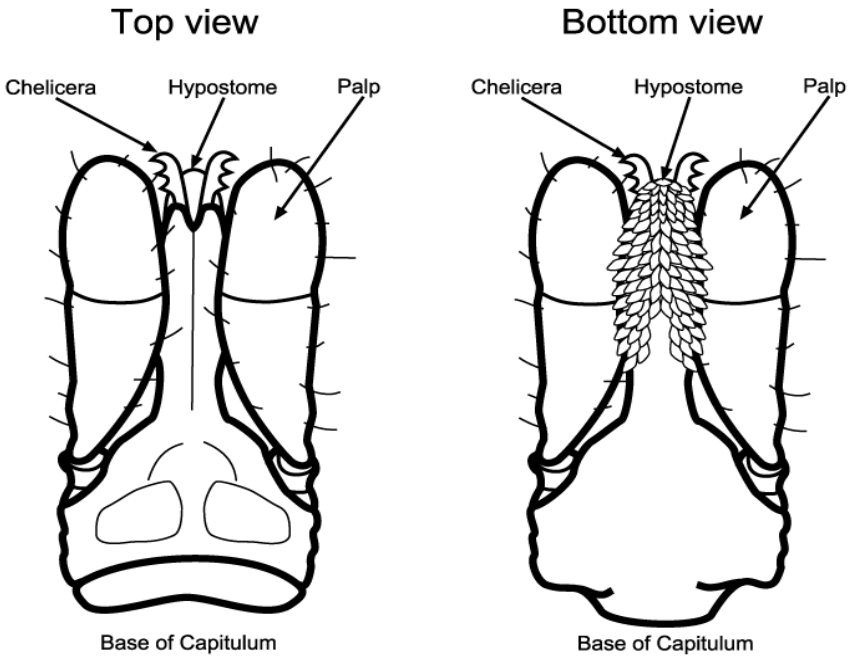

Figure 1. 3. The illustration of the mouthparts (ventral and dorsal aspect) of female *Ixodes ricinus* (illustrated by Charlesworth, 2008).

Figure 1.4 Demonstration of an engorged and an unfed female *Ixode scapularis* (www.inspq.qc.ca).

2.1.2.2 Argasidae (Soft Ticks)

The *Argasidae* (soft ticks) have insignificant differences when compared to the ixodid (Parola and Raoult, 2001) as it consist of a pear-shaped outline with an anterior body region that is broadly round (Figure 2.2). Although the external surface of soft ticks is able to expand, it is not designed to handle the large volumes of blood ingested during blood meals, as the final expansion may vary from 5-10 times more than the tick's unfed body weight. Argasids are very resistant to starvation and can even survive for several years without feeding (Díaz-Martín *et al.*, 2015; Manzano-Román *et al*., 2015).

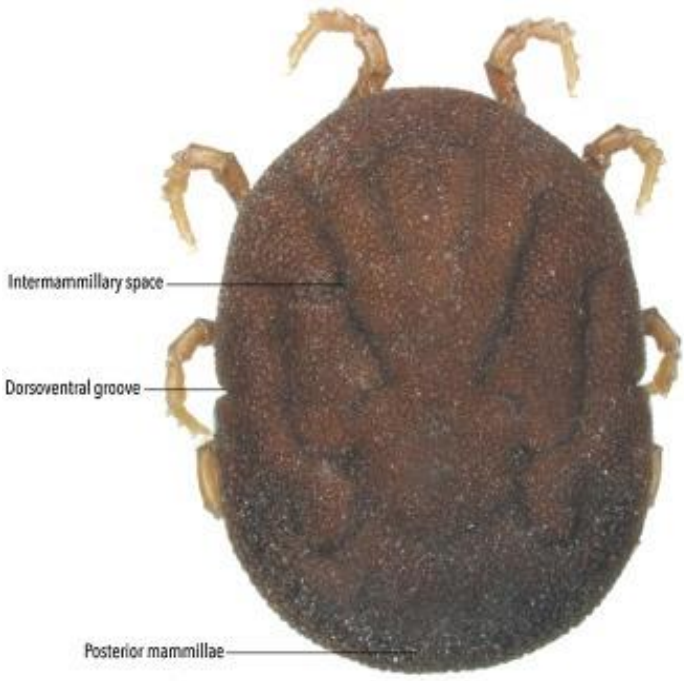

Figure 2. 1 The dorsal side of an *Ornithodoros* tick (Bakkes *et al.,* 2018).

Soft ticks tend to lack the scutum and possess a leathery cuticle. Most species of *Argasidae* have a dorsal plate located in the centre and is covered by tiny mammillae, ridge structures that protrude above the cuticle surface. Sub-circular ridges represent sites where muscles are attached and occur in distinctive patterns on top of the dorsal and ventral surfaces. While

feeding on their hosts, argasid ticks can expand their body weight 3-5 times (Figure 2.1). This is due to high folded integument that makes it possible for the ticks to extend and stretch without additional growth (Burger *et al*., 2014). In larvae stage, an oval dorsal shield is usually seen, however this organ is not the ixodid scutum. Eyes are seen on the lateral surface of the body when present in that specific species of soft ticks (Figure 2.3) (Farkas *et al*., 2012).

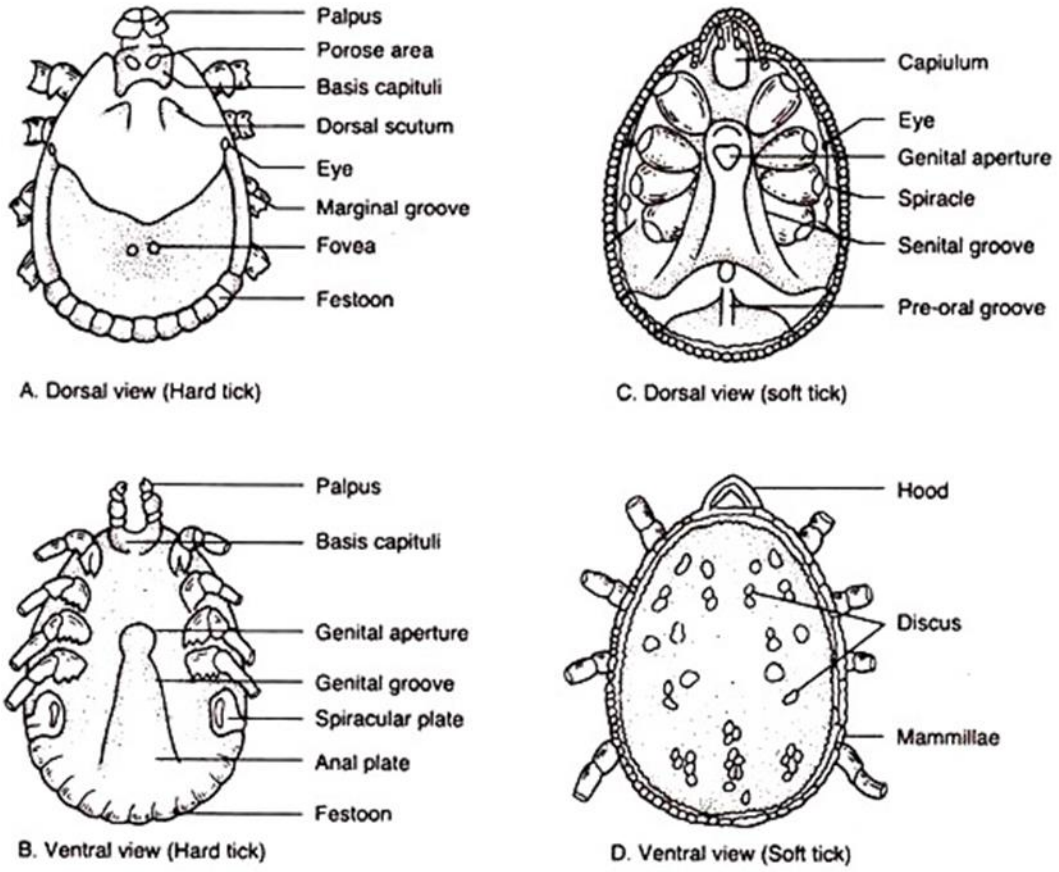

Figure 2. 2 The general morphology of ticks of Soft tick and hard tick. The views of Dorsal and ventral sides of hard and soft ticks. A and B are side of ixodid (hard tick) (*Dermacentor*), C and D are sides of Argasid (soft tick) (*Ornithodoros*) respectively.

2.1.3 Life cycle

All ticks experience four phases during their developmental cycles: the first stage involves the egg, followed by three active phases, the larva, nymphal and the adult phase. It is only during the adult stage where sexual dimorphism is evident. Soft ticks undergo several nymphal stages before reaching the adult form, while in hard ticks the development takes place at an accelerated rate, with only a single nymphal stage (Estrada-Peña *et al.*, 2012; Apanaskevich and Oliver, 2014). During life cycle, a tick searches for a host and attaches with each stage, where it feeds over a period of time, depending on whether the tick possesses a one host life cycle, two-host or three host life cycle (Pfäffle *et al*., 2013).

2.1.3.1 one-host life cycle

Larvae hatches from eggs laid by female adult. The developed larva finds and climbs on a suitable host, attaches, and feeds until engorged. Feeding and moulting proceeds until the adult stage is reached sequentially on the same host.The females feed until fully engorged, after which they detach from the host and fall off to the ground, where they lay a single large batch of eggs in a secure environment and die (Figure 2.4). The demographic structure of a parasitic population of one-host ticks has been estimated to be eight larvae, four nymphs, two males and one female (Apanaskevich and Oliver, 2014; Madder *et al.,* 2014). One-host life cycle is characterised by *Rhipicephalus* spp. (Apanaskevich and Oliver, 2014; Madder *et al.,* 2014).

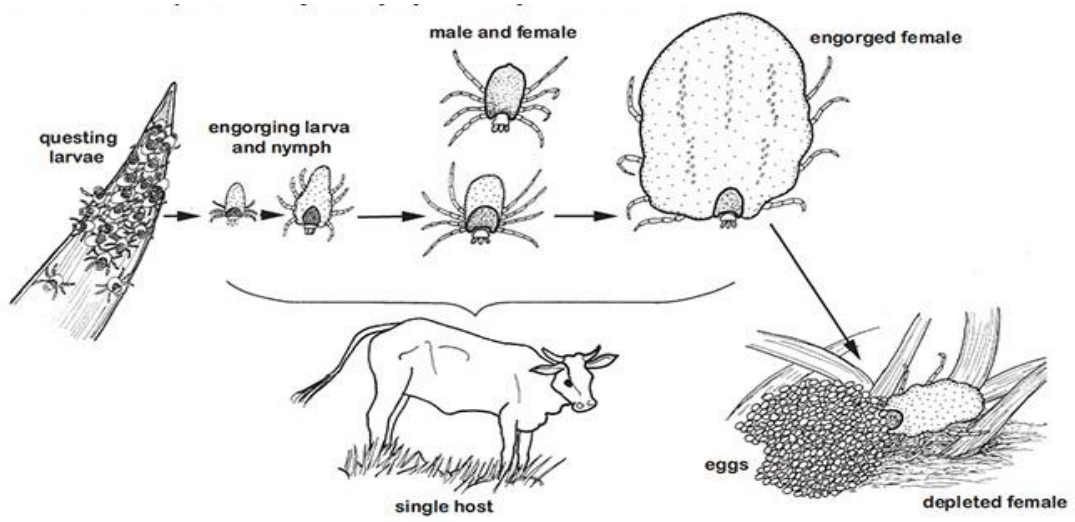

Figure 2. 3 One-host life cycle-the example is *Rhipicephalus decoloratus* (Walker *et al.*, 2003).

2.1.3.2 Two-host life cycle

The larvae hatch from eggs laid by adult female. Developing larvae find and climb on its first host, where it attaches and feeds. It then moult on the host to nymphs as it attaches to the same host, feeds until engorged. The nymph detaches from the first host, and drops to the ground as it develops into another stage (Figure 2.5). The adult tick climbs on to the next host, where it attaches, partially engorge and mate. The fully engorged female detach from the second host, drops to the ground, and lay a single large batch of eggs in a secure environment and dies subsequently. *Hyalomma* and *Rhipicephalus* spp. are characterised with two-host life cycle (Sonenshine and Roe, 2013).

Transmission of pathogens in ticks with two-host life cycles can either be transovarial or transstadial transmission (Mancin *et al*., 2014). Additionally, the one and two-host ticks have an advantage over the three host ticks, in terms of protection. Thus, the undeveloped stages are secured from hostile climatic conditions and reduces mortality rates (Baneth, 2014).

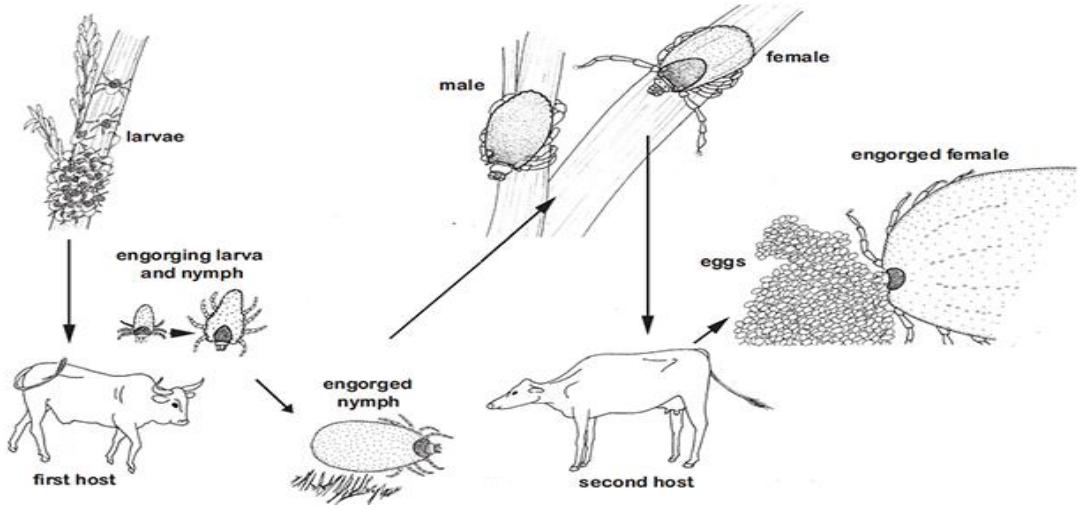

Figure 2. 4 A two-host life cycle with an example of *Rhipicephalus bursa* tick (Walker *et al.*, 2003).

2.1.3.3 Three-host life cycle

After oviposition by adult ticks, the eggs begin to hatch after a few weeks or a month reliant on the temperature of the environment they live in. The larvae are then distributed into near vegetation to find hosts. Developed larvae fnds and attaches to a host by chance and feeds for several days. With enough feeding, the larvae falls from the host and find a sheltered location. Moulting then begins after several days, and nymphs appear (Apanaskevich, 2014).

The nymphs seek for available host; once they find it they attach, feed and fall off from the host as engorged nymphs (Figure2.6). The nymphs then search and find a suitable place where they moult into adults. Male and female ticks begin questing for hosts and attach, feed and mate. Once engorged to repletion, female ticks drop off from the host and looks for an appropriate shelter to oviposit and then later die. The life cycle then start all over again. Transmission of pathogens on this cycle can be transovarial, transstadial and intrastadial through *Rhipicephalus appendiculatus* (Ememe *et al*., 2018).

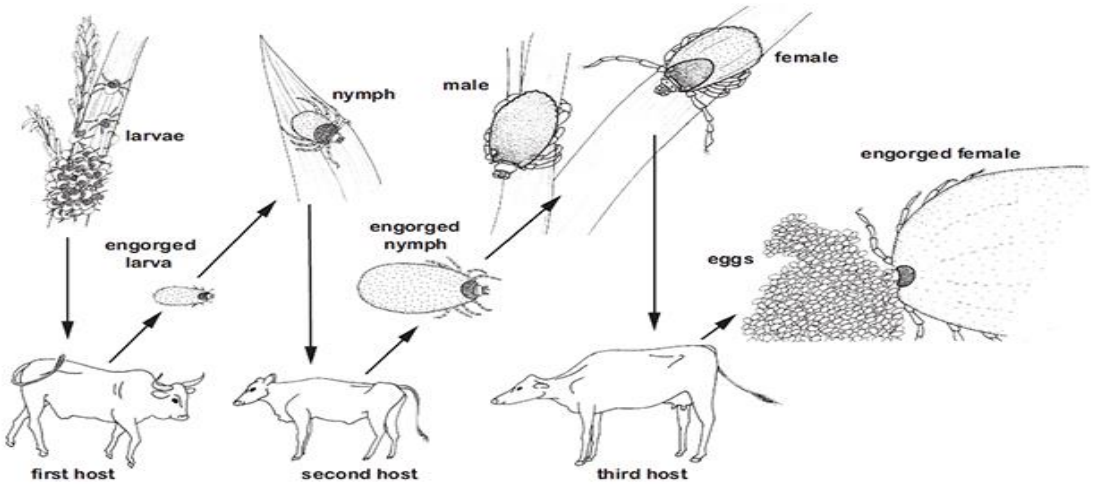

Figure 2. 5 A three-host life cycle with an example of *Rhipicephalus appendiculatus* (Walker *et al*., 2003)

2.1.4 Distribution

Anthropogenic land-use had been reported to play a significant role in facilitating tick outbreaks through a variety of mechanisms, including wildlife host population and community (Titcomb *et al*., 2017). Ticks just like any other arthropods are likely to respond to different weather conditions such as hot and mild temperature variables. The predicted trends on weather therefore subsequently determine the variety of ticks and their regional distribution (Estrada-Peña, 2015).

Human actions can result to changes in the landscape, modification of habitats in which vectors, hosts and pathogens coexistence have huge effects on the epidemiology of tick-transmitted pathogens than climate change (Estrada-Peña 2015). This has lead to the diversity of tick species identified in South Africa as Walker *et al*., (2014) recorded, *Haemaphysalis laechi* (*elliptica*), *Ixodes rubicandus, H. truncatum, R.* (*Boophilus*) *macroplus,Otobius megnini, Margaropus winthemi, Rhipicephalus* (*Boophilus*) *decoloratus, Ornithodorus moubata* complex, *R. evertsi evertsi* and *R. simus*. Meanwhile Iweriebor *et al*., (2017)

identified *Amblyomma herbraeum, Hyalomma marginatum rufipe, R. appendiculatus,* and *Rhipicephalus sanguineus* species in the Eastern Cape Province of South Africa.

