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OCCURRENCE OF TICK-BORNE HAEMOPARASITES IN SELECTED SOUTH AFRICAN
WILD RODENT SPECIES IN THE MNISI COMMUNAL AREA

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Liesl De Boni
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DECLARATION

I, Liesl De Boni, declare that this mini dissertation is my own original work. Where other people's work has been used (either from a printed source, internet or any other source), this has been acknowledged and referenced in accordance with University of Pretoria requirements. I have not used work previously produced by another student or any other person to hand in as my own. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

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Candidate: Dr Liesl De Boni
Supervisor: Professor Marinda C. Oosthuizen
Co-supervisor: Professor Kelly A. Brayton
Co-worker: Dr Agatha O. Kolo
Department: Veterinary Tropical Diseases
Degree: MSc (Animal/Human/Ecosystem Health)

SUMMARY

In 2013, a disease survey of people in the Mnisi community, Bushbuckridge, Mpumalanga, South Africa revealed a surprisingly high level of exposure to tick bite fever, *Bartonella*, Q fever and leptospirosis. This indicated the need to educate the community about ectoparasites, exposure to rodents and general hygiene. In addition, the potential contribution of rodent pathogen transmission to human febrile illnesses is considered a public health concern and warrants further investigation.

Most human tick-borne diseases are considered emerging diseases because the number of reported cases annually is increasing. This is probably due to ecological changes, increased awareness and an increased number of susceptible individuals in the global population. Conservation efforts have increased numbers and ranges of host animals which maintain the infected tick population and humans are increasingly encroaching into tick dense areas. Thus, humans are more exposed to tick-borne infectious diseases. Increased awareness by doctors and the public has also contributed to the increased reporting of human tick-borne disease. With major medical advancements, there is an increased proportion of the population that is more susceptible to these infectious diseases.

Vector-borne parasitic zoonoses are an important aspect of a constantly changing world because they are also constantly adapting to their new circumstances. Infectious organisms can change virulence, hosts and vectors. These diseases are, therefore, very much a part of

the future. With new advances in molecular biology, detection of infectious diseases is constantly improving.

The economic impact and losses due to vector-borne diseases are wide and felt at both the human health and animal health levels. Losses occur due to the cost of human illness, deaths, reduced animal production and costs to control or prevent disease. In developing countries where animals are relied on for work, transportation and nutrition, the losses are more profound.

The order Rodentia is the most abundant and diversified order. Rodents are distributed all over the world except for Antarctica. They often live in close contact with humans, their farm animals and their pets. Rodents pose a threat to humans in several ways. Not only do they act as reservoirs for infectious diseases and serve as hosts for vectors, they also compete globally with us for food, causing serious pre-harvest damage to cereals every year. Examples of rodent-borne human illnesses include rickettsiosis, babesiosis, human granulocytic anaplasmosis, leptospirosis, bartonellosis, tick-borne relapsing fever, Lyme disease and Q fever. More information about these diseases and their impact on public health in an African context is needed.

This mini dissertation is divided into two parts. The first is a narrative literature review on what is known about the common rodent-borne bacterial and parasitic pathogens which have an important impact on public health and their relevance in Africa. The current knowledge is described and recommendations for further research are given. The second part is a laboratory project which focused on determining the incidence of selected tick-borne pathogens found in wild rodent species in the Mnisi community, a livestock-wildlife-human interface area in South Africa. Forty wild rodent blood samples underwent a broad-based screening approach using reverse line blot (RLB) hybridization and a more specific approach using quantitative real-time PCR (qPCR) techniques.

The RLB results revealed that 7.5% (n=3) of the samples tested were positive for the presence of *A. bovis* DNA, while 2.5% (n=1) were positive for *A. phagocytophilum* DNA; none of the samples tested positive for *B. microti* (n=0). PCR products from 17.5% (n=7) of the samples hybridized with *Theileria/Babesia* group-specific probes, 17.5% (n=7) with the *Anaplasma/Ehrlichia* group-specific probes, 17.5% (n = 7) with both the *Babesia* 1 and 2

genus-specific probes and 7.5% (n = 3) with the *Babesia* 1 genus-specific probe. A total of 57.5% (n=23) of samples were negative (or below the detection limit of the test) for any species for which we tested.

Results of the *B. microti* SYBR green real-time PCR (qPCR) proved difficult to interpret due to the wide melting temperature range observed; nine of the rodent samples (22.5%) produced melting temperatures within 4°C of that of the positive control (i.e. 79.16 - 86.17°C). Going forward we propose to sequence the parasite 18S rDNA gene of these nine rodent samples to determine whether they are indeed South African variants of *B. microti* and/or a novel species.

The qPCR for *A. phagocytophilum* revealed 14 positive samples. This is an incidence of 35% (14/40) in a very small sample size which is relevant and warrants further surveillance for *A. phagocytophilum* in South Africa. Five of the 14 *A. phagocytophilum* PCR positive rodent samples were confirmed by gene sequence analysis. From the 12 partial *msp2* gene sequences obtained, two Msp2 sequence types were identified (designated Ap1 and Ap2); Ap1 differed by one amino acid from Ap2. Ap1 was identical to *A. phagocytophilum* human strain Webster and *A. phagocytophilum* strain Dog2, while Ap2 was identical to *A. phagocytophilum* human strain HZ as well as *A. phagocytophilum* strains previously described from various hosts (including deer, ticks and mite). It should, however, be noted that we could only obtain partial *msp2* gene sequences (334 bp) spanning the conserved area of the gene. Although valuable data, as we could confirm the presence of *A. phagocytophilum* DNA in our samples, full-length gene sequences will have to be obtained if we want to draw any clear conclusions on the genetic variability of the *A. phagocytophilum* strains circulating in the Mnisi communal area.