2.1.5 Control strategies

Complete eradication of ticks has not yet been successful; therefore, possible approaches are taken to limit the distribution of ticks that include the use of acaricides, biological controls, genetic manipulation and regulatory systems. Acaricides can be externally utilized on animal hosts to eliminate attached ticks and disrupt their feeding. However, regardless of such approaches, additional researches have emphasised on the importance of considering epidemiology of certain tick species in relevance to ecological habitat to develop effective strategies to control tick abundance (Jurisic *et al*., 2010).

2.2 Tick-borne pathogens

2.2.1 *Anaplasma* species

Anaplasma spp. are obligate intracellular Gram-negative bacteria located in the blood cells of infected mammals. This genus is capable of causing infection called anaplasmosis in both humans and animals. Dumler and Walker, (1994) reported the first human case of human granulotic *anaplasmosis* on patient who died after two weeks of being bitten by a tick.

However, this pathogen is associated with diseases in vertebrates and can be carried in animal as reservoirs (Rymaszewska and Grenda, 2008). Species of *Anaplasma* including *A. centrale, A. phagocytophilum, A. platys, A. bovis, A. ovis, A. marginale* etc and are of medical and veterinary importance thus infecting and causing diseases in humans and animals, specifically livestock (Table 2) (Liu *et al*., 2011, Berthelsson, 2017).

An *Anaplasma* infection related to human is identified as human granulocytic anaplasmosis, with remarkable cases found between May and October when tick vectors are most active,

particularly the nymphs, which are hard to detect because of their small size (Angelakis, 2010). The biological and ecological distinct sub-populations of *A. phagocytophilum* are adapted to particular reservoir hosts and tick species, which also exhibit varying pathogenicity (Angelakis, 2010).

2.2.2 Classification

Species of *Anaplasma* are found to be closely related to the genus *Ehrlichia,* with both genera belonging to *Anaplasmataceae* family, in *Rickettsiales* order (Dumler *et al*., 2001). Historically, taxonomic reorganization and reclassification of the genus using genomic studies of 16S rRNA gene has provided a vital role to the systematics of the *Anaplasma* spp. as it was incorrectly classified from viruses to protozoa (Hove *et al*., 2018).

2.2.3 Epidemiology

A. phagocytophilum bacterium is known as the main causative agent of disease in domesticated animals (Europe) (Foggie, 1951) for years. Lately, this infection has also been discovered with quite a number of species of mammals including humans, in different countries with Ixodes ticks as vectors (Table 2) (Stuen *et al*., 2013).

The most reported species of *Anaplasma* identified to infect cattle, dogs and African buffalo in Southern Africa include *A. centrale* and *A. marginale* (Figure 2.7) (Hove *et al*., 2018). *A. centrale* infection normally result in insignificant disease while *A. marginale* generally cause clinical bovine anaplasmosis in South America, South Africa, Australia and Israel (Debeila *et al*., 2012; Bell-Sakyi *et al*., 2015; Hove *et al*., 2018).

Recently, Byaruhanga *et al*., (2018) confirmed the prevalence of *A. marginale* and *A. centrale* in prom cattle in Uganda. Moreover, *A. marginale* has been recovered from ten wild ruminants including baffaloes in South African Provinces (Debeila *et al*., 2012).

Table 2 Species of genus *Anaplasma* with probable diseases and common vectors globally (Rar and Golovljova, 2011).

Species	**Common name of disease(s)**	**Common natural host(s)**	**Primary vector(s)**	**Distribution**
A. platys	Canine cyclic thrombocytopenia	Dogs	*R. sanguineus* *Dermacentor* spp.	Australia, (Mediterranean), Southern Europe , Southern USA, South America, Asia,
A. bovis	Bovine ehrlichiosis	Cattle	*Rhipicephalus* spp., *Hyalomma* spp., *Ixodes* spp., *Haemaphysalis* spp.,	South America, Africa and Asia,
A. phagocytophilum	Canine anaplasmosis, Tick-borne fever, Human Granulytic Anaplasma and Equine ehrlichiosis	Cattle, Dogs, Sheep, goats, , wild ruminants and Humans	*Ixodes scapularis, I. ricinus* , *I.pacificus, I. persulcatus D. silvarum,* *I. trianguliceps, I. hexagonus, H. longicornis*	Africa , Northern hemisphere (America and America Europe), and Asia

Figure 2. 6 Epidemiology of cases reported on bovine Anaplasmosis in South Africa (Hove *et al*., 2018).

2.2.5 Life cycle and transmission

The life cycles of *Anaplasma* species include the reproductive stages that take place in both ixodid and vertebrate hosts. *Anaplasma* spp. are capable of causing a persistent infection in host vertebrates. Therefore, *Anaplasma* spp. is transmitted transstadially rather than transovarially by ixodid ticks. The process of sucking blood from previously infected animals allows uninfected ticks to become infected thus transmitting the infectious agent to next animals (Rar and Golovljova, 2011).

2.2.6 Vectors and reservoirs of *Anaplasma* spp.

Reports have investigated about 20 different species of ticks that are incriminated as vectors of *Anaplasma* globally (Hamou *et al*., 2012). Waal, (2000) reported five most significant ticks species which have been known to transmit *A. marginale* in South Africa which include *H. marginatum*, *R. evertsi evertsi*, *R. microplus*, *R. simus* and *R. decoloratus*. Reports in Botswana

have shown that tick vectors such as *Amblyomma* and *Rhipicephalus* species collected on goats are infested with *Anaplasma* spp. (Mushi *et al*., 1996, Berthelsson, 2017).

However, *I. ricinus* tick in Central and Northern Europe is the main vector for *A. phagocytophilum*, while *I. pacificus* and *I. scapularis* are the leading transmitting vectors in North America. *R. sanguineus* and *Dermacentor* spp. are the vectors responsible for transmitting *A. platys* (CDC, 2016). In Europe, the most dominant vector is the *I. ricinus*, while *I. scapularis* and the *I. pacificus* are recognized vectors transmitting the pathogens in North America (Dantas-Torres *et al*., 2012).

Although cattle are identified reservoir hosts of *Anaplasma* spp., recent reports have confirmed the diverse array of reservoirs, as *Anaplasma* infection have been detected on buffaloes and wild animals (Battilani *et al.,* 2017). Chisu *et al*., (2018) further elucidated that *Anaplasma* spp. also infect domestic and wild animals. Therefore, Anaplasma infections depend on competent and available suitable host for transmission to take place effeciently. With the exception of dogs, *A. phagocytophilum* can be found in an extensive variety of warm-blooded animals, including cattle, cats, goats, sheep, horses, wild animals and people (CDC, 2016).

2.2.7 Clinical characteristics

The possible clinical characteristics of *A. phagocytophilum* disease after a ten to fourteen days of incubation period, include anorexia, fever (>39°C), lethargy, rarely lymphadenomegaly or hepatomegaly and splenomegaly development. In certain animals, they tend to be reluctance to move, weakness and lameness occur and with some signs of respiratory difficulties (CDC, 2016). Severe diseases in livestock are characterized by low milk production, weight loss, fever, abortion and can lead to mortality (Atif *et al.*, 2013; Mutshembele *et al*., 2014).

2.2.8 Diagnosis

Laboratory diagnosis include the use of PCR for molecular diagnosis and using EDTA or citrate-anticoagulated for confirmation of anaplasmosis (Dumler *et al.*, 2007). Standard serological tests procedures are essential as indirect immunofluorescence antibody (IFA) analyses for immunoglobulin G (IgG) with the use of *A. phagocytophilum* antigen. Culture isolation and immunohistochemical (IHC) assays, could also be employed in diagnosing infections with the pathogen. However, blood smear evaluation is more beneficial for HGA diagnosis.

2.2.9 Treatment of *Anaplasma* infection

Testing of antimicrobial susceptibility of *A. phagocytophilum* shows levofloxacin, doxycycline and rifampin to be the best effective treatment. For children under the age of one, chloramphenicol can be used to avoid yellowing of teeth. During the first week of the disease, a microscopic analysis of blood smears may expose morulae in the cytoplasm of white blood cells (CDC, 2016). Anaplasmosis can spread rapidly and could be fatal, and if treatment is delayed, so treatment should be given at its early stages and doxycycline treatment must be used immediately an observed diagnosis has been performed (CDC, 2016).

2.3 Ehrlichia spp.

2.3.1 Bacteriology

Genus *Ehrlichia* is a small group of Gram-negative obligate intracellular bacteria which can primarily invade and attack bone marrow cells (Figure 2.8), particularly leukocytes within the body of humans. The first ehrlichial disease was initially recognized in South Africa during the nineteenth century, while its tick-borne nature was confirmed in 1900 (Parola and Raoult, 2001).

Ehrlichia species undergo cell division through binary fission and develop from elementary to initial bodies and eventually forming colonies that are bound by vacuole. Once the morulae is formed, it signifies a defining trait of this group of bacteria (Parola and Raoult, 2001). The evolutionary changes in the outer membrane proteins have led to the occurrence of new strains of *Erhlicia*, which infect a larger diversity of hosts. (Frutos *et al*., 2007).

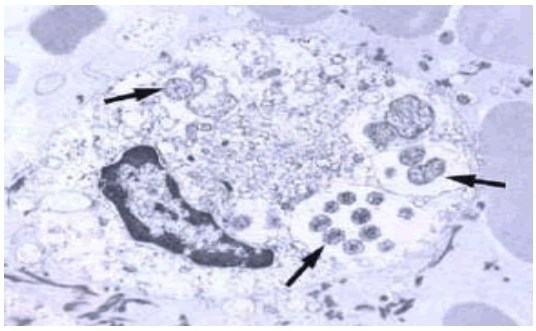

Figure 2. 7 This is an electron photo micrograph which shows morulae in a bone marrow leukocyte of a patient that is diagnosed with ehrlichiosis. The arrows show individual Ehrlichiae.

2.3.2 Classification of *Ehrlichia* species.

Ehrlichia spp are in the family *Anaplasmataceae*, under the order *Rickettsiales* and comprises of four genera of medical significance: *Neorickettsia, Aegyptianella, Anaplasma* and *Ehrlichia.* The genus *Ehrlichia* has been reclassified by genetic analysis of 16S rRNA genes. Currently, *Ehrlichia* consist of the following species *E. muris, E. chaffeensis, E. canis, E. (Cowdria) ruminantium* and *E. ewingii* (Table 3). Members of this genus are linked with pathogens of ruminants, horses, cats and dogs (Dumler *et al.*, 2015).

2.3.3 Epidemiology

The distribution of *Erhlichia* around the world reflects the tick abundance and relative mammalian reservoirs present (Figure 2.9). However, species of *Erhlichia* such as *E. chaffeensis* are predominatly identified to cause human erhlichiosis with about 1,518 cases

reported in 2013 particularly in South Eastern, South-central and mid-Antlantic states in America. Other species such *as E. ewingii* are mainly prevalent in white-tailed deer and dogs in the United States, with the first human cases identified in 1999 in Missouri in four patients, with of three patients who were immunosuppressed (Abuhammour, 2018).

Furthermore, human monocytic ehrlichiosis (HME) initiated by *E. chaffeensis* is considered as regularly identified tick-borne infection in the southern US, as the number of HME cases reported annually has gradually increased from two hundred patients in the year 2000 to more than nine-hundred patients in 2009 (Christou, 2011). Illnesses caused by *E. ewingii* are reported in both dogs and humans in the Southeastern and South-Central United States (Beall *et al*., 2012).

Similarly, in South Africa in the Eastern Cape Province, *Erhlichia* spp. Including *E. canis, E. chaffeensis*, and *E. muris* were detected in ticks collected from sheep, goats and cattle (Iweriebor *et al*., 2017) while *E. canis* has been reported in dogs and cats in other provinces of South Africa, including KwaZulu-Natal, Mpumalanga and Free-state Province (Mtshali, 2017).

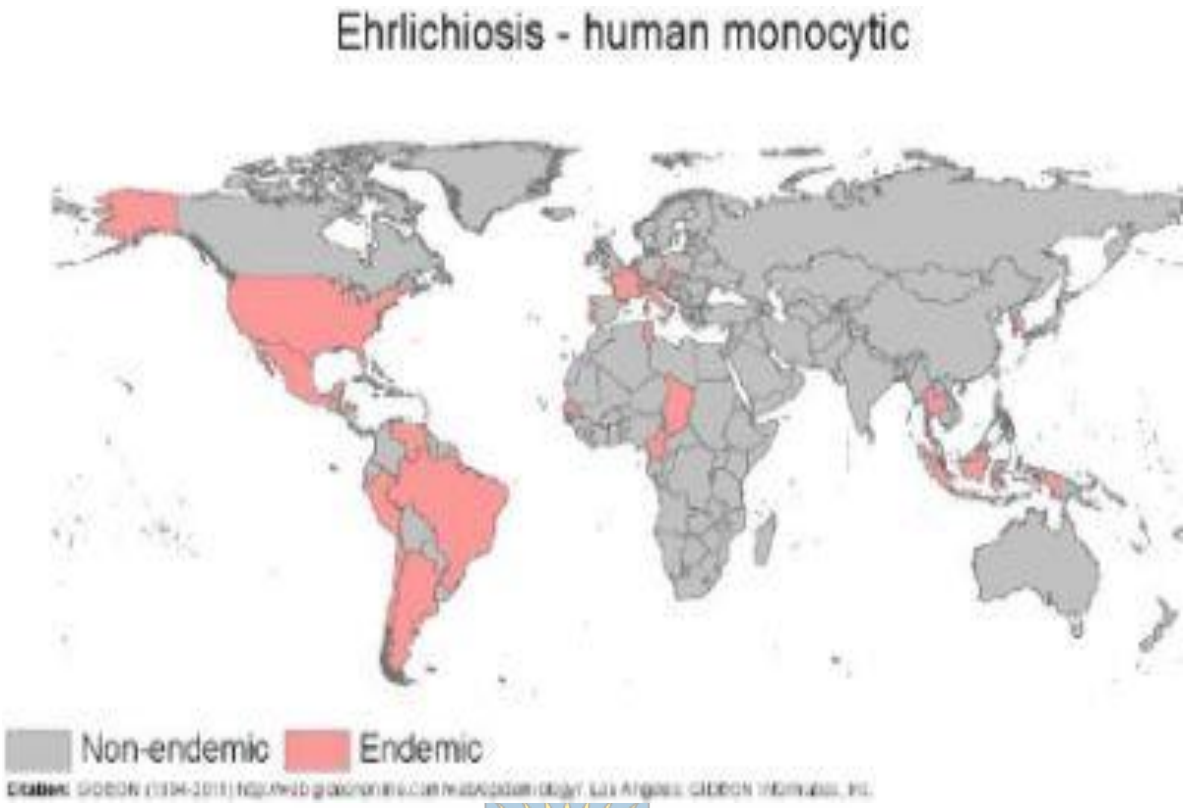

Figure 2. 8 The distribution of ehrlichiosis disease around the world (Hay *et al*., 2013).

Table 3 Distribution of *Ehrlichia* spp. with common diseases around the world (Nair *et al*., 2014).

Species	Common name of disease(s)	Common natural host(s)	Cells most commonly infected	Primary vector(s)	Distribution
E. chaffeensis	Human monocytic ehrlichiosis	Humans, dogs, deer horses and rodents	Monocytes, macrophages	*A. americanum*, *Derma centor variabilis*	South and Central America, Africa USA, Europe

E. ewingii	Human Granulocytic Ehrlichiosis Canine Granulocytic Ehrlichiosis,	Humans and dogs	Primarily neutrophils and eosinophils	*A. americanum, Otobius megnini*	United States of America
Ehrlichia canis	Canine monocytic ehrlichiosis (CME)	Humans, Dogs, wolves, jackals	Primarily mononuclear cells	*Dermacentor variabilis*	Worldwide, primarily tropical and temperate climates
E. muris	Not linked with any disease	Rodents, humans	Mononuclear cells	*Haemaphy- salis* spp.	Japan
E. ruminantium	Heartwater disease	Ruminants	Endothelial cells	*Amblyomma*spp.	Africa, Caribbean

2.3.4 Vectors of *Ehrlichia* spp.

Ehrlichia spp. are transmitted by tick vectors by replicating within blood cells of ticks while infecting animals or humans through a tick bite (Socolovschi *et al*., 2012). The dominant zoonotic cycle of *E. chaffeensis* and *E. ewingii* comprises of many persistently infected white-tailed deer *A. americanum* ticks, normally found widely distributed across United States, and are identified as the most common reservoir of human erhlichiosis agents (Table 3) (Harris *et al.*,2016). Other tick vectors include *I. ricinus, I. pacificus, A. testudinarium, Haemophysalis yeni* and *D. variabilis* may also have a limited contribution in human transmission (Harris *et al.*, 2016).

2.3.5 Clinical characteristics

Human monocytic ehrlichiosis caused distinct bacteria that circulate in different host cells with human ehrlichioses presents predominantly as undifferentiated illnesses. Paradoxically, human ehrlichiosis include clinical symptoms such as headache, myalgia, anorexia, leukopenia and malaise (Parola and Didier Raoult, 2001).

Rarely, rashes can be observed and hospitalization is required during the infection, which often lasts 13 weeks. Particularly, a patient with underlying immunosuppression may have a severe ehrlichioses, which may even be fatal. Frequent laboratory abnormalities including thrombocytopenia, leukopenia and elevated liver enzymes may occur (Parola and Didier Raoult, 2001).

2.3.6 Diagnosis of *Ehrlichia*

PCR and IFA assays are most effective tools for diagnosis of infections caused by *Erhlichia*. Ehrlichiosis diagnosis uses observation of morulae in leukocytes after staining with Wright's or Giemsa staining of blood smears. Serological testing on paired blood samples by isolation of organisms in cell culture systems are crucial (Dumler *et al.,* 2007). Doxycycline treatment is commenced immediately an empirical clinical diagnosis is being determined since human ehrlichiosis is fatal and relatively progressive

2.3.7 Treatment for *Ehrlichia*

Tetracyclines is considered as an effective treatment for ehrlichioses. Although *E. chaffeensis* susceptible to rifampin, it is resistant to cotrimoxazole, erythromycin, quinolones and penicillin. It is suggested that tetracycline treatment be sustained for at least seven days or for three to five days after starting the medication (Parola and Raoult, 2001).

2.4 *Rickettsia*

2.4.1 Bacteriology

Rickettsia is a genus of strictly intracellular, Gram-negative bacteria with short rods that maintain basic function when stained by Gimenez technique. These zoonotic pathogens cause infections that disseminate in the blood to many organs (Parola and Raoult, 2001; Jensenius *et al*., 2004; Walker, 2007)

2.4.2 Classification of Rickettsia

Genus *Rickettsia* is part of the bacterial tribe *Rickettsiae,* and in the family *Rickettsiaceae* under the order *Rickettsiales*. It comprises of three antigenically distinct groups; Spotted Fever Group (SFG), Thyphus Group (TG) and Transitional group (TGR) (Merhej, 2011; Peniche-Lara *et al*., 2013).

Table 4 Species of Rickettsia with common host (Monir, 2017)

Organism	**Target**	**Disease**	**Distribution**	**Vector**	**Reservoir**
R. rickettsii	Vascular endothelium	Rocky Mountain spotted fever	North , Central, and South Ameria	Tick	Rodents, dogs
R. conorii, R. africae, R. australis	Vascular endothelium	Other spotted fevers	Worldwide	Tick	Rodents, dogs
R. akari	Vascular endothelium	Rickettsialpox	Worldwide	Tick, Mite	Mouse

R. prowazekii	Vascular endothelium	Typhus	United State of America	Tick, body louse	Human
R. typhi	Vascular endothelium	Murine (endemic) typhus	Worldwide	Tick, Flea	Rodents, rats

2.4.3 Vectors and Reservoirs

Species of *Rickettsia* have been detected in diverse tick species. As reported by Milhano *et al*., (2014), *Rickettsia massiliae* is transmitted by *Rhipicephalus sanguineus,* while *R. raoultii* DNA was confirmed from questing *I. ricinus* and in *D. silvarum* ticks (Jia *et al*., 2014). Similarly, Olivieri *et* al., (2018) detected transmission of *R. massiliae* and *R. raoultii* DNA in *R. sanguineus* and *D. reticulatus* ticks (Olivieri *et al*., 2018)

Although *Ambylomma* spp. are associated with *R. africae* infection, *Rhipicephalus* and *Haemophalis* spp. are also considered as vectors of *R. africae* pathogen. As confirmed by Portillo *et al*., 2007) who detected *R africae* DNA from *R. decoloratus* tick collected in oryx. Other vectors include *H. parva, H. adleri* and *H. dromedarii* ticks collected form different animal hosts including sheep, goats, dogs, a tortoise, a wolf, camels and a horse (Ereqat *et al*., 2016)

Studies have confirmed rodents as one of the reservoirs of SFG in the wild, thus contributing to the distribution and epidemiology of these pathogens in wildlife (Gajda *et al.,* 2017). Recently birds have been identified as one of the vectors of *Rickettsia* as documented by Amoêdo-Lima *et al*., (2018).

2.4.4. Epidemiology

In Africa, there are several reports of human-pathogenic *Rickettsia* species detected thus far and they include *R. conoriii*, *R. aeschlimannii*, *R. africae*, *R. parkeri*, *R. massiliae*, *R. akari*, and *R. sibirica mongolotimonae* (Parola *et al.*, 2001, Fournier, 2003, Mura *et al*., 2008). Humans in Cameroon, Burkina Faso, Ivory Coast, Mali and in Senegal and Ethiopia are frequently infected with *Rickettsia* species (Figure 2.10) (Mediannikov *et al*., 2010; Eldin *et al*., 2011).

Rickettsioses caused by *R. conorii* and *R. africae* are frequently reported pathogens in sub-Saharan Africa. *R.conorri* species are of concern tourism industry of South and there were several reports of illnesses and infections from tourists going back to their home countries later after visiting nature game reserves in South Africa (Kelly 2006; *Ndip et al*., 2004; Rutherford *et al.* 2004).

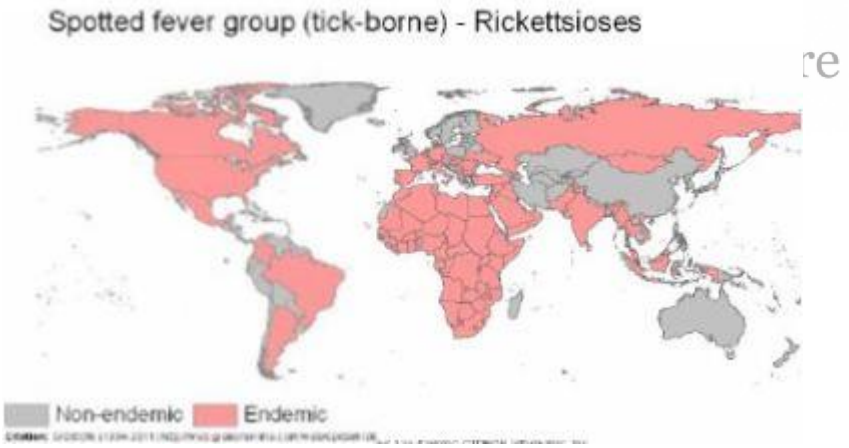

Figure 2. 9 An epidemiology of rickettial species around the world (Hay *et al*., 2013)

2.4.6 Clinical characteristics

The clinical symptoms differ with the difference species of *Rickettsia* involve as these signs begin to show within six to ten days subsequently after the tick bite.Clinical symptoms include

headache, fever, rash and muscle pain. Leukocyte and thrombocytopenia count defects are common and hepatic enzyme levels are frequently raised (Parola and Raoult, 2001).

2.4.7 Diagnosis and treatment

Doxycycline is used for treatment for tick-borne rickettsial disease in patients, which should be taken immediately when a patient shows symptoms and sign indicating rickettsial disease (Chapman *et al*., 2008). Treatment choices for rickettsial pathogens must never be late while waiting for laboratory approval. However, late treatment can result to severe disease and long-term sequelae or death (Dahlgren *et al*., 2012).

2.5 *Borrelia* species

The genus *Borrelia* is a group of bacterial spirochete which consist of more than thirty species of vector-borne pathogens. *Borrelia* can be categorized into two main groups, *Borrelia burgdorferi* sensu lato that contain nineteen species of *Borrelia*. This group was confirmed since the year 1984 and is transmitted through blood meal from a host during the blood meal of ixodes tick. It is associated with a disease called Lyme borreliosis (LB). The second group comprises of twenty-five confirmed species of *Borrelia*, however these species are related to human relapsing fever (RF) and is commonly transmitted by soft ticks (Wang, 2015)

2.5.1 Epidemiology

Tick-borne relapsing fever (TBRF) is an identified bacterial agent which is normally caused by species of *Borrelia.* *B. duttonii* is the main causative agent in humans and is prevalent in Eastern Africa (Figure 2.11) and *B. crocidurae* in western Africa. *B. duttonii* is transmitted through a bite by *Ornithodoros moubata* in Tanzania (Cutler, 2010). The most widespread reports of Lyme disease (LD) are associated with North America and Europe (Obiegala *et al.*, 2012).

Although tick-borne relapsing fever is prevalent in Tanzania and in Democratic Republic of Congo, but it has not been reported in further South. It is listed as one of the top ten killer diseases in children under the age of five years, and is associated with perinatal death of up to 436/1000 in Tanzania (McConnell 2003). While in the Democratic Republic of Congo, infection is associated with different results, including mortalities, particularly in pregnant women (Chitanga, 2014).

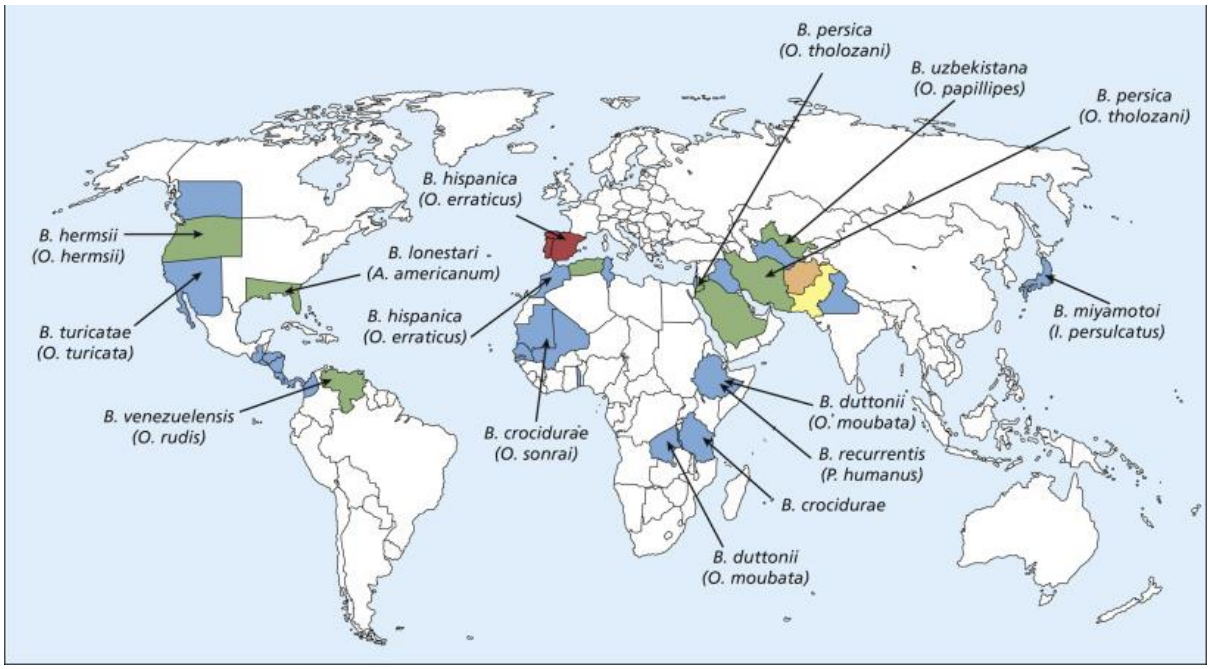

Figure 2. 10 Epidemiology of relapsing fever borreliae across the globe with their relative tick vectors**.**

2.5.2 Life cycle

In order for transmission to occur, *B. burgdorferi* pathogen must migrate to the salivary glands where they are transmitted through tick bite. During feeding, *B. burgdorferi* spirochetes in the skin of a host pass through to their colonization sites to cause a disease. After settling in the reservior for sometime, spirochetes within the skin infect feeding ticks to complete the cycle (Sultan, 2013). Immediately after it feeds on its host, it molts and falls off to the second host while growing to the next stage of life. Furthermore, the next host on nymphal stage of tick

could be a human thus infesting human with the *B. burgorferi* pathogen (Figure 2.12) (CDC, 2015).

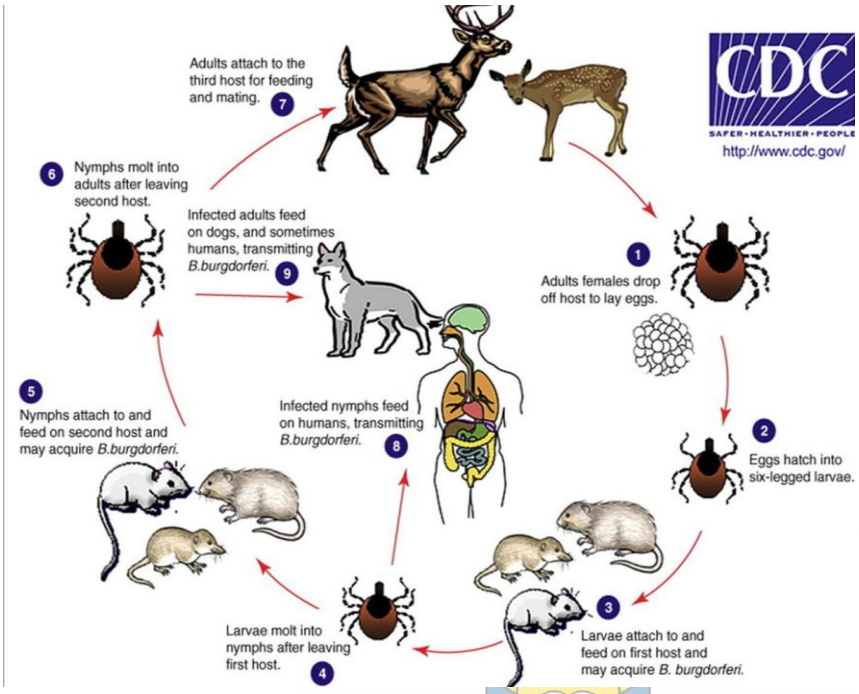

Figure 2. 11 A life cycle of Bor*relia burgorferi* from tick species to human and animal hosts (CDC, 2015).

2.5.3 Clinical characteristics

The clinical characteristic primarily includes fever >39°C with polyalgia chills. Lyme disease may cause severe signs with indicators in the skin, nervous system joints and heart tissue in humans and in companion animals, mainly dogs. Abdominal signs may occur, including abdominal pain, diarrhea and vomiting. *B. duttonii* is lead to miscarriage in pregnant women (Mégraud *et al.*, 2017; Obiegala *et al.*, 2012).

2.5.4 Diagnosis and treatment

The severity of relapsing fever primarily depend on the etiologic *Borrelia*, ranging from mild fever to malaria-like symptoms and could ultimately lead to death if not promptly treated. Physical examination may find splenomegaly, hepatomegaly and inconstant rash. The

preferred parenteral drug for Lyme borreliae is ceftriaxone because it is highly active against Lyme borreliae *in vitro and* alternative drugs are intravenous penicillin and cefotaxime. Parenteral antibiotic treatment is recommended for late Lyme neuroborreliosis (Stupica, 2014).

2.6 *Babesia* species.

Babesia is a protozoan apicomplexan parasite proficient in infecting red blood cells and it is transmitted by ticks to various hosts. Babesiosis is a malaria-like infectious disease caused by protozoa of the genus *Babesia (*Chauvin *et al.,* 2009*; Krause et al.,* 2017*).* It's prevalence is increasing in various parts of the world with its zoonotic potential, particularly in the tropical and subtropical countries along with its severe economic impacts. The first confirmed case of human fatal babesiosis caused by *Babesia divergens* was recorded in 1956. Since then, the significance of babesiosis as potentially life threatening zoonotic infection in humans have been reported globally (Anderson *et al*., 1974; Laha *et al.,* 2015; Khan, 2015).

However, in mammals, this parasite is capable of undergoing schizogony and multiply by fission, developing a characteristic of piroplasm stage with a small pear shaped structure (Figure 2.13) in blood cells. It then forms a distinctive paired body, which form a several schizonts that leads to formation of many sporozoites in salivary glands of the tick. This parasite is passed on from female ticks to their offspring by transovarian transmission. (Chauvin *et al.,*2009; Gholamreza *et al.,* 2017).

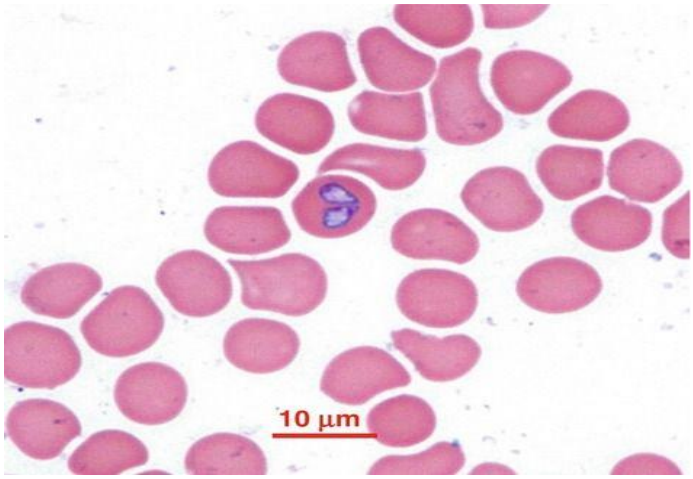

Figure 2. 12 Appearance of an intraerythrocytic pair of pear-shaped *Babesia* spp. (in color) (Amy *et al*., 2014).

2.6.1 Morphology

Two identified groups of *Babesia* known are small babesias group (1.0-2.5 µm long) which include *B. rodhaini, B. gibsoni, B. microti B. bovis etc, while* large babesias (2.5–5.0 µm long), include *B. Canis*, *B. bigemina, B. caballi*, etc (Rossouw, 2015).. The size normally determine the orientation of babesia parasite in red blood cells (RBCs). However, more than 100 species of *Babesia* have been recognized to infect many mammalian species (Laha *et al*., 2015).

2.6.2 Epidemiology

Several species of *Babesia* have lead to human infections throughout the world. In United State *B. microti* is detected to be the primary agent of human babesiosis, particularly in Northeast and upper Midwest where it is endemic. Nearly all cases in Europe have been recognized for *B. divergens*, but the infection is sporadic (Homer *et al*., 2000). *B. venatorum* is endemic in North Eastern China (Figure 2. 14) (Krause, 2017).

More than one hundred species have been stated, however, only a few have been identified to cause human infections, including *B. microtti, B. divergens, B. duncani*. Babesiosis has become one of the most important canine diseases in South Africa as reports have shown the

detection of *B. bicorns* in black rhinoes, which subsequently died in Tanzania and South Africa (Oosthuizen *et al*., 2009).

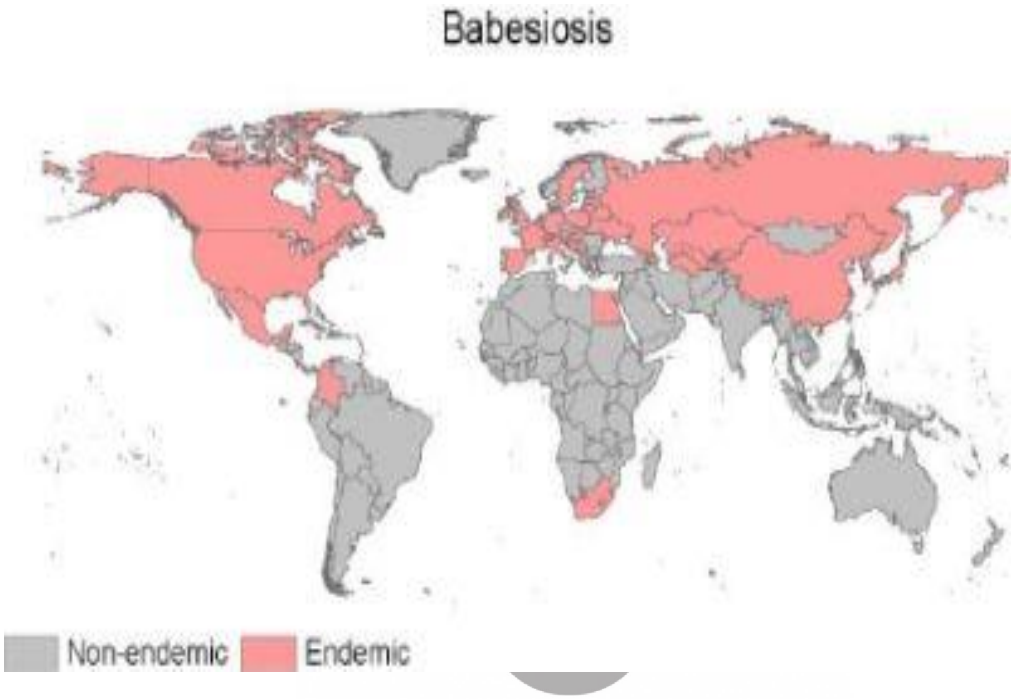

Figure 2. 13 An epidemiology of disease babesiosis worldwide (Hay *et al*., 2013).