These findings suggest that *A. phagocytophilum* has already been introduced into South Africa and that it could cause febrile illness in ruminants, horses, dogs and people. The competency of the rodents as reservoir hosts in South Africa and the presence of suitable vectors still needs to be studied. Also, further research is needed to determine if the strain found is a public health threat because there are many variants of *A. phagocytophilum* and not all variants infect humans.

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ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ARDS	Acute Respiratory Distress Syndrome
ATBF	African tick bite fever
<i>A. phagocytophilum</i>	<i>Anaplasma phagocytophilum</i>
ApMSP2 f	Forward primer for <i>A. phagocytophilum</i> R-T PCR
ApMSP2 p	Taqman probe for <i>A. phagocytophilum</i> R-T PCR
ApMSP2 r	Reverse primer for <i>A. phagocytophilum</i> R-T PCR
<i>B. microti</i>	<i>Babesia microti</i>
CCHF	Crimean Congo haemorrhagic fever
CDC	Centers for Disease Control and Prevention
CSF	Cerebrospinal fluid
CFT	Complement Fixation Test
°C	degrees Celsius
DAFF	Department of Agriculture, Forestry and Fisheries
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of the Congo
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic Acid
EhrF	<i>Ehrlichia/Anaplasma</i> forward primer
EhrR	<i>Ehrlichia/Anaplasma</i> reverse primer
ELISA	Enzyme-linked immunosorbent assay
HGA	Human granulocytic anaplasmosis
HIV	Human Immunodeficiency Virus
HGE	Human granulocytic ehrlichiosis (now renamed as HGA)
IFAT	Immunofluorescent antibody testing
IFA	Immunofluorescent Assay
IHC	Immunohistochemical staining
IPA	Immunoperoxidase assay
MIF	Microimmunofluorescence

MSF	Mediterranean spotted fever
M	molar
mg	milligram
μl	microliter
μM	micromolar
ml	milliliter
mM	millimolar
NGS	Next generation sequencing
NICD	National Institute of Communicable Diseases
PCR	Polymerase chain reaction
qPCR	Quantitative PCR
RLB	Reverse Line Blot
RLB-F2	<i>Theileria/Babesia</i> forward primer
RLB-R2	<i>Theileria/Babesia</i> reverse primer
rRNA	ribosomal Ribonucleic Acid
RT PCR	Real Time PCR
SFG	Spotted fever group
SDS	Sodium Dodecyl Sulphate
Smba_F	Forward primer for <i>B. microti</i> R-T PCR
Smba_KR	Reverse primer for <i>B. microti</i> R-T PCR
SSPE	Sodium Chloride Sodium Phosphate EDTA
TAPP	Tick-associated Pathogen Panel Test
TBF	Tick-borne Fever
TBRF	Tick-borne Relapsing Fever
UDG	Uracil DNA Glycosylase
UK	United Kingdom
USA	United States of America

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INTRODUCTION

1. General introduction

In the one hundred and twenty years since it was discovered that arthropods could transmit pathogens causing human diseases, a wealth of viruses, bacteria, protozoa and helminths have been found to be transmitted by hematophagous arthropods (Gubler, 1991; Gubler, 1998). Not only did vector-borne diseases cause global health concerns, they were such a serious problem that they hindered the development of large areas in the tropics, for example, the Panama Canal (Gubler, 1998). Historically, control was quickly achieved by elimination of arthropod breeding sites, and by the 1960s vector-borne diseases were no longer considered a major public health threat outside of Africa (Gubler, 1998). However, this respite was short-lived and by the 1970s vector-borne diseases began to re-emerge. Because of a de-emphasis on control programs and changes in public health policies at this point, the international community found itself ill-prepared for this re-emergence (Gubler, 1998). Vector transmitted diseases remain of major importance to the health of humans and animals globally (Harrus and Baneth, 2005).

Rodents are thought to play a pivotal role when it comes to infectious diseases. Not only do they play a role as hosts for vectors such as ticks, they also act as reservoirs of many infectious diseases. Rodents (and their associated vectors) are widespread and abundant. Examples of rodent-borne illnesses include rickettsiosis, babesiosis, human granulocytic anaplasmosis, leptospirosis, bartonellosis, tick-borne relapsing fever and Lyme borreliosis (Durden et al, 2006; Colwell et al, 2011). In the United States, Lyme disease and human granulocytic anaplasmosis have been identified as two of the most common vector-borne bacterial diseases (Courtney et al, 2004).

The Mnisi community is a rural area of high poverty where inhabitants live livestock-dependent lifestyles. It is in the north-eastern corner of the Bushbuckridge municipal area in Mpumalanga province, South Africa. It is considered a livestock-wildlife-human interface as it shares 75% of its boundary with wildlife conservation reserves. There are approximately 40 000 people living in 8 500 households in the community (Statistics South Africa, 2012). A recent survey (Berrian et al, 2016) showed that 76% of households sampled in 2013

reported seeing rodents in or around their homes. Of those who reported seeing rodents, 62% saw them daily. This indicates the close level of contact between people in this community and rodents. In the same study, they found that 72% of the surveyed households reported owning at least one animal and 68% of households reported owning livestock indicating regular contact with animals which are potential reservoirs for rodent-borne diseases.