2.6.3 Vectors of *Babesia* spp.

Babesia spp. are transmitted naturally from animal to animal by tick bites and through transovarian transmission from adult female ticks to their eggs. However, ticks are generally distributed all over the world predominantly in humid and subtropical countries (Jongejan and Uilenberg, 2004). The ticks' genera including *Rhipicephalus*, *Boophilus*, *Hemaphysalis*, *Hyalomma*, and *Ixodes* acts as vector in transmission of *B. bigemina*, while *Boophilus*, *Rhipicephalus*, and *Ixodes* are transmitters of *B. bovis*. However, *Babesia* spp. in dogs is transmitted by the *R. sanguineus*, *H. longicornis*, *H. leachi*, and *D. marginatus (Table 5)* (Laha *et al*., 2015).

Table 5 Some of Babesia spp. listed with their possible tick vectors and diseases they are likely to cause within the hosts as well as their distribution (Solano-Gallego *et al*., 2016)

Babesia *spp.*	**Vectors**	**Hosts**	**Pathogenicity**	**Disease**	**Distribution**
B.bovis	*Ixodes Rhipicephalus* (*Boophilus)*	Cattle, deer	High	Redwater fever	Europe, Africa, Australia, South & Central America
B. bigemia	*HaemaphysalisRhipicephalus (Boophilus)*	Cattle deer	Moderate	Redwater fever	Asia Australia North and South America, Southern Europe and Africa,
B. divergins	Ixodes	Cattle	Moderate	Redwater fever	western & central Europe
B.ovis	*Boophilus* , Ixodes	Goats sheep	Low	Babesiosis	Southern Europe , Asia,Africa Tropical America
B. microtti	Ixodes	Human	Low	Babesiosis	Worldwide

2.6.4 Life cycle

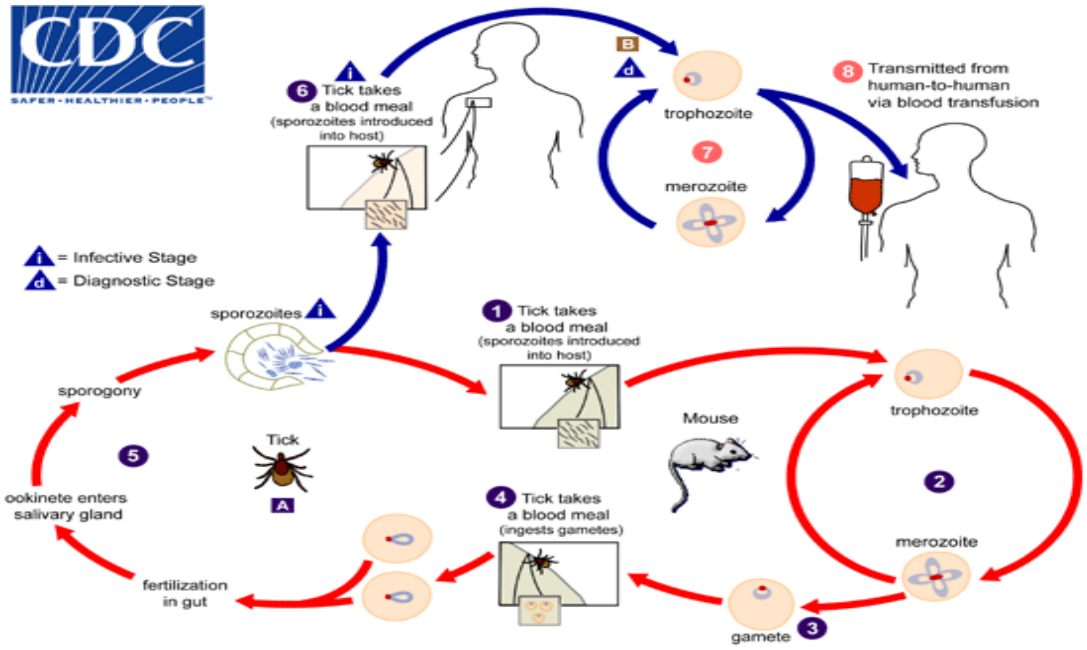

Figure 2. 14 Life cycle of Babesia microti within two hosts (CDC, 2016)

Two hosts involved in the life cycle of *B. microti* are *Peromyscus leucopus*, a rodent and an ixode tick. During feeding, infected tick transmits sporozoites into the rodent and the transmitted sporozoites migrate to erythrocytes where they undergo asexual reproduction. In human, after asexual reproduction, parasites multiply in the blood, thus leading to clinical manifestations of the disease. Human to human transmission is through blood infusion. However, large *Babesia* spp. are subjected to transovarial transmission (Figure 2.15) (CDC, 2016).

2.6.5 Clinical Presentation

Manifestations of disease occur immediately after 1-4 weeks of infection and could lead to sweating, fever, chills, fatigue, hepatosplenomegaly, hemolytic anemia, and myalgias. Moreover, infections caused by *B. divergens* are likely to be more severe due to *B. microti*, where clinical recovery commonly take place (CDC, 2017).

2.6.6 Diagnosis and treatment

Molecular assays, including qPCR on EDTA blood and serological tests provide the most sensitive and precise diagnostic examinations. These can provide an indication of the desired pathogen load and allow identification of the species, which gives applicable prognostic information. Internal positive controls should be considered for a successful DNA isolation from the sample tested (CDC, 2016).

Precise diagnosis is highly effective when several diagnostic analyses are used, which comprises of microscopic analysis, molecular and serology assays. The incidence of co-infections can complicate the diagnosis (Villiers, 2016). Hence treatment needs antiparasitic drugs, for instance those used for malaria. Atovaquone and azithromycin are used to treat such cases and is normally taken for seven to ten days (Krause *et al*., 2017).

2.7 *Theileria* species

Theileria species are tick-transmitted intracellular protozoan pathogens of wild animals and domesticated ruminants. These species are in the phylum *Apicomplexa* that consist of a large complex group of Eukaryotic organisms. *Theileria* spp. are found to be closely related to *Babesia*, although they differ by developmental stage in leukocytes prior. Although *Theileria* have been reported in several mammalian species, the vast majority of *Theileria spp.* described to date are found in ruminants (Morrison, 2015).

2.7.4 Epidemiology

The two major significant species in livestock and water buffaloes are the *T. parva*, which normally result to East Coast fever (Thompson, 2008; Yusufmia, 2010), and *T. annulata*, which result to Mediterranean theileriosis across North Africa and central Asia (Yusufmia, 2010). Other *Theileria* species such as *T buffeli, T. mutans, T. taurotragi,* and *T. sergenti* and *T. velifera* are typically confined in Africa or Asia corresponding to the environmental spreading

of their tick vectors, except for the global distribution of the pathogenic *T. buffeli.* (Uilenberg, 2001;Valli *et al*., 2016)

Theileriosis is endemic through Africa, and has been reported in eleven countries in the region of Tanzania, Kenya, Burundi and Uganda (Lawrence *et al.*, 1992). However, East Coast fever was also documented in Comoros between 2003 and 2004 for the first time (Deken *et al*., 2007).

2.7.1 Vectors and resevior

Theileria spp. infect an extensive variety of both domesticated and wild animals and are conveyed by tick vectors of the genera *Amblyomma, Rhipicephalus, Haemaphysalis* and *Hyalomma*. In many parts of Africa, *T. mutans*, *T. velifera* and *T. orientalis* are widely distributed over Africa and are transmitted by an *Amblyomma* tick. *T. taurotragi* and *T. parva,* are transmitted predominantly by *R. appendiculatus. T. taurotragi* is transmitted by *R. pulchellus*. *Hyalomma* species are the vectors for *T. lestoquardi* and *T. annulata* as confirmed by Mohammadi, (2017).

2.7.2 Life cycle

Using *Theileria parva* as an example, this species experiences a sequence of developmental modifications in ticks and bovine host (Figure 2.16). *Rhipicephalus appendiculatus* is primarily a vector involved in the transmission of *T. parva* species that occurs trans-stadially as reported by Norval *et al.*, (1992). Infection of larvae and nymphs is obtained from the piroplasmic stage which is present in red blood cells (RBCs) in infected cattle. Infected nymphs and adults transmit sporozoites during feeding to the next host (Nene *et al.*, 2016).

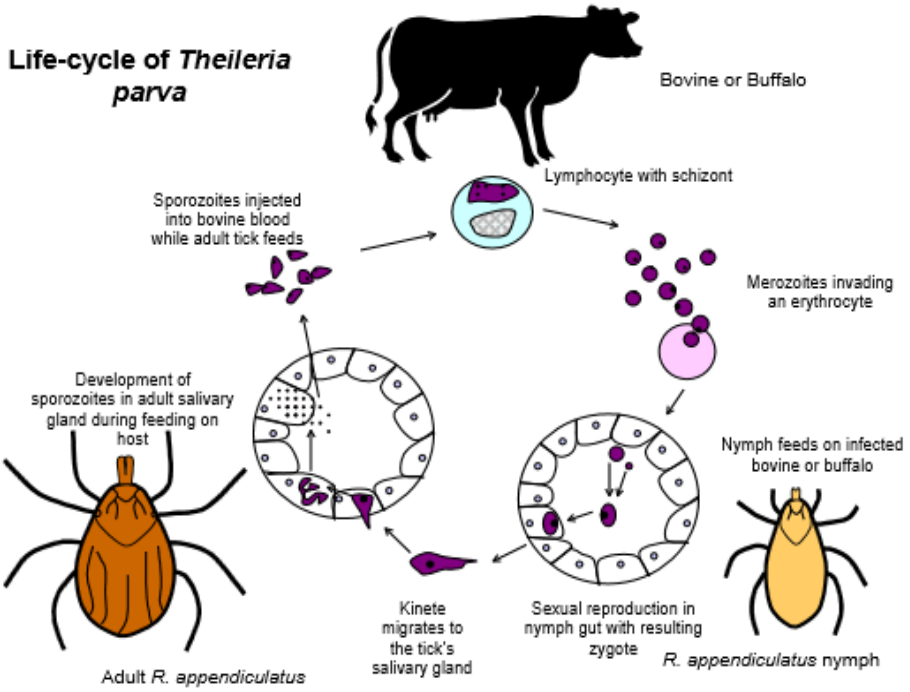

Figure 2. 15 A life cycle of Theileria parva on cattle host (CDC, 2016).

2.7.5 Diagnosis

Theileria pathogens are diagnosed using the different methods including microscopy, which is done on Giemsa-stained blood smears. Xenodiagnosis method is used to approve disease-causing species and their tick vectors respectively. The direct injection is done in order to differentiate species that are responsive to spread in the piroplasm stage (Mans *et al.*, 2015).

Serological diagnosis is accomplished by complement fixation and indirect fluorescent antibody test (IFAT). Preparation of antigen is done from schizont or piroplasm antigen, as it is commonly resultant from infected a cell culture or animal. Thereafter, cell culture of *Theileria* might be beneficial in diagnosis processes, so as to identify the forming parasites with an aim of generating IFAT antigen to study distinct parasite populations using monoclonal antibodies. Lastly, detection using molecular methods is also useful to allow direct confirmation of the presence of parasite genomic material (Mans *et al.*, 2015).

2.7.6 Prevention and control

Tick control is found as the most signficant factors affecting the epidemiology of bovine theileriosis. This is attained by the use of acaricides to eliminate ticks in both free living and parasitic stages. However, more sustainable and reliable methods for the control of theileriosis involve a combination of strategic tick control and vaccination; these have yet to be successfully applied on a large scale in endemic areas (Lawrence *et al*., 2005).

CHAPTER THREE

3.1 Materials and Methods

Ethical clearance

Ethical clearance was acquired from the University of Fort Hare Ethics Committee before the study was conducted. Upon arrival at the sampling sites, authorisation was attained from the farmers before any tick collection from their animals could commence. Tick collection was done with assistance of veterinary worker and animal health specialists responsible for handling and treating the animals.

3.1.1 Study area, tick collection and identification.

Figure 3. 1 Google map satellite image showing the geographical locations Raymond Mhlaba local municipality (www.googlemaps.com).

3.1.2 Sample collection

The study was conducted from May 2018 to September 2018 in Raymond Mhlaba local Municipality in the Eastern Cape Province (Figure 3.1). The investigation was carried out within the following sites, from Debe, which is located at the following coordinates: 32.836°S 27.154°E, under Raymond Mhlaba local Municipality, and Fort Beaufort located at 32° 47' 0" S, 26° 38' 0" E as indicated on Figure 3.1.

Collection of ticks was done manually from domesticated animals (sheep, goats, horses and cattle). Tick samples were collected using forceps and placed into 50 mL Nalgene tubes that contained 70% ethanol. The samples were transported to the laboratory of Applied and Environmental Microbiology Research Group in the Microbiology Department at the University of Fort Hare. The ticks were separated on the basis of the animal hosts from which they were collected, and the location as well. The collecting tubes were properly labelled accordingly and were stored at 4°C until further processing (Iweriebor *et al*., 2017).

3.1.3 Ticks identification and processing for DNA extraction

All tick species collected were identified using the morphological criteria and appropriate taxonomic keys (Manilla, 1998; Estrada-Peña *et al.,* 2004). The ticks were sorted according to species, collection site, and stage of development (Yoontae *et al.,* 2016). Also, molecular approach targeting the 12S mitochondrial DNA was amplified by PCR and obtained amplicons sequenced and derived sequences were edited and phylogenetically analysed.

3.1.4 DNA extraction

Ethanol conserved samples were rehydrated and washed twice in sterile distilled water for twenty minutes and then left to dry for additional twenty minutes. This was done in order to get rid of all the ethanol residues from the tick samples. The ticks were placed into different petri dishes according to the previously mentioned separation basis. DNA was extracted from

the minced samples using ReliaPrep DNA Tissue miniprep system ZYMORESEARCH Quick DNA Universal Kit in accordance to the manufacturer's instruction.

Briefly, the ticks were cut into smaller bits of pieces using sterile razor blades. 200 uL of nuclease free water added onto each petri plate containing chopped of ticks. The mixture was collected into 1.5 mL Eppendorf tube, thereafter the sample was vortexed using a vortex machine. In tubes containing the mixture, 20 uL of Proteinase K (PK) solution was added. After PK was added, 200 uL of cell lysis buffer (CLD) was also added onto the mixture. The tube caps were put back on, and then the sample was mixed by vortexing for 10 seconds. The samples were then placed onto a heating block that had been set at 56^{o} C and were incubated overnight.

The tube was then taken out from the heating block machine. A measure of 250 uL of the binding buffer (BBA) was added into the tube, the tube content was then vortexed for ten seconds. ReliaPrepTM Binding Columns were set inside collecting tubes for each sample. The supernatant from each sample was transferred into the binding columns and centrifuged at maximum speed for 1 min. The collecting tubes that contained the flow through were discarded. The binding columns were set onto fresh collecting tubes. A measure of 500 uL of column wash solution (CWD) was included onto the column, after, the samples were centrifuged for 2 minutes at most extreme speed. The flow through was disposed of. This progression was redone twice for a total of three washes. After the last wash, the column was then set onto clean 1.5ml micro-centrifuge tubes.

Nuclease Free water of about 50 uL was added to the columns. These samples then were centrifuges for 1 minute at maximum speed. The ReliaPrep^{TM} Binding Columns were then discarded, and the eluent that was contained inside the micro-centrifuge was kept ice. The eluent assumed to be containing the DNA was later stored at -20^{o}C until further processing.

3.1.5 PCR detection of bacteria in ticks

Each tick specimen was screened using PCR for both tick identification and detection of of the following bacterial patohgens which are *Anaplasma* spp., *Rickettsia* spp., *Ehrlichia* spp., *Borrelia* spp., as well as protozoan species including *Babesia* and *Theileria* spp.. Confirmation of tick species identification was done by PCR targeting the mitochondrial 12S rDNA gene (Pesquera *et al.,* 2015). Screening of bacterial and protozoan tick-borne was also carried out using PCR assay with specific primers targeting fragment genes for each genus as shown in Table 3.1 of primers used.

PCR amplification was carried out in a 15 µL reaction cocktail in a 200 µl tube with 8 µL of Master Mix, 1 µL of forward and reverse primers, 3 µL of nuclease free water and 2 µL of the template DNA. The amplification was performed using thermal cycler (BIORAD, MycyclerTM thermal cycler). Primers that were used for the detection of genetic materials of the tick hosts, bacterial and protozoan pathogens in the PCR amplification are listed in table 3.1. The PCR cycling conditions were as follow; initial denaturation at 94 °C for 3 min, followed by denaturation at 93 °C for 30 sec, different annealing temperatures depending on the pathogen type (as listed in the table 6 below) for 30 sec with an elongation of 1 min at 72 °C and a final elongation at 72 °C to infinite. The target genes for all the pathogens amplified in this study are also listed. The PCR amplifications were visualized by gel electrophoresis on 1% agarose gel stained with 2 µL ethidium bromide (Alliance 4.7, UVITEC, UK). Appropriate DNA ladders were used as genetic markers (Aktas, 2013).

Table 6 Primer sequences used in PCR amplification for the molecular identification of genes of interest of ticks and pathogens.