To better understand the burden of zoonotic diseases in South African rural communities, the National Institute for Communicable Diseases (NICD) conducted a zoonosis survey of people living in Mnisi. Adult patients with non-malarial acute febrile illness presenting to the Hluvukani community health care centre were offered enrollment into the study. Patients then answered a questionnaire about exposure to vectors, acute blood samples were taken for IgM and IgG serological testing and they were requested to return after two weeks for convalescent blood samples to be taken. Patients were enrolled from October 2012 to June 2013 (n = 119) (Quan et al, 2013) and September 2014 to December 2016 (n = 70) (Anon, 2015; Quan et al, 2017). Very high levels of recent/previous exposure to zoonoses was found. More than 80% of patients had had one of the following zoonoses: African tick bite fever (57-73%), Q fever (9.5-19%), Leptospirosis (1.4-6.8%) and arboviruses (4.2-19%).

During the first study period, 9.5% of patients were suffering from acute bartonellosis and 1.35% of patients had recent brucellosis. In addition, 64 healthy herders and or veterinary staff were included in this study period. In this group 98% showed exposure to at least one zoonosis that was tested for. They found African tick bite fever (92%), Q fever (59%), leptospirosis (22%) and arboviruses such as chikungunya and Sinbis (6.3%) (Quan et al, 2013). From information collected during the second study period, 90% of patients reported having been bitten by a tick during the survey period or previously in their life. Children were not included in the survey but information on 25 children presenting with non-malarial acute febrile illness was gathered (Anon, 2015).

It is known that human febrile illness is a common presenting sign in patients seeking medical care in Africa (Amos et al, 2012). Febrile illnesses are frequently misdiagnosed and understanding of aetiologies is often limited. This leads to misclassification as malaria and subsequent delays in appropriate treatment (Amos et al, 2012; Amexo et al, 2004).

Therefore, the potential contribution of rodent pathogen transmission to human febrile illnesses is a public health concern and should be further investigated.

Recently, wild rodent blood samples (n=423) from four provinces in South Africa were screened for tick-borne haemoparasites using the Reverse Line Blot hybridization assay (dissertation by Milana Troskie, submitted to the Department of Environmental, Water and Earth Sciences, Faculty of Science, Tshwane University of Technology for MSc: Environmental Management). The results revealed the presence of *Babesia microti* (11.3%), *Anaplasma phagocytophilum* (1.8%), *A. bovis* (3.5%), and *Ehrlichia ruminantium* (3.5%) either as single or mixed infections. The presence of a *Babesia microti*-like 18S rDNA sequence was confirmed in the four striped mice *Rhabdomys pumilio* (29%), and *R. dilectus* (5.5%) species. *Anaplasma phagocytophilum* was also recorded in *R. pumilio* (2.3%) and in *R. dilectus* (3.1%). In another investigation, 141 dog blood samples collected from the Mnisi community were screened using the RLB assay (Kolo et al, 2016); 16S rDNA sequences sharing 99% similarity with *Anaplasma* spp. South Africa dog (Inokuma et al, 2005) were detected. In addition, one dog blood sample had a DNA sequence with a 99% sequence similarity to three different strains of *A. phagocytophilum* (Kolo et al, 2016). The detection of these emerging zoonotic agents from domestic dogs and rodents in South Africa highlights the potential risk of human infection that may occur with these pathogens.

2. Aim and objectives

The aim of the study was to do an extensive review of the literature on the occurrence of rodent-borne diseases in Africa and the possible risks for public health. Also, the prevalence and diversity of tick-borne pathogens present in wild rodent species trapped in the Mnisi community, Mpumalanga Province, South Africa have been determined using the Reverse Line Blot (RLB) hybridization assay and real-time quantitative PCR (qPCR) assays for the specific detection of *A. phagocytophilum* and *B. microti*.

The specific objectives:

(i) Perform a narrative literature review that will provide information on rodent-borne bacterial and parasitic pathogens of potential zoonotic importance present in Africa, mainly focusing on their geographical distribution and host range, transmission and arthropod

vectors, as well as clinical signs, diagnosis and treatment. Research gaps will be identified and recommendations for further research will also be provided.

(ii) Screening of wild rodent blood samples (n=40) collected in the Mnisi community, Bushbuckridge, Mpumalanga, South Africa for the presence of haemoparasites using the RLB hybridization assay.

(iii) Specific detection of *A. phagocytophilum* and *B. microti* infections using previously described qPCR assays.

LITERATURE REVIEW

BACTERIAL AND PARASITIC RODENT-BORNE DISEASES OF ZOO NOTIC IMPORTANCE AND THEIR RISKS FOR PUBLIC HEALTH: AN AFRICAN CONTEXT

This literature review describes the major known bacterial and parasitic tick-borne diseases that are found in rodents. Their zoonotic importance in Africa is described as well as geographical distribution, host range, mode of transmission, arthropod vectors, clinical signs, diagnosis and treatment. Due to their public health importance, some rodent-borne diseases (i.e. leptospirosis, bartonellosis, Q fever) are included even though they are not necessarily tick-transmitted.