Species	Target gene	Primer name	Primer sequence 5' to 3'	Tm	Reference
Tick spp.	12S rRNA	T1B T2A	AAACTAGGATTAGATACCCT AATGAGAGCGACGGGCGATGT	51	Pesquera *et al.*, 2015
Rickettsia spp.	*glt*A(5 end)	CS-78 CS-323	GCAAGTATCGGTGAGGATGTAAT GCTTCCTTAAAATTCAATAAATCAGGAT	50	Pesquera *et al.*, 2015
	*omp*A (semi-nested)	Rr190.70p Rr190.701n	ATGGCGAATATTTCTCCAAAA GTTCCGTTAATGGCAGCATCT	46	Pesquera *et al.*, 2015
	*omp*B (nested)	*romp*B	GTAACCGGAAGTAATCGTTTCGTAA GCTTTATAACCAGCTAAACCACC	56	Pesquera *et al.*, 2015
Ehrlichia spp./ *Anaplasma* spp.	16S rRNA	EHL dsb-728 EHLdsb-330	CTGCTCGTCTATTTTACTTCTTAAAGT GATGATGTCTGAAGATATGAAACAAAT	47	Pesquera *et al.*, 2015
Borellia spp.	flaB (nested)	Outer1- Outer2-	AAGCATTTTCWATTTTAGCAAGTGATG RGAATTGGCAGTTCAATC	52	Pesquera *et al.*, 2015
Babesia spp. *Theileria* spp.	18S rRNA	BTF1 BTR1 BTF2 BTFR2	GGCTCATTACAACAGTTATAG CCCAAAGACTTTGATTTCTCTC CCGTGCTAATTGTAGGGCTAATAC GGACTACGACGGTATCTGATCG	58	Jefferies *et al* ., 2007

3.1.6 DNA sequencing and sequence editing

The amplified PCR products were sequenced in a commercial sequencing facility. The alignment and sequence editing was achieved using Geneious Prime 2019.0.3 version. The generated nucleotide sequences were subjected to homology search using the BLAST 2.0 database program in the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/blast/ Blast.cgi), where the edited sequences were compared with genetic sequences of previously characterized bacterial and protozoan species curated in the GenBank NCBI (Altschul *et al.*, 1990).

3.1.7 Phylogenetic analysis

Edited sequences were concatenated and aligned and compared to other species to construct a maximum possible phylogenetic tree using Mega 7 version software with 1000 boost trap replication. Phylogenetic analysis was performed using BioEdit Tree Builder. Sequence data sets for positive samples were submitted to NCBI GenBank for accession numbers.

CHAPTER FOUR

4. 1 Results

4.1.1 Tick prevalence within the two sites of interest

A total of 962 tick samples were collected from the designated localities in Raymond Mhlaba Local Municipality of Eastern Cape Province and analysed for the prevalence of tick-borne bacterial and protozoan pathogens in the sampling sites.

Morphological and molecular identification methods were used to delineate the collected ticks and six species belonging to three genera of ticks, identified as, *Rhipicephalus*, *Amblyomma* and *Haemophysalis* are reported. Amongst them were *Amblyomma hebraeum* 38,7 % (n = 373 adult), *R. appendiculatus* 17.3% (n =167 adult), *R. microplus* 13,6% (n =131 adult), *R. simus* 11,6 % (n =112 adult), *R. eversti eversti* 8,9 % (n =86 adult), and *H. longicornis* 9,6 % (n =93 adult) in decreasing order of their prevalence (Figure 4.1). *A. hebraeum* was the most prevalent species in both study sites with cattle serving as the common host. The two designated sites were compared based on the abundance of ticks collected as represented in Figure 4.2.

Furthermore, edited sequences for tick identification were subjected to BLAST for confirmation of species using NCBI BLAST Nucleotide, and showed all the generated sequences having more than 97% sequence identity to previously reported partial 12S mitochondrial DNA sequences from tick species.

4.1.2 Homology data analysis

When searching against species-specific sequence databases, analysed data revealed T46 sequence identical with 97.1% similarity to the partial 12S rDNA gene of *A. hebraeum* with GenBank Accession number AF150049.1. T44 sequence was identical to *Rhipicephalus microplus* spp. (MK332391) obtained from BLAST with 97.1% similarity.

4.1.3 Phylogenetic analysis

Phylogenetic tree was constructed (Figure 4.3) using MEGA6 version for statistical maximum likelihood of the tick samples obtained with reference sequences from NCBI GenBank. Tick samples were randomly selected for phylogenetic analysis. Phylogenetic analysis clearly showed T44, T43 and T45 clustering with *A. triste* (KU284929), *A. parvitarsum* (KU284920) and *A. triginum* (KU284864), while T46 clustered with *R. annulatus* (KY676832) and *R. australis* (KY676830).

It can be concluded that cattle in this study commonly serve as tick host, most probably due to their larger volume and more space for ticks' attachment. Similarity within the diversity of ticks collected at each study site was identified. The respective species collected in different animals and their number per each site are summarised in Table 7 (Debe Location) and Table 8 (Fort Beaufort). Comparison of tick abundance between the two study sites are shown in Figure 4.2.

Table 7 Proportion and distribution of collected tick species in Debe Location

Animal	**Tick species**	**Developmental stage**	**Number**
Cattle	*R. eversti eversti*	Adult	41
	A. hebraeum	Adult	115
	R. microplus	Adult	12
	R. appendiculatus	Adult	63
	R. simus	Adult	54
	H. longicornis	Adult	35
Goat	*R. eversti eversti*	Adult	0

	A. hebraeum	Adult	60
	R. microplus	Nymph	66
	R. simus	Adult	19
	H. longicornis	Adult	13
	R. appendiculatus	Adult	40
Sheep	*R. eversti eversti*	Adult	3
	A. hebraeum	Adult	27
	R. microplus	Adult	9
	R. simus	Adult	4
	H. longicornis	Adult	8
Horse	*A. hebraeum*	Adult	13

Table 8 Proportion and distribution of collected tick species in Fort Beaufort

Animal	**Species**	**Developmental Stage**	**Number**
Cattle	*R. eversti eversti*	Adult	36
	A. hebraeum	Adult	57
	R. simus	Adult	13
	R. microplus	Adult	15
	H. longicornis	Adult	20
	R. appendiculatus	Adult	31
Sheep	*R. eversti eversti*	Adult	6

	A. hebraeum	Adult	40
	R. simus	Adult	17
	R. microplus	Adult	18
	H. longicornis	Adult	10
Goat	*R. appendiculatus*	Adult	22
	A. hebraeum	Adult	55
	R. simus	Adult	5
	R.microplus	Adult	13
	H. longicornis	Adult	7
Horse	*R. eversti eversti*	Adult	0
	A. hebraeum	Adult	15
	R. simus	Adult	0
	R. microplus	Adult	0
	H. longicornis	Adult	0

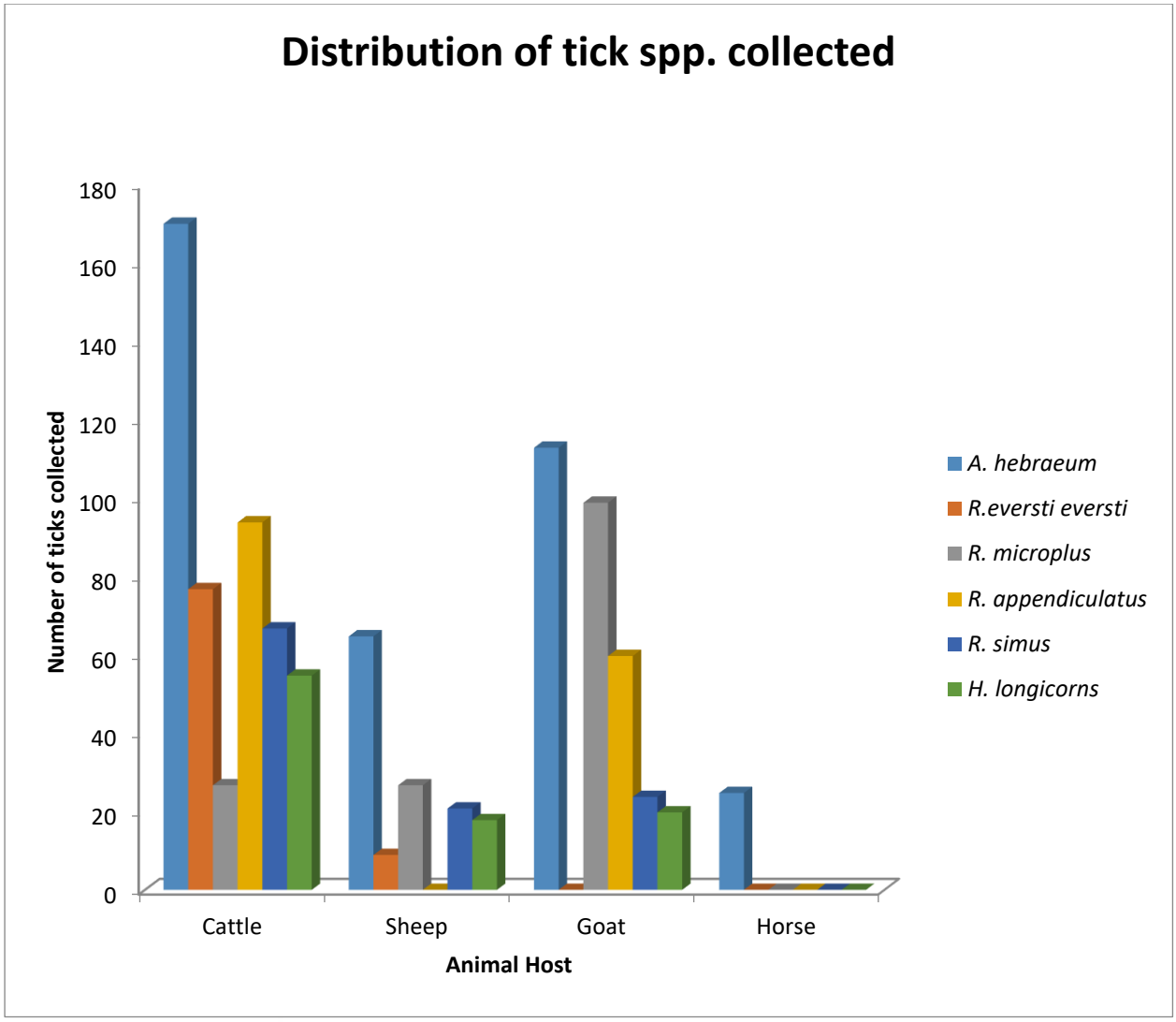

Figure 4. 1. The distribution of tick species. in each domestic animal from the two study regions.

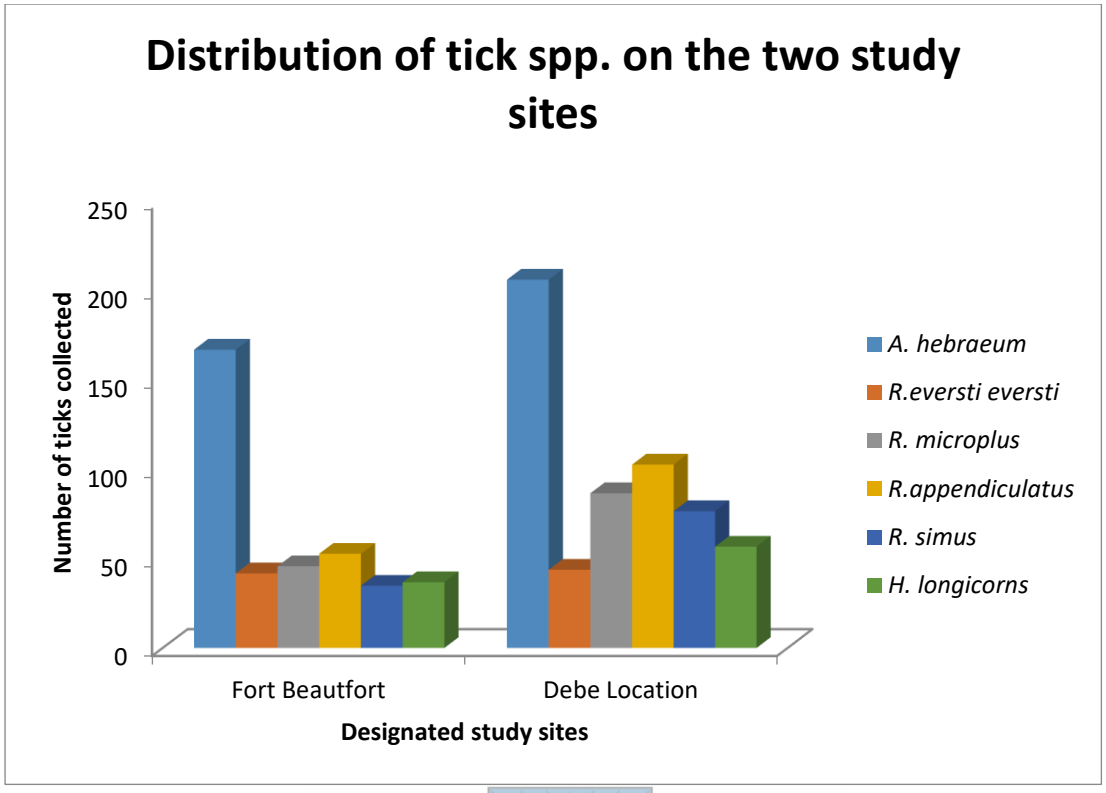

Figure 4. 2 The distribution of tick species, from domestic animals in the study sites in Fort Beaufort and Debe location, Eastern Cape, South Africa.

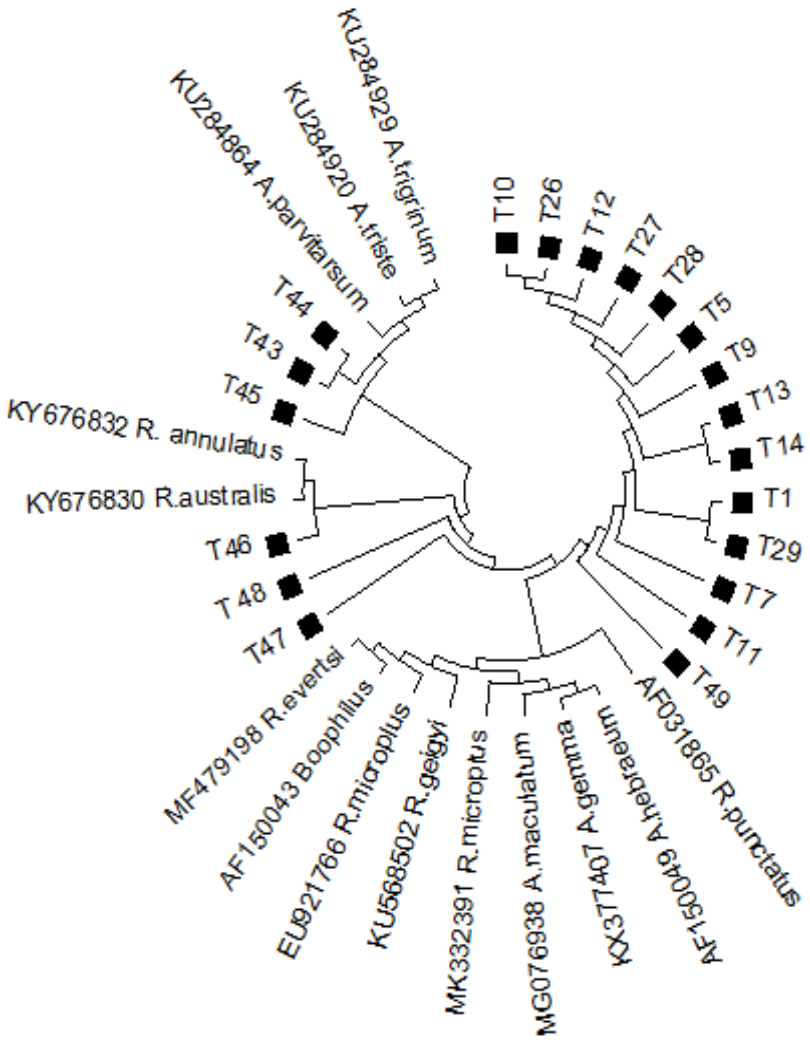

Figure 4 3 Neighbour-joining phylogenetic tree of 12S mitochondrial rDNA of tick species generated from the study with the reference sequences from GenBank. Tree was constructed by using the neighbour-joining method. Black dots show tick species identified from this study.

Table 9 Reference strains used in phylogenetic analysis of tick species.

Strain Accession Number	**Species**	**Geographic of origin**
KU284929	*A. trigrinum*	Brazil
KU284920	*A. triste*	Uruguay
KU284864	*A. parvitarsum*	Argentina
KY676832	*R. annulatus*	Israel
KY676839	*R. australis*	South Africa
MF479198	*R. evertsi*	DRC
AF150043	*Boophilus*	Jordan
EU921766	*R. microplus*	Mozambique
KU568502	*R. geigyi*	Guinea-Bissau
MK332391	*R. microplus*	Uganda
MG076938	*A. maculatum*	Mexico
KX377407	*A. gemma*	Ethiopia
AF150049	*A. hebraeum*	Zimbabwe
AF031865	*R. punctatus*	Australia

4.2 Detection, identification and Prevalence of *Rickettsia* spp. in the study sites.

Molecular screening for tick-borne bacterial and protozoan pathogens showed the detection of *Rickettsia* spp. and *Theileria* spp. only while infection with *Anaplasma, Babesia* and *Ehrlichia* and *Borrelia* were not detected among the ticks screened.

A total of 60 (6%) positive samples were detected for *Rickettsia* spp. from the 994 ticks analysed (Table 9). Screening for *Rickettsia* spp. by PCR assays was achieved using specific primers targeting three protein-coding genes, citrate synthase *glt*A gene (401bp), *ompA* (631 bp) (Figure 4.5) and *ompB* (511 bp) (Figure 4.6) which were sequenced and analyzed to delineate the *Rickettsia* spp. down to the species level.

Detection of *Rickettsia* was positive from the three genera of ticks collected in this study and predominatly obtained from *Amblyomma hebreaum.* Also, a higher proportion of the pathogen (55%) was obtained in ticks collected from cattle thus inferring that cattle are most common hosts of *Rickettsia* pathogen in the study sites. Among the ticks collected from goats, 23% prevalence of *Rickettsia* was observed while the remaining 22% were detected in ticks collected from sheep. The occurrence of *Rickettsia* spp. based on animal and its tick vector are summarised in Figure 4. 4.

The positive samples identified from the respective tick species analysed are summarised in Table 10. A homology search for generated *Rickettsia* sequences showed that they had a high sequence similarity of above 97% with homologous sequences of *Rickettsia* in GenBank. Comparison of *omp*A sequences by BLAST analysis showed ≥98% high homology with different rickettsial species, including *R. africae.* While *omp*B sequences were homologous to *R. africae,* with ≥97% identity.

However, sequence B188 showed higher degree to closely related SFG species of *Rickettsia parkeri* (KY113111) with 99, 16% identity. While sequence B209 was 100% homologous to published *R. tamurae* (DQ113910).

Reference sequences used for phylogenetic analysis were randomly selected and analysed with generated sequences of *omp*A and *omp*B genes obtained from this study. The phylogenetic tree obtained for *ompA* gene showed that test sequences clustered with reference sequences (Table 11) from NCBI GenBank nucleotides database as illustrated in Figure 4.6. All ompA sequences clustered with *R. africae* (U834362). Similarly, in *ompB* gene, phylogenetic analysis showed that all study sequences clustered with *R. africae* (KX227791.1) (Figure 4.7).

Table 10 Prevalence of rickettsial pathogens in different species of ticks.

Host vector	**Tick vector**	**Total positive for each host %**
Cattle	*R. eversti eversti* *A. hebraeum* *R. simus* *R. microplus* *H. longicornis*	55%
Goat	*A. hebraeum* *R. simus* *R. microplus* *H. longicornis*	23%
Sheep	*A. hebraeum*	22%

	R. microplus	

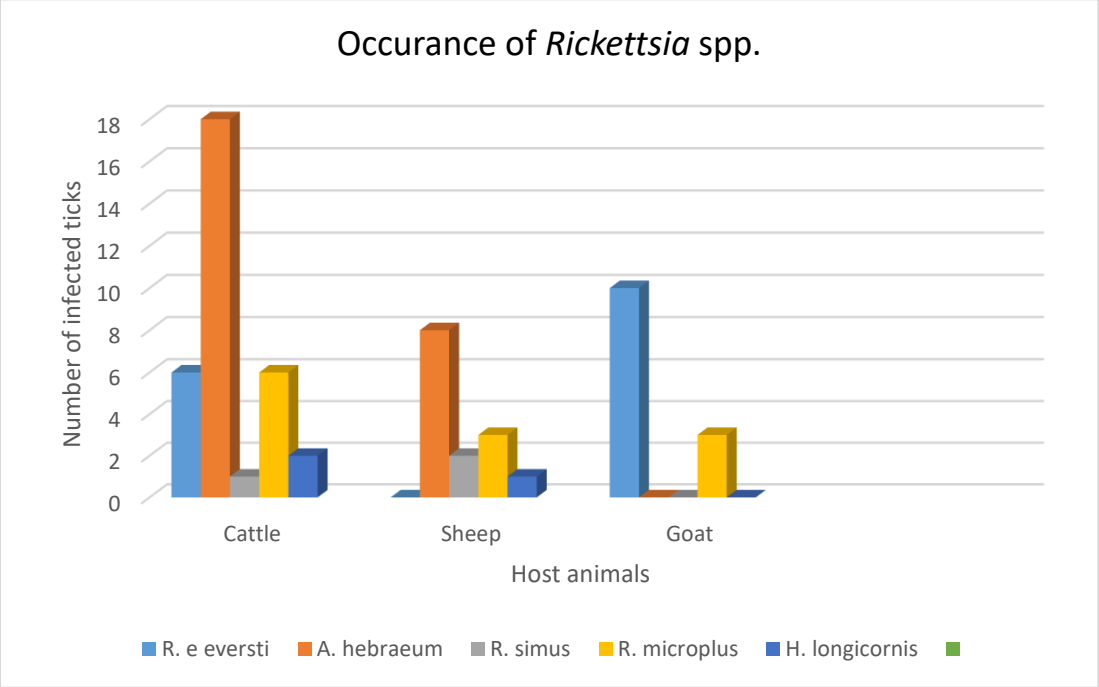

Figure 4. 4 Occurrence of *Rickettsia* species in domestic animals in Raymond Mhlaba Local Municipality in Eastern Cape, South Africa.

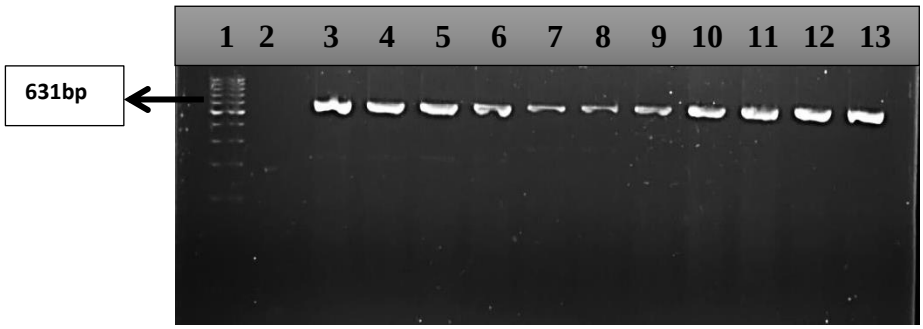

Figure 4. 5 An electrophoretic gel picture of the 631bp of a rickettsial ompA gene. Lane one represents the 100bp m/w marker while lane 2 is the negative control (nuclease free water was negative control) and lanes 3- 13 are representatives of the positive samples.

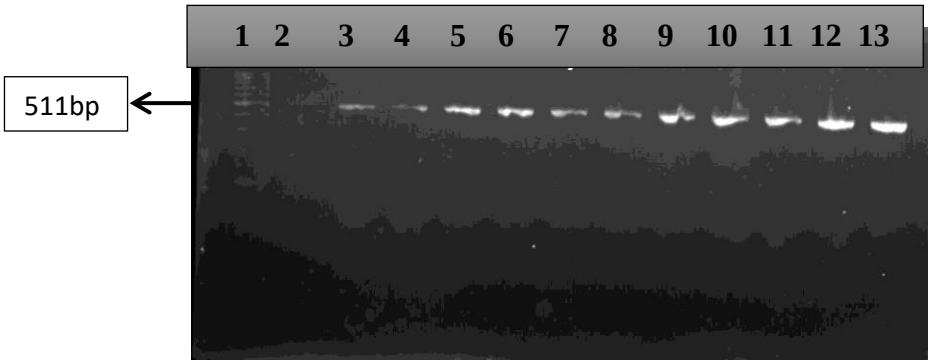

Figure 4. 6 An electrophoretic gel of 511bp rickettsial ompB gene. Lane one is the 100bp m/w marker while lane 2 is the negative control (nuclease free water was negative control) and lanes 3- 13 are representatives of the positive samples based on the OmpB gene of Rickettsia.

4.2. 1 Phylogenetic analysis for Rickettsial spp.

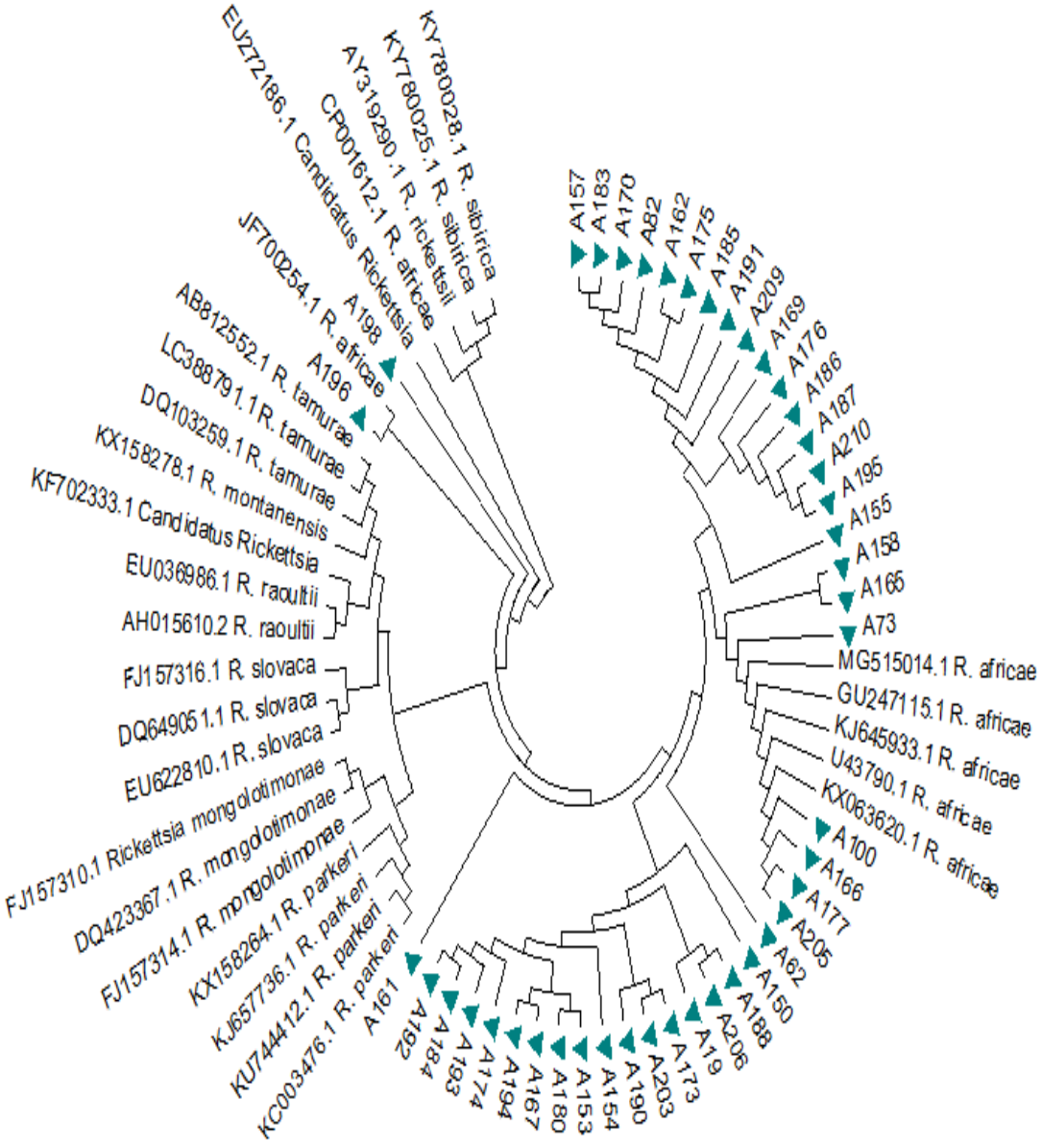

Figure 4. 7 A phylogenetic tree constructed based on the ompA gene of *Rickettsia*. The phylogenetic tree was drawn with MEGA 6 using neighbor-joining tree method. Construction was based on sequences generated from the study with other Rickettsia reference sequences obtained from GenBank.The test sequences in bold clustered with other Rickettsia references.

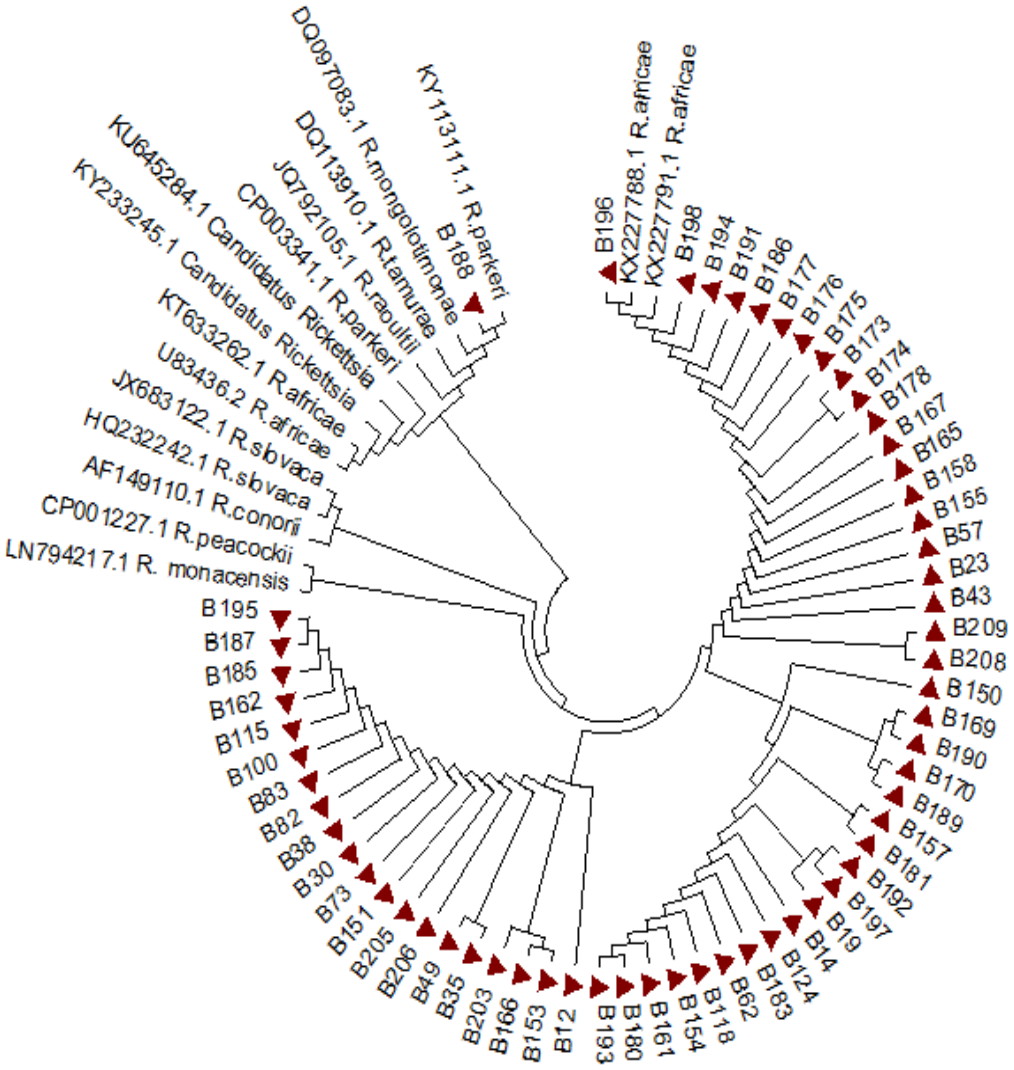

Figure 4. 8 Phylogenetic tree of *omp*B gene sequences generated from the study with the related reference sequences obtained from NCBI GenBank. Tree was constructed by using the neighbor-joining method. All study sequences clostered monophyletically with *R.africae* sequences from GenBank with the exception of sequence B188 which clustered with *R .parkeri*.

Table 11 Rickettsial reference strains used to construct ompA phylogenetic tree

Accession Number	Species	Geographic origin
CP001612.1	*R. africae*	Ethiopia
AH015610.2	*R. raoultii*	France
AY319290.1	*R. rickettsii*	USA
DQ423367.1	*R. mongolotimonae*	Portugal
KF702333.1	*Candidatus Rickettsia*	Costa Rica
KY780025.1	*R. sibirica*	Russia
KX158278.1	*R. montanensis*	USA
EU272186.1	*Candidatus Rickettsia*	India
EU036986.1	*R. raoultii*	France
FJ157314.1	*R. mongolotimonae*	Spain
JF700254.1	*R. africae*	Israel
MG515014.1	*R. africae*	Brazil
KJ645933.1	*R. africae*	Madagascar
U43790.1	*R. africae*	France
KX063620.1	*R. africae*	Ethiopia
DQ103259.1	*R. tamurae*	France
LC388791.1	*R. tamurae*	Japan
AB812552.1	*R. tamurae*	Japan
KX158264.1	*R. parkeri*	USA

KJ657736.1	*R. parkeri*	Uruguay
KC003476.1	*R. parkeri*	USA
KU744412.1	*R. parkeri*	Argentina
KY780028.1	*R. sibirica*	Russia
FJ157310.1	*R. mongolotimonae*	Spain
EU622810.1	*R. slovaca*	Slovakia
FJ157316.1	*R. slovaca*	Spain
DQ649051.1	*R. slovaca*	Portugal

Table 12 Rickettsial reference strains used to construct *ompB* phylogenetic tree

Accession number	**Species name**	**Geographic origin**
DQ113910	*R. tamurae*	Japan
DQ097083	*R. mongolotimonae*	Algeria
AF123706	*R. africae*	France
KY113111	*R. parkeri*	Brazil
KY233245	*Candidatus Rickettsia*	Lebanon
U83436	*R. africae*	France
KU645284	*Candidatus Rickettsia*	China
JX683122	*R. slovaca*	Romania
HQ232242	*R. slovaca*	Germany

KX227788	*R. africae*	Kenya
KX227791	*R. africae*	Kenya
JQ792105	*R. raoultii*	China
AF149110	*R. conorii*	Australia
LN794217	*R. monacensis*	USA
CP003341	*R. parkeri*	USA
CP001227	*R. peacockii*	USA

4.3.1 Detection and Prevalence of *Theileria* spp. identified in the study sites

Members of *Theileria* spp. were detected in 10 (1%) out of 994 tick samples assessed for the presence of *Theileria* and *Babesia*. The obtained sequences were subjected to BLAST analysis which showed that they had sequence similarity with homologous *Theileria* sequences curated in GenBank. *Theileria* sequences were detected in *Rhipicephalus* ticks.

Statistically, 5% of 86 *R. eversti eversti* ticks were infested with *Theileria*, while 1% of 131 *R. microplus* harboured the pathogen. The overall tick infestation in cattle was 80% prevalence, while 20% prevalent in both goats and sheep. It can be clearly pointed that cattle are predominant host of *Theileria* infection (Table 10).

4.3.2 Homology data analysis

BLAST analyses of piroplasm sequences showed that all ten sequences were *Theileria* spp. with sequences C18, C22, C24, C65, C76, C77 and C81 belonging to *T.orientalis* with homology above 98% while sequences C69 and C208 were assigned to *T.buffeli* and *T.velifera* with percentage homology of 99 and 96% respectively.

4.3.3 Phylogenetic analysis

Sequences of 18S rDNA *Theileria* were subjected to Phylogenetic analysis using neighbor-joining tree method as implemented in MEGA version 6. The phylogenetic tree constructed with published 18S rDNA gene sequences of *Theileria* spp. (Table 13) and obtained sequences is represented in figure 4.9. Phylogenetic analysis has shown the clustering of C18 sequence with *T. buffeli* (KY355137.1). While C21 and C65 sequences were on the same clade with *T. sergenti* (GU143088.1) and lastly, sequence C76, C81 and C77 clustered on the same clade with *T. orientalis*.

All the sequences obtained in this study and the sequences from NCBI showed high percentage homologies which ranged from 90%- 99.0%.