1. Global Relevance

The order Rodentia is an abundant and diversified order. Rodents are distributed all over the world except for Antarctica. They often live in close contact with humans, their farm animals and their pets. In some places, they provide a connection between wildlife and humans and expose them to zoonoses occurring in natural ecosystems (Meerburg et al, 2009). Rodents pose a threat to humans in several ways. Not only do they act as reservoirs for infectious diseases and serve as hosts for vectors, they also compete globally with us for food causing serious pre-harvest damage to cereals (Stenseth et al, 2003).

Approximately 20% of emerging infectious diseases recorded between 1940 and 2004 are caused by arthropod-borne pathogens (Jones et al, 2008). Ticks are considered one of the most important vectors of both human and veterinary disease. This includes diseases that are both infectious and/or toxic (Andreotti et al, 2011). A literature search of online databases found that the most common reported tick-borne infectious agents that are contracted during recreational activities (such as camping and hiking) include *Babesia* species, *Borrelia* species, *Rickettsia* species, *Ehrlichia* species, *Francisella tularensis*, *Coxiella burnetii*, Crimean-Congo haemorrhagic fever virus and tick-borne encephalitis virus (Jacobs and Schutze, 2004; Natarajan and Miller, 2017; Bratton and Corey, 2005).

Most human tick-borne diseases are considered emerging diseases because the number of reported cases has increased over the last few decades. The reasons for this are

multifactorial, most likely due to a combination of ecological changes, increased awareness and increased susceptible individuals in the population. The increasing desirability of rural living and outdoor activities means that humans encroach on tick dense areas. In addition, conservation efforts have allowed expansion of the range of certain host animals that help maintain the infected tick population (Kjemtrup and Conrad, 2000). This increases human exposure to these diseases. Awareness by physicians, medical doctors and even the public may have contributed to the number of reported cases of human tick-borne disease. An important factor that may have resulted in increased incidence is the expanding number of susceptible individuals. These are: the elderly (people are living longer due to better healthcare), HIV positive people, splenectomised individuals and people on immunosuppressive drugs for immune mediated disease/cancer/after an organ transplant (Kjemtrup and Conrad, 2000).

Colwell et al (2011) examined the factors that influence interactions leading to the emergence of vector-borne zoonotic diseases. Drivers for interaction between humans and parasite vectors include environmental changes (urbanisation), climate change, global travel/trade, catastrophic events (earthquake, tsunami), demographics (increased proportions of young people in developed economies), human behaviour (movement from urban to rural environments) and changes in land use (Harrus and Baneth, 2005; Colwell et al, 2011). Moreover, the global economic crisis and climate change (specifically the worsening of droughts in Sub-Sahara Africa) has resulted in changes in human habits and increased movements of people from developing to industrialised areas (Ramin and McMichael, 2009). Vector population numbers and distribution can change because of ecological and climatic changes (Rosenthal, 2009), international commerce, global transport (van der Weijden et al, 2007), insecticide/acaricide/drug resistance (Takken and Knols, 2007) and changes in land use (Gubler, 1998). Vector-borne parasitic zoonoses are an important aspect of a constantly changing world because they are also constantly adapting to their new circumstances. Infectious agents can change virulence, hosts or vectors (and their distribution). These diseases are, therefore, very much a part of our future. On a practical level, awareness is the first step to early diagnosis. With new advances in molecular biology, diagnosis of these infectious diseases is constantly improving (Colwell et al, 2011).

Interestingly, despite the global abundance of humans and their presence in all types of climates and ecological conditions, they are not major reservoirs of tick-borne infectious agents. In comparison, some of the most important human diseases transmitted by flying insect vectors (malaria, leishmaniasis) target humans as the main host. Tick-borne infections of humans typically arise secondary to an infringement of a circulation between wildlife animal reservoirs and tick vectors. Some agents, for example *B. burgdorferi* and *A. phagocytophilum*, can infect hosts belonging to more than one category but all these zoonotic agents are associated with wildlife reservoirs, wild rodents in this case (Baneth, 2014). The causative agents of tick-borne diseases have probably been circulating for centuries among the rodent and tick populations (Baneth et al, 2014). Many vector-borne diseases of humans existed as zoonotic infections long before our ancestors were recognised as humans (Gwadz, 2016). The three most feared vector-borne diseases (malaria, bubonic plague and epidemic typhus) had major influences on the outcomes of our evolution and our human history (Dobson, 2013).

The impacts of vector-borne infections are wide and felt at both the human health and animal health levels with associated economic consequences. While some of these diseases are quite deadly (i.e. malaria, etc.), the main human burden is financial because of increased health costs and increased expenses (due to absence from work). In developing countries, the additional costs are enough to push low-income families further into poverty and starvation. While Lyme disease is rarely fatal, in 2005 it was estimated that the national expenditure for therapy over 5 years in the USA was 2.5 billion dollars. Losses due to costs associated with treatment, animal mortalities, acaricides and reduced production are a major burden on the animal health front. However, in developing countries where animals are relied on for work, transportation and nutrition, the losses are more profound (Harrus and Baneth, 2005).