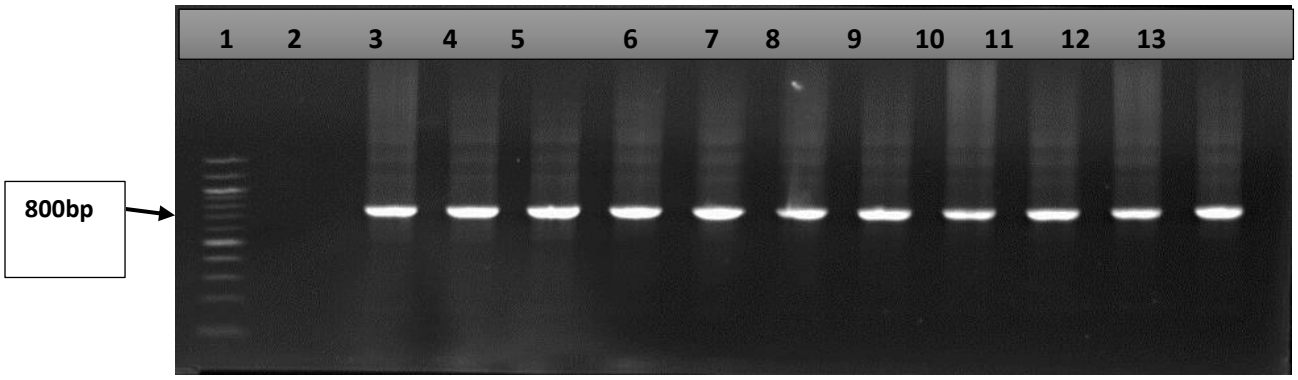

Figure 4 9 An electrophoretic gel of 800bp of *Theleria*. Lane one is the 100bp m/w marker while lane 2 is the negative control (nuclease free water was negative control) and lanes 3-13 are representatives of the positive samples based on *Theileria*.

Table 13 Prevalence of *Theileria* spp. in different tick species on animals collected Debe Location

Animal Host	**Tick & *Theileria* spp.**	**No. of positive samples**	**% of +ve tick spp.**	**Total *Theileria* spp. for each host**

Cattle	***R. eversti eversti***			
	T. orientalis	3	5%	
	T. buffeli	1		
				80%
	R. microplus			
	T. velifera	1	5%	
	T. orientalis	3		
Goat	***R. microplus***			
	T. orientalis	1	1%	10%
Sheep	***R. microplus***			
	T. orientalis	1	1 %	10%

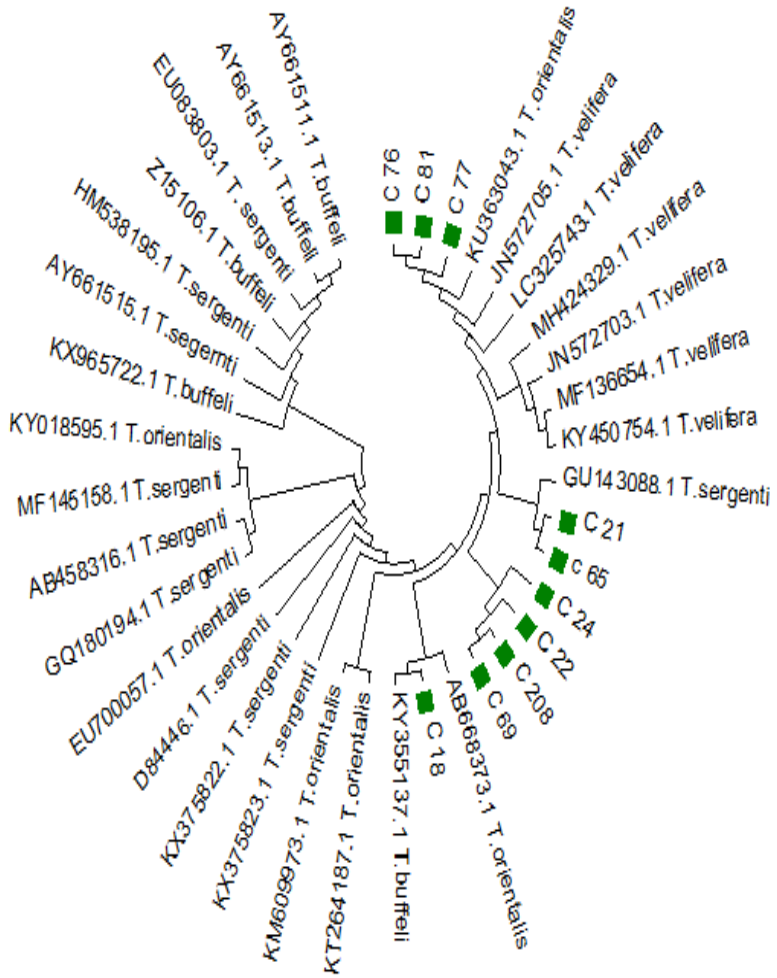

Figure 4. 10 Phylogenetic tree of 18S rDNA gene sequences of *Theileria* spp. Sequences in greed dots are rfofm this study with related reference sequences from Genbank. Phylogenetic tree was constructed in MEGA 6 by use of the neighbor-joining method.

Table 14 Reference strains used to construct a phylogenetic tree of *Theileria* spp.

Accession Numbers	**Species**	**Geographic of origin**
KU363043	*T. orientalis*	China
JN572705	*T. velifera*	South Africa
LC325743	*T. velifera*	Putao

MH424329	*T. velifera*	DRC
JN572703	*T. velifera*	South Africa
MF136654	*T. sergenti*	Mozambique
KY450754	*T. orientalis*	Sudan
GU143088	*T. buffeli*	Taiwan
AB668373	*T. orientalis*	Japan
KY322137	*T. orientalis*	Oman
KT264187	*T. orientalis*	Thailand
KM609973	*T. sergenti*	Kerala
KX375823	*T. sergenti*	Italy
D84446	*T. sergenti*	Japan
EU700057	*T. orientalis*	India
GQ180194	*T. sergenti*	China
AB458316	*T. sergenti*	Japan
MF145158	*T. sergenti*	China
KY018595	*T. orientalis*	South Korea
KX965722	*T. buffeli*	South Korea
AY661515	*T. sergenti*	Japan
MH538195	*T. sergenti*	Nigeria
Z15106	*T. buffeli*	South Africa

EU083803	*T. sergenti*	China
AY661513	*T. buffeli*	USA
AY661511	*T. buffeli*	USA

4.3.4 GenBank accession numbers

Sequences obtained in this study have been deposited in the GenBank database under the following accession numbers: MK347206-MK347215 (tick identification), MK405447-MK405477 (rickettsia *ompA* gene), MK405386-MK405446 (rickettsia *ompB* gene) and K405375- MK405385 (theilerial species). Reference strains used for phylogenetic analysis in this study are represented in the phylogenetic trees constructed.

CHAPTER FIVE

Discussion

5.1 Identification of Distribution of ticks from the designated study sites

Ticks are known to convey and transmit remarkably diverse range of zoonotic agents such as bacterial and protozoan pathogens. Besides causing skin damage, discomfort and reducing productivity, ticks have been rated the second as transmitters of vector-borne diseases, as they cause serious limitation on exportation of animal products.

Furthermore, there has been a growing burden over the past decades concerning their distribution and emergence in ecological areas where they hitherto have not been found (Mtshali, 2012). The global distribution and redistribution of ticks has been spurred by climate change, migratory animals and increased in the global trade of animals.

In this study, 994 ticks were collected in a period of 4 months (May to September 2018) from different animals including horses, cattle, goats and sheep around the Raymond Mhlaba Local Municipality in the Eastern Cape of South Africa. Ticks collected belonged to six species distributed among 3 genera which were *A. hebraeum* (37,5 %), *R. appendiculatus* (16%), *R. microplus* (13,1%), *R. simus* (11,2 %), *R. eversti eversti* (8,6 %), and *Haemaphysalis* spp. (9,3%).

Few investigative researches done in the Eastern Cape Province are supporting the finding of the study, as Nyangiwe *et al.,* (2013) reported the displacement of *R. decoloratus* by an introduced invasive species of *R. microplus* predominantly collected from cattle and goats in two communally grazed fields in Eastern Cape Province.

This report strongly supports the finding of this study, as the collection of *R. microplus* was obtained frequently in cattle, goats and in sheep as summarised in Figure 4.1. Nyangiwe *et al.,*

(2017) further reported the *R. microplus* in different regions of South Africa including North West, Free State and Western Cape.

However, in Western African regions, *R. microplus* was confirmed to colonize more than a half of the West African countries in less than a decade (de Clercq *et al.,* 2012). According to literature, this species was recently introduced (Nyangiwe *et al.,* 2017) and furthermore this study shows its distribution as it is now colonizing the Eastern Cape of South Africa.

A study done by Iweriebor *et al.,* (2017) detected the presence of *R. eversti eversti* and was found to be the most prevalent followed by *R. sanguineus*, *R. appendiculatus*, *A. hebraeum* and *H. marginatuni rufipes* in the Eastern Cape Province. The findings are partially agreeing to the obtained ticks, although in contrast with the absence of *H. marginatuni rufipes* and *R. sanguineus*.

A conceivable reason for the absence of these species in this study could be factors such as seasonality and favourable environmental conditions, as the collection sites were on different regions of the Province. The absence of *R. sanguineus* can be explained by the fact that it is identified as an exclusive parasite of domestic dogs according to the finding of Dantas-Torres *et al.,* (2017), of which during the collection in this study, no ticks were collected from dogs.

R. appendiculatus is known as a significant rhipicephalid tick distributed in eastern and Southern Africa where it occurs on a wide variety of domestic and wild ruminants and has been confirmed in many Provinces of South Africa including Gauteng, Free-State, Eastern Cape and Kwazulu-Natal (Mtshali, 2012; Wanzala *et al*., 2014).

According to Horak *et al.,* (2002), *R. appendiculatus* is identified to cause paralysis in sheep, although in the present study, it was collected from cattle and goats. Similarly, recent report made in the three provinces of South Africa including Eastern Cape, has confirmed the

collection of *R. appendiculatus* from cattle and goats (Guo *et al*., 2019). *R. appendiculatus* has been recorded as one of the rhipicephalid ticks that habitually feed on humans with eighteen records reported in 2000, globally (Horak *et al.*, 2002). This makes it possible for humans to be bitten by ticks as they keep their livestock close to human dwellings mainly in the villages of Eastern Cape Province.

Another species that has newly drawn attention in livestock industry is the *R. eversti eversti*.In this study *R. eversti eversti* was collected from cattle, sheep and goats, although Horack *et al.*, (2017) reported this species in all horses from the nine provinces of South Africa. However, its distribution is mostly reported in sub-saharan Africa and is best know as the main leading vector of protozoan pathogens including *Babesia* and *Theileria* spp. (De Waal and Potgieter, 1987). Iweriebor *et al*., 2017, have further reported *R. eversti eversti* as a vector of *Erhlichia*.

R. simus is an identified ixod tick capable of feeding on humans as it has been collected in horses and donkeys as reported by Horack *et al.*, (2017). This species is associated with different tick-borne pathogens including *Anaplasma centrale* (Bell-Saky *et* al., 2015) and this tick species has been found in Free State, Gauteng, Limpopo and in Eastern Cape (Horack *et al.*, (2017).

The distribution of *R. microplus* is confirmed in many continents including Asia, Africa and in Australia (Ali *et al*., 2016; Baron *et al*., 2018). This species has been associated with many pathogenic diseases causing agents including viruses as documented by Maruyama *et al*., (2014) in a study that analysed a salivary gland transcriptome from female tick *R. microplus* collected in cattle and confirmed the presence of viral genes of flavivirus. Also, bacterial pathogens such as *Ehrlichia ruminantium* was recently detected from *R. microplus* tick in the western region of Africa (Biguezoton *et al*., 2016).

Based on the morphological and genetic identification of the ticks collected from this study, it is confirmed that *A. hebraeum* (37,5%) is the most prevalent species. However, this species has been reported for its adverse economic role across the African continent including South Africa, Swaziland, Botswana, Zimbabwe and southern Mozambique (Akanyang *e al.*, 2013; Madder *et al.*, 2014; Sungirai *et al*., 2015; Halajian *et al*., 2016).

In the South African country, Horak *et al.*, (2015) found the spreading of introduced adult *A. hebraeum* species collected from white rhinoceroses on four privately owned farms, in Free State Province and further noticed *A. hebraeum* detected from rhinos in Gauteng, Limpopo and North West Provinces. However, *A. hebraeum* existence is present along the Southern and Eastern seaboard of South Africa, from around Port Elizabeth in the west to Southern Mozambique in the East (Horak *et al*., 2017).

Regarding the concern on the distribution of this species, Bournez *et al.*, (2015) have suggested that it is in south-east African regions where there is a unimodal annual rainfall pattern, as it is mainly during the rainy season (September to April) that *A. hebraeum* adults are observed on hosts. Therefore, this report is supporting the findings of this study, where adult tick of *A. hebraeum* was collected around the same season, thus suggesting that, the distribution of this tick species is associated with the changes in climatic conditions and availability of their host.

Moreover, *A. hebraeum* species in this study was detected to parasitize and obtain its blood meal on cattle, sheep and goats. Similarly, Maina *et al.*, (2014) also encountered *Ambylomma* spp. in domesticated animals. Several reports have confirmed *A. hebraeum* as a vector for many tick-borne pathogens including *Erhlichia ruminantium*, which is linked to heartwater disease that is frequently fatal in infected cattle, goats, sheep and some wild ruminants (Allsopp, 2015).

Previous studies conducted by Lubinga *et al.*, (2014) have provided with evidence of transstadial and mechanical transmission of Lumpy Skin Disease caused by *Capripoxvirus* by

A. hebraeum. Fever, enlarged lymph nodes and multiple nodules all over the body of an animal characterize this disease, though it is not pathogenic to humans.

Immature stages of *A. hebraeum* have the propensity to infest small mammals and birds, of which they play a significant role in dispersing the tick (Jongejan and Uilenberg, 2004). The nymph and adult stages of *A. hebraeum* are implicated in the transmission of the etiologic agent of heartwater disease which affects various species of domestic animals and wild ruminants.

The majority of tick species in this study were collected from cattle, hence suggesting that cattle could be possible communal reservoirs of tick species and diseases. Reports of Pesquera *et al.,* (2015) agrees with the finding of this study, as they detected the presence of tick-borne pathogens (*Anaplasma, Erhlichia* and *Rickettsia*) in cattle.

Subsequently, the epidemiology of zoonotic diseases has been associated with availability of their tick reservoirs (Estrada-Pena, 2015). This however enhances knowledge concerning the spread of zoonotic pathogens in this Province, as the study revealed an abundance of different tick species capable of vectoring these pathogens.

The province of Eastern Cape in South Africa is particularly reputed with small-scale farming with animals being kept in close proximity to each other and to households. This leads to continuous spread of diseases, with one tick infecting more than one host. Nonetheless, distribution of tick species with diseases could also be attributed to natural migration of birds and animals leading to transportation of ticks and tick borne pathogens into new areas. Export of animals for economic purposes from one place to another has lead to introducing of new exotic tick species.

5.2 Prevalence of Rickettsia in ticks collected from the study

In the present study, genetic analysis obtained showed the occurrence of *Rickettsia* spp. (60) which are of medical importance and are members of spotted fever Group Rickettsiae. The identified species have added to the epidemiological records of emerging zoonotic tick-borne pathogens in the Eastern Cape, South Africa. In the present study, rickettsial DNA was detected from four genera of ticks namely, *Amblyomma, Rhipicephalus, Haemaphysalis* and formerly *Boophilus*, collected from domesticated animals.

The findings obtained indicate that *Rickettsia* is present in ticks from Raymond Mhlaba Local Municipality at the Eastern Cape, South Africa as previously reported by Iweriebor *et al*., (2017), who detected the incidence of Spotted fever Group rickettsiae species in ticks from different localities of Eastern Cape, South Africa, although in contrast with the rickettsial species detected in this study. This however reveals a diversity of species of *Rickettsia* emerging and infecting animals and humans is an increasing rate in the Eastern Cape Province.

Members of Spotted fever group rickettsiae have been reported globally in different cases of illnesses in both humans and animals with the first report of pathogenic spotted fever in Africa, isolated in a patient from a tick bite in Zimbabwe (Kelly *et al*., 1994).

New epidemiological data of spotted fever have been reported in different parts of Africa and in the Baltic regions of Europe for the first time with *Ixodes ricinus* and *Dermacentor reticulatus* being the vectors of *Rickettsia helvetica* and *Rickettsia raoultii* (Radzijevskaja *et al*., 2015). SFGR causes diseases with various symptoms ranging from mild to life-threating illnesses and complications such as renal failure, purpura fulminant, and severe pneumonia as reported from three patients in Australia (McBride *et al.*, 2007).

According to Walker (1996), after the pathogen enters the targeted cells and reproduces by binary fission within the cytosol, it spreads through the bloodstream to infect the endothelium,

and ultimately damage severely parasitized cells directly. Hence rickettsial pathogens cause several diseases such as rickettsial pox, Rocky Mountain spotted fever (RMSF) and African tick bite fever (ATBF) in humans. General clinical characteristics associated with SFG include severe headache, fever, characteristic rash and malaise (Gajda *et al.*, 2017).

Several studies have determined the role played by rodents as reservoirs of SFGR. Gajda *et al.*, (2017) conducted a study using the spleen and blood of wild-living rodents for the detection of Spotted Fever Group Rickettsiae in Southwest Poland (Gajda *et al.*, 2017) with other studies comparing the sensitivity of conventional PCR and real-time PCR assays for the detection of rickettsial DNA in tissue samples from *Rickettsia*-infected laboratory rodents (Zemtsova *et al.*, 2015).

These studies confirmed that rodents are one of the reservoirs of SFG in the wild, leading to distribution and epidemiology of these pathogens in wildlife. Nonetheless, species of SFG Rickettsiae are a threat to animals and humans all around the world with some species emerging in new areas, while increasing the distribution and epidemiology of these pathogens as these pathogens are recently detected on new host reservoir.

Genetic analysis of sequence data obtained in this study estimated that partial DNA sequences are 98.2% similar to *R. africae*, while one sequence (B209) rickettsial species of spotted fever group had 100% homologous with *R. tamurae* (DQ113910) and another (B188) sequence had 99.1% homologous to *R. parkeri* (KY113111). Consequently, phylogenetic data analysis are agreeing with genetic analysis as all the sequence of *omp*A gene are clustering with *R. africae* (Figure 4.5), while in *omp*B gene one isolate clustered with *R. parkeri* as illustrated in figure 4.6.

R. africae is the agent of African tick-bite fever, which has been identified as an increasing incidence of acute febrile illness in pastoral and diverse farming communities across the

African continent (Faburay *et al*., 2015). Historically, it was demonstrated that a strong geographic relationship exists between the prevalence of *R. africae* illness and incidence of *Amblyomma* species (Fournier *et al*., 1998), although molecular analysis in this study has shown that the prevalence of *R. africae* is not only observed among *Amblyomma* spp. but also in other genera such as *Haemaphysalis* and *Rhipicephalus*.

Further, this finding shows that the prevalence of *R. africae* is not restricted to a specific tick vector, but depends on the availability of ticks and host-interaction. Studies in Botswana support the findings from this study on the abundance of *R. africae* from different tick spp. including *R. decoloratus* tick with 77% prevalence and 20% prevalence in *Ambylomma* collected in oryx (Portillo *et al*., 2007).

Also in the West bank, *R. africae* infection was detected in *Rh.sanguineus, Rh. turanicus, H. parva , H. adleri, H. impeltatum* and *H. dromedarii* ticks collected from sheep, goats, dogs, camels, a tortoise, a wolf and a horse (Ereqat *et al*., 2016). Similarly, Ogo *et al*., (2012) reported the detection of *R. africae* from various tick species of *A. variegatum, R. (Boophilus) decoloratus*, and *R. sanguineus* with the prevalence ranging from 4%- 8%. These reports are in support of the results obtained in this study as the *R. africae* was found in different genera of ticks collected from different domestic animals.

Several studies have confirmed that this pathogen is of veterinary importance as it causes severe loss in economy especially in the earning of local populations and equally causing infections among humans. According to previous studies, *R. africae* is widely distributed in all the regions of the African continent (Ogo *et al*., 2012) and has been detected by PCR in many regions, including Burundi, Mali, Sudan, and Niger and in most sub-tropical countries and South Africa. Recently, Vanegas *et al.,* (2018) confirmed *R. africae* in Cameroon.

Spotted fever caused by *R. africae* has become prevalent in the past 30 years and is identified as African tick bite fever illness in humans (Mediannikov *et al.*, 2010).

The first confirmed human case of African tick-bite caused by *R. africae* in Africa was documented in a man from Zimbabwe in the year 1992 (Stephany *et al*., 2009) and within a period of 5 years, there was an outbreak of 13 human cases of *R. africae* in South Africa (Fournier *et al.*, 1998). Eight patients, from age 63–75 years showed African tick bite fever symptoms after a trip to South Africa and the diseases was confirmed by eschar biopsy specimens obtained from the patients (Roch *et al.*, 2008). Symptoms and clinical characteristics from the patients included swollen lymph nodes, muscle aches, headache, reactive arthritis and fever, although these symptoms are typically common in other diseases. Patients with severe rickettsial disease may experience oliguria and anaemia (Roch *et al.*, 2008).

Historically, sero-prevalence of *R. africae* has been detected and found to be endemic around African countries, although recently; it is distributed to other continents including North America, where it was detected to be 99.6% homology from 576-bp *omp*A sequence from *A. ovale* ticks (Vogel *et al*., 2018).

Herbert *et al.*, (2014) reported the prevalence of *R. africae* in *A. naponense* in Brazil. Furthermore, in the Oceanica, DNA isolated from *A. loculosum* collected from human and birds was found containing rickettsial DNA which had 100% similarity with the relevant genes of strain ESF-5 *R. africae* from Ethiopia with the Accession number CP001612.1 (Eldin *et al*., 2011). The distribution of African tick-bite to other continents can be due to unlimited trading of animals and migratory birds from Africa to other continents. Furthermore, travelers from United States have been diagnosed with African tick bite fever after visiting Africa (Binder *et al*., 2015; Cherry *et al*., 2018).

R. parkeri is a bacterium, which is identified as a human pathogen. Molecularlly, *R. parkeri* sequence (188) obtained in this study was 99.1% similar to strains of *R. parkeri* (KY113111) detected in Brazil. This species has not been reported in the African continent previously, thefore the *R. parkeri* reported in the present study, represents its first molecular confirmation in the entire African continent. *R. parkeri* is prevalent in South and North America where its causes American tick bitr fever that have similar characteristic symptoms like the ATBF. *R. parkeri* has been found in other countries including Brazil (Weck *et al.,* 2017) and Uruguay where a human cases were reported and the patients ususally suffer from headache, malaise, fever and arthralgia (Faccini-Martíne *et al*., 2018). Additionally *R. parkeri* infection was diagnosed by eschar biopsy in the Unites States of America (Kelman *et al*., 2018).

In the present study, *R. parkeri* DNA was detected in *Ambylomma* spp.. The findings are therefore congruent with the previous published studies, which confirmed *R. parkeri* in different *Amblyomma* spp. (Venzal *et al*., 2004; Melo *et al*., 2015; Weck *et al.*, 2017). Although recently Paddock *et* al., (2017) detected this species on *Dermacentor* tick and this data is further supported by Sánchez-Montes *et al*., (2018) who also found *R. parkeri* on the same species of *Dermacentor*. In addition, *R. parkeri* is also reported to be vetcored by *Rhipicephus microplus* tick as confirmed by Cordeiro *et al*., (2017). Nonetheless, *R parkeri* has no specific tick vector.

Partial sequence of *omp*B B209 obtained was 100% homology to *R. tamurae* (DQ113910) isolated from Japan from an *Amblyomma testudinarium* tick. Most epidemiological reports on *R. tamurae* are based on Asia; and the pathogen is identified as the human etiological agent in different countries of Asia including Southeastern Asia (Aung *et al*., 2014), Japan (Thu *et al*., 2019), China (Nooroong *et al*., 2018) and in Malaysia (Kho *et al*., 2018). However, this is the only study confirming the presence of *R. tamuare* in the African Continent.

The distribution of *R. tamuare* in the Asian continent is explained by factors such as land use, which is said to be a driver of emerging infections and increased access to forest areas (Satjanadumrong *et al*., 2019). However, in the Eastern Cape Province, the livestock normally graze on the forest; this could be the reason behind the emergence of *R. tamurae* in the locality of Raymond Mhlaba Municipality.

Amblyomma testudinarium is a common identified vector of *R. tamurae* that infects humans which is characterised by the following clinical characteristics like skin wounds, erythema, and muscular pain in human (Nakao *et al*., 2017). *R. tamurae* has been associated with different *Amblyomma* spp. as reported by Blanco *et al*., (2017) who collected and screened nymphs of *A. ovale* tick from small mammals such as wild rodents and marsupials in Brazil. In contrast, recent reports have stated the new vector of *R. tamurae*, as it was isolated from a *Haemaphysalis megaspinosa* tick (Nooroong *et al*., 2018).

Furthermore, it can be concluded that, the bacterial pathogens detected in the present study are distributed amongst available tick species, and could possibly lead to distribution of these pathogens in the Eastern Cape region.

The finding of this study therefore highlights the perception that rickettsioses might be under reported and most of the time it could be misdiagnosed as other infections with symptoms of fever globally, hence clinical diagnosis is essential.

According to South African statistics, Eastern Cape is regarded as the poorest province in terms of GDP per capita income. However, agricultural practice such as small-scale farming dominates its economy so tick-borne pathogens and their diseases have the capacity to negatively affect subsistence agriculture thus, leading to poor yields and less profit for survival. The cost associated with ticks prevention using acaricides and treatment of associated diseases is relatively high, and this raise food security concerns.

Additionally, with regards to the study sites where ticks were collected, animal shelters were in close proximity to human dwelling, yet the animals were allowed to freely graze from the forest during the day. This explains how infected ticks from wild animals can be transferred to domestic animals, and possibility of spreading the pathogens from domestic animals to humans. Normally, symptoms of tick-borne illnesses are sometimes misdiagnosed as malaria, and consequently leading to chronic complications and public health impact, hence diagnosis is essential.

5.3 Prevalence of Theileria spp. in ticks collected in the study

In the present study, occurrence of *Theileria* spp. in ticks was investigated in a total of 994 ticks collected in the Raymond Mhlaba Local Municipality at Eastern Cape Province of South Africa. The overall prevalence of *Theileria* detected was 1% (10/994) in ticks collected from sheep, cattle and goats on one study site (Debe Location). This could be due to free-ranging animals in that location as they were permitted to graze in the wild, while in Fort Beautfort location animals were restricted on enclosures and therefore remained un-infected.

According to Kocan *et al*., (1991) piroplasmid pathogens are frequently associated and infecting free-living animals globally. Correspondingly, *Theileria* spp. was confirmed in Korea from grazing indigenous cattle with a high infection rate of 54.7% compared to non-grazing Cattle (Kim *et al*., 2017), thus supporting the findings of this study.

Amplification and DNA sequencing of 18S rDNA gene was crucial for the identification of *Theileria* species and for delineation to the species level, obtained sequences were subjected to BLAST and we obtained ≥98% homology to published sequences of *T. orientalis, T. buffeli* and *T. velifera.*

An alignment of the generated 18S rDNA sequences with those representatives of *Theileria* spp. by BLAST showed that all ten sequences were *Theileria* spp.. Sequences C18, C22, C24,

C65, C76, C77 and C81 belonging to *T. orientalis* with homology above 98%, while sequences C21, C69 and C208 were assigned to *T. buffeli* and *T. velifera* with percentage homology of 99 and 96% respectively.

Phylogenetic analysis indicated high likelihood of the obtained C18 sequence clustering with 18S rDNA *T. buffeli* (KU355137) detected in buffaloes from Brazil, while sequence C77 clustered with *T. orientalis* (KU363043) obtained from infested cattle from China. C61, C21 sequences were on the same clade with *T. sergenti* (GU143088) detected in horse from Taiwan (Figure 4.8).

Respectively, the high homology obtained from the 18S rDNA sequences and previously isolated nucleotide sequences of *Theileria* suggests that the parasite is widely circulating among domestic and wild animals across the globe. Over the past decades, reintroduction and trades in animals could have played a huge role on the observed high homology internationally, hence the high homology exist between *Theileria* species detected from this study with those of other *Theileria* spacies prevalent across the globe.

Theileria spp. are habitually known for their severe economic impacts on the livestock industry. The detected *Theileria* spp. have been reported in several parts of the world, with the significant impacts in game reserve and animal husbandry in general. The first described cases of *T. orientalis* and *T. velifera* was in eastern Siberia, while *T. buffeli* was first confirmed from the Asian water buffalo as reported by Fujisaki *et al*., (1994).

T. velifera is a protozoan pathogen recognised to cause illnesses such as enlargement of glands, loss of milk production and loss of appetite in cattle. *T. velifera* has been detected by Githaka *et al*., (2014) in cattle from Kenya and this pathogen is transmitted by the ixodid tick, *Rhipicephalus appendiculatus*. Yusufmia *et al*., (2010) also identified species of *T. velifera* with 70.0% prevalence in cattle from KwaZulu-Natal Province in South Africa.

Several epidemiological studies on *T. orientalis* have been reported in many countries such as Turkey, France and Spain (Aktas *et* al., 2006; Altay *et* al., 2008; Gimenez *et al.,* 2009). Although in African countries, few studies have reported *T. orientalis* infesting cattle from Ngong Farm in Kenya (Moumouni *et al*., 2015). Strains of *T. orientalis* were also documented in Ethiopia (Gebrekidan *et al.,* 2016).

In South Africa, strains of *T. buffeli* were detected in few Provinces from blood samples of roan antelope with an infection rate as high as 100% (Berggoetz *et al*., 2014). A co-infection of this species with other theilerial species (*T. ovis* and *T. bicornis*) and *Anaplasma* spp. (*A. centrale, A. platys* and *A. ovis*) (Berggoetz *et al*., 2014) have equally been documented. Although the incidence of *T. buffeli* is quite low in South Africa, Chaisi *et al*., (2014) managed to identify *T. buffeli* in Kwazulu-Natal buffaloes in South Africa. Nevertheless, this is the first study reporting the occurrence of *Theileria* species in Raymond Mhlaba local Municipality at Eastern Cape Province.

Based on molecular data from various studies, *T. buffeli, T. sergenti* and *T. orientalis* have been determined to be closely related and therefore have been reclassified as the same species of *Theileria*. Due to similarity in morphology, geo-graphical distribution, serology, vector transmission and incompletely understood life cycles, major Piroplasm Surface Protein (MPSP) and 18S rDNA sequences studies have chosen these parasites as the *T. sergenti/T. buffeli/T. orientalis* group of benign *Theileria* (Kamau *et al*., 2011). Therefore, *T. orientalis/T. sergenti/T. buffeli* is considered as *T. orientalis* complex.

From the six species of ticks collected only two species vectored *T. orientalis* complex. *Theileria* spp. in this study were confirmed to be vectored by *Rhipicephalus* spp. and *R. microplus.* The findings are congruent with that of Kakati *et al*., (2015) in India, who detected the presence of *T. orientalis* from ten *R. microplus* spp. with an overall infection of 83%.

However, Kukhuu *et al*., (2011) found none of the common vectors species, *Amblyomma* , *Haemaphysalis* spp. and *Dermacentor* spp. as carriers of *T. orientalis*. Although previous studies have reported that *T. orientalis* is transmitted transtadially by different species of *Haemaphysalis* ticks including *H. longicornis* (Fuujisaki, 1992; Hammer *et al.,* 2015) which is in contrast with the findings of the study as there was no sequence of *T. orientalis* in *H. longicornis*. This could be attributed to non-host specificity of *T. orientalis* and thus confirming that *T. orientalis* is equally vectored by *Rhipicephalus* spp. and the availability of any tick species. Conclusively, this is the first study in Africa confirming the presence of *T. orientalis* from *R. microplus* tick.

T. sergenti/buffeli/orientalis group is identified as a benign bovine protozoan species that occasionally lead to serious economic loss in the livestock industry. Several studies have reported the infestation of *T. orientalis* in cattle and buffaloes around the world (Kamau *et al.,* 2011) and it correlate with results obtained in this study where *T. orientalis* strains were obtained from ticks infesting cattle, sheep and goats.

Reports by Yang *et al*., (2014) are strongly consistent with the findings of this study, as the prevalence of *T. sergenti/buffeli/orientalis* DNA was detected in 30.8% of the cattle, 53.2% of sheep and 44.4% of the goats in China.

Also, Elsify *et al*., (2015) detected the occurrence of *T. orientalis* in cattle, sheep and buffaloes. Similarly, strains of *T. orientalis* have been reported in many animals including wild animals such as ungulates and red foxes, (Najm *et al*., 2014). This study suggests that there is a correlate existence of a common epidemiological cycle among wildlife and sympatric domestic animals.

T. orientalis has been identified to cause bovine theileriosis. Few studies reported *T. orientalis* as asymptomatic in livestock as it caused mild anaemia. Recent outbreaks have presented this pathogen as an emerging parasite as evidenced by a number of clinical outbreaks characterized

by fever, anaemia, jaundice and abortion and even mortality recorded from several countries (Kakati *et al*., 2015; Gebrekidan *et al*., 2017).

The majority of *T. orientalis*-infected cattle become chronic carriers of the parasite, occasionally developing severe or fatal anaemia under certain unfavourable illnesses. Furthermore, prenatal infection due to intrauterine transmission of *T. orientalis* may be a chronic risk factor that threatens cattle health from generation to generation (Kim *et al*., 2017). Much attention has been paid recently, as records of fatal disease due to *T. orientalis* in crossbred adult bovines infested with *Haemaphysalis bispinosa* has increased considerably in Southern India (Kakati *et al*., 2015).

One of the major significant reasons for emergence of these *Theileria* species from other continent to Africa is uncontrollable animal migration, trading, and transportation of animals for economic reasons; although increased human mobility, population growth, and climate change might probably constitute major risk factors for geographic expansion to new areas of sub-Saharan Africa. As determined by the study, distribution of tick species in Eastern Cape have become a growing burden on the transmission of new *Theileria* pathogens being introduced which could later become increasingly ubiquitous.

Limited literature is available concerning the epidemiology and prevalence of these theilerial species in South Africa. This study was therefore necessary in order to add knowledge on the importance of protozoan tick-borne pathogens in the epidemiological database of the country. Increased wildlife-human interactions due to socio-economic changes could enhance the risk of contracting theleriosis.

Thus, it is therefore significant to conduct additional studies to establish the correlation between the pathogenic types and clinical signs of diseases they may likely cause among humans. Further studies are therefore necessary to precisely detect and distinguish these novel

genotypes in hosts and tick vectors in South Africa. Nevertheless, this study provides beneficial genetic data towards the correct classification of this very complex group.

In conclusion, we have established the phylogeny of *T.buffeli*/ *orientalis* and *T. sergenti* species occurring in cattle, sheep and goats with the corresponding vectors of ticks from the Local Municipality of Raymond Mhlaba in the Eastern Cape Province.

5.4 Conclusion

Ambylomma, Rhipicephalus and *Haemophalis* were the three genera of ticks collected in this study, and found infesting cattle, sheep, goat and horses. Five targeted genes were investigated in this study; however, two pathogens were successfully detected by PCR. Sequencing of the purified amplicons played a role in delineation of the different species of the isolated pathogens, and compelling confirmation showed prevalence of *R. africae, R. tamuare, R. parkeri,* and *T. orientalis* complex in Raymond Mhlaba Local Municipality at Eastern Cape Province.

The hypothesis of the study is nullified because tick vectors documented in the Municipality harboured a number of tick-borne pathogens. The finding of the study expands the knowledge on the epidemiology and distribution of tick-borne pathogens around localities of the Eastern Cape Province in South Africa. Thus, the findings highlight the importance of theilerial and rickettsial species as emerging pathogens for the first time in Raymond Mhlaba Local Municipality, Eastern Cape Province of South Africa.

5.5 Recommendations

The findings are of great significant in raising awareness in local communities on the incidence of emerging tick-borne pathogens. This study recommends that farmers should avoid keeping their livestock in close proximity to their dwellings to avoid transmission of these pathogens

to humans. It is necessary to enhance the knowledge about prevention of such diseases, as it is impossible to eradicate ticks.

A frequent dipping is crucial in animals to minimize the distribution of ticks. Clinicians and Veterinarians should be aware of the clinical signs of tick-transmitted diseases and infections among humans and domestic animals. Application of diagnostic assays must be considered to determine the prevalence of infections. For tourist visiting game reserve in South Africa should be aware of prevention measures for instance wearing clothes that cover the entire body to avoid tick bites.

It is therefore necessary to enhance further studies on the distribution of ticks on the study sites and genetic screening for prevalence of other tick-borne diseases on other Municipalities around Eastern Cape and to all nine Provinces of South Africa.

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