Migration of refugees from unstable countries is an age-old phenomenon that results in significant challenges for infectious disease control. Most of the world's displaced people or refugees live in refugee camps or temporary shelters. These environments are often overcrowded and have poor provision of sanitation, clean water, food and healthcare. Barriers to vectors and animals carrying infectious diseases are often absent or insufficient and person to person contact is amplified. These situations commonly result in epidemics

which can be devastating (Saker et al, 2004). For example, in 1994 after almost one million people fled from genocide in Rwanda for safety in Zaire, approximately 50 000 people died in the first month due to epidemics of cholera and dysentery (Centers for Disease Control and Prevention, 1995). Migration of people also favours vector-borne diseases as they might move from non-endemic regions and may not have any immunity to local pathogens (Saker et al, 2004). Malaria epidemics in refugee camps are well documented in Afghanistan and Pakistan (Molyneux, 1997). Louse-borne infections, for example epidemic typhus and relapsing fever, are classically associated with refugee camps and can cause high mortality (Centers for Disease Control and Prevention, 1994). Countries experiencing mass migration influxes most likely also have illegal immigration and one must ask the question: if an infectious tropical disease is imported into a non-endemic country this way, are doctors prepared enough and is there enough awareness to achieve timely diagnosis and treatment for these patients?

2. The African Context

The African continent suffers from the highest burden of infectious diseases of humans and animals in the world while at the same time having the least capacity for disease detection, identification and monitoring (Rweyemamu et al, 2013). Studies focusing on the risk of spread of emerging infectious diseases all point to Africa and Asia as being likely to have the endemic settings for emerging diseases at human-livestock-wildlife interface areas (Rweyemamu et al, 2013). The majority (72%) of the disease burden in Africa correlates with poverty, interactions between socio-economic opportunities and the health of animals, people and ecosystem compared to 27% in the rest of the world (Rweyemamu et al, 2013). Other factors to consider are climate variation, displacement due to natural disaster or civil instability that encourage the spread and socio-economic impact of disease and trapping communities in chronic poverty (Rweyemamu et al, 2013). The importance of *Coxiella*, *Rickettsia*, *Ehrlichia*, *Anaplasma* and *Borrelia* species as a cause of illness in sub-Saharan Africa is poorly characterised because laboratory capacity to diagnose these infections in humans is often lacking in developing countries (Kelly et al, 1996). The Southern African Centre for Infectious Disease Surveillance (SACIDS) that was set up by academic and research institutions in the Southern African Development Community (SADC) aims to

strengthen Africa's detection, surveillance and research capacity for emerging and re-emerging infectious diseases.

Armed conflict, changes in land use and a rapid growth in human population size have been some driving forces for people moving and establishing communities in previously uninhabited areas in Africa (Kock et al, 2002). In Southern Africa, the concept of Transfrontier conservation parks has come to life since the 2000s. The idea of these areas is to limit barriers between countries and promote free roaming of wildlife in the name of conservation (Department of Environmental Affairs, 2015). However, increased movement of wild animals across boundaries presents some challenges for animal health and the adjacent communities (Bengis et al, 2005). According to the South African Department of Agriculture, Forestry and Fisheries (DAFF), South Africa is largely dependent on livestock productivity for subsistence. The country also boasts large and diverse game reserves which can be considered as safe havens for ticks. Consequently, tourists and locals are often bitten by ticks and fall victim to the diseases they carry (Raoult et al, 2001).

In South Africa, the role of ticks in disease transmission, especially among livestock, has been widely reported, however, their role as vectors of human diseases is not as clear. In the review on the prevalence, distribution and burden of neglected tropical diseases, Hotez and Kamath (2009) noted that there was a lack of information on tick-borne zoonoses in sub-Saharan Africa making it difficult to assess their impact. Horak et al (2002) performed a study of ixodid ticks that feed on humans in South Africa and discovered 20 different tick species in the country. In South Africa, several tick-borne zoonoses have been reported namely Crimean-Congo haemorrhagic fever virus, tick-borne relapsing fever caused by *Borrelia duttonii*, ehrlichiosis caused by *Ehrlichia ruminantium*, *Ehrlichia canis* and *Anaplasma phagocytophilum*, babesiosis caused by *Babesia microti* and rickettsioses caused by *Rickettsia africae*, *R. aeschlimannii* and *R. conorii* (Chitanga et al, 2014). A survey investigating the occurrence of zoonotic diseases among South African veterinarians indicated that 63.6% had suffered from one or more zoonoses (Gummow, 2003). African tick bite fever (ATBF) and Q fever were amongst the 22 zoonotic diseases listed. For diagnosis of many of these diseases, general practitioners required assistance from the veterinarian which shows that zoonotic diseases are difficult to diagnose and are frequently misdiagnosed or missed. Gummow (2003) hypothesized that the incidence and severity of

certain zoonotic conditions are likely to increase at rates like the HIV epidemic, compounding an already serious situation. Testing for zoonotic pathogens in 1634 ticks collected from livestock in four SA provinces showed infection rates of 7% for *A. phagocytophilum*, 7% for *Coxiella burnetii*, 28% for *E. ruminantium*, and 27% *Rickettsia* species, while *B. burgdorferi* could not be confirmed. Based on finding zoonotic pathogens in ticks in South Africa, the authors recommended that these pathogens be considered as routine screening for patients presenting with acute febrile illness who have recently been exposed to ticks or livestock (Mtshali et al, 2015). Furthermore, studies on these pathogens as well as their vectors should be conducted to characterize and determine their prevalence in the country and factors influencing their epidemiology. A recent study examined 205 ticks collected from nine wildlife species and domestic animals from two provinces in South Africa and found *R. massiliae* (5.4%) in *Amblyomma sylvaticum* and *Rhipicephalus simus*, and *R. africae* (3.4%) in *A. hebraeum*. The authors recommended further research to assess the impact of these microorganisms in wildlife and the risk of transmission to domestic animals and humans (Halajian et al, 2016).

The prevalence of zoonotic agents in rodents was higher in informal settlements with poor sewerage systems and housing according to Taylor et al (2008). Rural settlements in sub-Saharan Africa are surrounded by habitats suitable for harbouring large numbers of rodents in some seasons leading to increased human-rodent interactions. With increasing human populations and encroachment into natural rodent habitats, regular interactions could increase the risks for contracting several zoonotic diseases resulting in serious public health challenges (Taylor et al, 2008). In Africa, rodent-borne zoonoses either cause disease and go undiagnosed or are misdiagnosed due to lack of information on the prevalence of the causative agent (Begon, 2003). Another barrier to healthcare in rural areas in South Africa is the cost of transportation to clinics followed by being too ill or debilitated to travel (Berrian et al, 2016).

In sub-Saharan Africa, acute febrile illness is still a common cause of adult hospital admissions (Rajaratnam et al, 2010). There is evidence that malaria is significantly over diagnosed in sub-Saharan Africa and this impacts mortality as other diagnoses are overlooked (Reyburn et al, 2004). In a prospective study of adult hospital admissions in Tanzania, it was found that in patients with febrile disease, malaria is over diagnosed and

bacterial disease is often overlooked. The under prescription of antibiotics had a negative impact on the mortality rates in these patients. They found that non-malarial diagnoses are often found if a wider range of diagnostic modalities are used. This highlights the importance of increased availability of microbiological diagnostics (Nadjm et al, 2012). For example, a systematic review of data in Africa demonstrated that acute leptospirosis is an important cause of febrile illness in hospital patients across Africa (Allan et al, 2015). Another study found that the similarity between symptoms of malaria, African tick-bite fever, human granulocytic anaplasmosis and human babesiosis together with the use of non-specific and non-sensitive diagnostic tools suggests a possibility for misdiagnosis in Nigeria and other African countries (Ogo et al, 2012).

3. Public Health Implications

In parts of Africa where malaria is endemic, people often diagnose themselves and undergo treatment at home using traditional remedies or drugs bought from shops. Only when this treatment fails do these people seek help at health centers. Because of the potential severity of the disease if not treated early and lack of resources for correct diagnosis, many international aid organizations have advocated home treatment of all childhood fevers as malaria. This, together with the general reluctance to seek medical care, has led to high rates of over-diagnosis and over-treatment of malaria. While affordable monotherapies were available and effective in the past, this was not a big problem. Unfortunately, malaria is becoming more difficult to treat and often requires more expensive combination treatments with more potential side effects. This makes the need for correct diagnosis even more important (Amexo et al, 2004). Effort must be made to overcome cultural barriers and develop trust in the healthcare system and providers so that people seek medical care and take the treatments given. But this also means that doctors and health care workers must be better informed and equipped to diagnose and treat other causes for febrile illness.

The consequences of disease misdiagnosis are felt at individual, household and national levels. Poor patients spend a higher proportion of their income on medical care than the wealthy. Poor households who rely on certain family members to provide for them suffer terribly if that member is unable to work. To restore health and continue working as soon as possible they often must sacrifice basic needs such as food and education to pay for medical

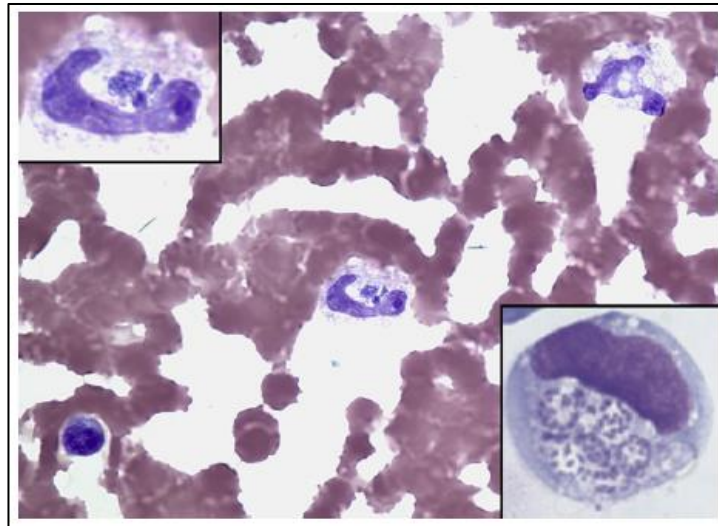
care. This pushes them further into a downward spiral of increasing poverty and vulnerability. It is also far more cost effective and in the interest of public health to improve diagnostics for febrile diseases because of more expensive, less safe drug regimens available to treat malaria and the risk of death due to malaria misdiagnosis (Amexo et al, 2004).

4. Rodent-borne bacterial and parasitic pathogens which have an important impact on public health

The following rodent-borne illnesses will be discussed: human granulocytic anaplasmosis, human babesiosis, rickettsiosis, Lyme borreliosis, tick-borne relapsing fever, leptospirosis and bartonellosis.

4.1 Human Granulocytic Anaplasmosis

Granulocytic anaplasmosis, caused by *Anaplasma phagocytophilum*, is an emerging tick-borne zoonosis worldwide. It is a small (0.5-1.5 µm in diameter), gram-negative, obligate intracytoplasmic coccus (Figure 1), from the family Anaplasmataceae. The bacterium infects neutrophils in humans and animals, resulting in clinical symptoms ranging from asymptomatic seroconversion to mild, severe, or fatal disease (Dumler et al, 2007). It causes tick-borne fever (TBF) in sheep, cattle and goats, granulocytic anaplasmosis (HGA) in humans, equine granulocytic anaplasmosis (EGA) in horses and canine granulocytic anaplasmosis (CGA) in dogs (Stuen et al, 2013; Carrade et al, 2009; Woldehiwet, 2010).



*Figure 1: Wright stained human peripheral blood smear showing *A. phagocytophilum* morulae in an infected band neutrophil (top left) and *A. phagocytophilum* bacteria within vacuoles of a human promyelocyte cell (bottom right) (Bakken & Dumler, 2015).*

The organism was first described in sheep from Scotland (Gordon et al, 1940; Foggie, 1951). During an experimental study on louping-ill, sheep contracted an unknown febrile reaction on tick-infested pastures. The disease was given the provisional name “tick-borne fever” (TBF) (Gordon et al, 1940). This infectious agent was subsequently renamed and reclassified several times over the years. Eventually based on phylogenetic studies, the granulocytic *Ehrlichia* group was renamed *Anaplasma phagocytophilum* (Dumler et al, 2001). This group contains the organism affecting ruminants - *E. phagocytophilum* (Philip, 1974), the organism affecting horses - *E. equi* (Gribble, 1969) and the agent causing human granulocytic ehrlichiosis (Chen et al, 1994) which are used as subjective synonyms for *A. phagocytophilum* (Dumler et al, 2001).

For decades *A. phagocytophilum* has been known to cause disease in domestic ruminants in Europe (Foggie, 1951). In horses, EGA was documented as early as 1968 in California, USA (Gribble, 1969), while infection in dogs was first identified in 1982, also in California, USA (Madewell and Gribble, 1982). In humans, HGA was first reported in the USA in 1994 (Chen et al, 1994). Subsequently, infections were reported in different parts of Europe, China and Japan (Lotric-Furlan et al, 1998; Strle, 2004; Zhang et al, 2008; Ohashi et al, 2013). Several

genetic variants of *A. phagocytophilum* have been characterized (Scharf et al, 2011) but not all infect humans.

Epidemiology and reservoir hosts

There is a distinction between rodent reservoirs of *A. phagocytophilum*. In a study in western USA they found that white-footed mice (*Peromyscus*) and voles (*Microtus*) only contributed by supporting the adult tick population but not *A. phagocytophilum* infection. Other hosts such as tree squirrels (*Sciurus*) and woodrats (*Neotoma*) are often infected with *A. phagocytophilum* and they also support the ticks (Foley et al, 2008). A large variety of reservoir hosts are implicated, and this depends on the geographic location. Mice (*Mus*), woodrats, chipmunks (*Tamias*), voles, shrews (*Sorex*), raccoons (*Procyon*), squirrels (*Sciurus*) and deer (*Odocoileus*) have been implicated. The role of birds as reservoirs has not been clearly established. Dogs and humans are accidental hosts and are not important in disease transmission to other host species (Carrade et al, 2009; Woldehiwet, 2010). Dogs act as sentinels for human exposure and can also be a source of infection by bringing infected ticks into contact with people (Carrade et al, 2009).

The pathogenesis of *A. phagocytophilum* infection in domestic animals in Africa still needs major investigation. In South Africa, the first report of the molecular detection of a bacterium (designated *Anaplasma* sp. South Africa dog strain) closely related to *A. phagocytophilum* (with 99% sequence identity) was in whole blood specimens from three dogs in Bloemfontein (Inokuma et al., 2005). More recently, the same *Anaplasma* species (South Africa dog strain) as well as a partial 16S rRNA sequence with 99% nucleotide identity to *A. phagocytophilum* strains ApGDr1, ApGDr2 and GDR4 (accession numbers: KC800963, KC800964 and KC455366) were detected in several dog samples collected in the Mnisi community area, Mpumalanga province, South Africa (Kolo et al., 2016).

Elsewhere in Africa, *A. phagocytophilum*-like DNA has been detected in cattle in Uganda (Muhanguzi et al, 2010); in Tunisia, a study found 40 (67%) horses seropositive for *A. phagocytophilum* exposure and eight (13%) of the horses positive for *A. phagocytophilum*-DNA using PCR (M'ghirbi et al, 2012); three genetic variants of *A. phagocytophilum* were identified in cattle in Algeria using a new qPCR tool (Dahmani et al, 2015); 12 (13.6%) spleen DNA samples from baboons and monkeys in Zambia tested positive for *A. phagocytophilum*

DNA using PCR and sequencing analysis (Nakayima et al, 2014); *A. phagocytophilum*-DNA was also found in a lion, a serval and a Southern African wildcat during a PCR survey of blood collected from wild captive felids in Zimbabwe, 13% of wild cats and 50% of servals in South Africa (Kelly et al, 2014).

Transmission and arthropod vectors

The most common mode of transmission of HGA is via a tick bite but there are alternate routes. Transplacental transmission has been documented (Horowitz et al, 1998b). Several butchers have contracted HGA after butchering large numbers of white-tailed deer carcasses during the hunting season. Since none of the butchers reported a preceding tick bite, it is not clear if transmission was acquired by direct exposure to infected blood through cuts in their skin or inhalation of aerosolized blood or contact of infected blood with mucous membranes (Bakken et al, 1996). *Anaplasma phagocytophilum* remains viable in refrigerated stored blood for up to 18 days which allows for transmission via blood transfusion (Kalantarpour et al, 2000). Although nosocomial transmission of HGA was reported in a Chinese hospital (Zhang et al, 2008), it was later shown to be a case of co-infection. Liu and Yu (2016) clarified that all the patients in the nosocomial transmission report were infected with both *A. phagocytophilum* (confirmed by PCR and positive seroconversion) and the severe fever with thrombocytopenia syndrome virus (confirmed by seroconversion, PCR and DNA sequencing). They explained that co-infection is possible as both infectious agents have been detected in *Haemophysalis longicornis* ticks in Asia (Luo et al, 2015; Kim et al, 2003). *Anaplasma phagocytophilum* is maintained in a zoonotic cycle between infected mammalian host reservoirs and ticks, mainly those of the *Ixodes persulcatus* complex (Thomas et al, 2009). *Ixodes ricinus* is the main vector in Europe, *I. scapularis* in mid-western and north-eastern USA and *I. pacificus* in Western USA (Parola, 2004; Woldehiwet, 2010). In Asia and Russia, it is transmitted by *I. persulcatus* and *Dermacentor silvarum* (Cao et al, 2000; Dumler et al, 2007). Other species of *Ixodes* ticks reported to maintain enzootic cycles of *A. phagocytophilum* include *I. spinipalpis* in the USA and *I. trianguliceps* in north western England (Zeidner et al, 2000; Bown et al, 2003). A study in France showed that the vector for EGA in Europe is also *I. ricinus* and this tick tends to be distributed in more humid areas near woods or prairies (Maurizi et al, 2009). Although the *Ixodes persulcatus* group of ticks - which are the known vectors for HGA - are absent in

Africa, the findings of pathogen DNA in various animals in Africa demonstrates that there already are other ticks which act as vectors for the organism in Africa. However, there have been no reports yet of clinical HGA in humans in South Africa (Mtshali et al, 2017).

A South African study sampling ticks collected from livestock, discovered *A. phagocytophilum* infection rates of 6%, 17% and 1.25% in ticks collected from cattle, goats and sheep respectively (Mtshali et al, 2015). In the absence of the recognised vectors, Mtshali et al (2015) recommend considering *Rhipicephalus* species (*R. e. evertsi* & *R. decoloratus*) and *Amblyomma hebraeum* as possible vectors or reservoirs. In another South African study by Mtshali et al (2017), they found that 18% of the 318 ticks collected from cats and dogs were positive for *A. phagocytophilum*-like bacteria. The infection rates in the Northwest Province were particularly high, up to 63%. The potential vectors identified here were *R. sanguineus* (50% of the positive ticks) and *H. elliptica* (15% of the positive ticks) but the actual incidences and identity of the true hosts in South Africa remain unknown (Mtshali et al, 2017). Another study of dogs and their ectoparasites found *Anaplasma* species South Africa dog strain 98% similar to *A. phagocytophilum* in four samples and one dog blood sample had a DNA sequence with a 99% sequence similarity to three different strains of *A. phagocytophilum* (Kolo et al, 2016).

An Egyptian study reported higher *A. phagocytophilum* infection rates in ticks collected from dogs and sheep (13.7%) and from goats (5.3%). Teshale et al (2016) reported finding *A. phagocytophilum* DNA in *R. pulchellus* ticks in Ethiopia. A molecular survey of 4297 questing ticks collected in Shimba Hills Nature reserve in Kenya and arranged into 270 tick pools, found *A. phagocytophilum* DNA in one of the *R. maculatus* pools demonstrating this pathogen-vector relationship for the first time (Mwamuye et al, 2017). It has also been detected in *R. ripicephalus* in North Africa (Ghafar & Amer, 2012), *A. cohaerens* in Ethiopia (Hornok et al, 2016) and *H. marginatum* in Tunisia (M'ghirbi et al, 2012).

Importance and incidence

By 2010, just over 1700 cases of HGA had been reported to the CDC in the USA (Centers for Disease Control and Prevention website, accessed 28 May 2017). Although sero-surveys in individuals bitten by ticks in USA showed 8.9% to 36% seroprevalence rates (Aguero-Rosenfeld et al, 2002), Bakken et al (1998) reported seroprevalence of 14.9% among healthy

individuals living in HGA endemic regions with no history of a recent tick bite suggesting that infections can often be subclinical. Interestingly, HGA occurs at much lower incidence in Europe and Scandinavia with increasing cases being described in eastern Asia especially China, South Korea and Japan (Bakken & Dumler, 2015). Maurizi et al (2009) found a seroprevalence of 16.0% to 20.1 % for equine anaplasmosis in France and none of the horses that were tested from Africa were positive.

Reports of human *A. phagocytophilum* cases in Africa are scarce with only one published in Egypt (Ghafar et al, 2005). Confirmed cases of HGA in sub-Saharan Africa are yet to be reported (Cabezas-Cruz et al, 2012).

Clinical signs, diagnosis and treatment

The incidence and severity of the disease varies between regions. This is largely dictated by the strain or variant, the hosts it has adapted to and the capacity of the vectors present (Woldehiwet, 2010). HGA causes an acute influenza-like febrile illness of varying severity. Men are slightly more affected than women. The median age of patients is 50 years of age. The most common clinical signs are fever, myalgia, headaches, malaise and chills, but anorexia, arthralgias and coughing may also occur (Dumler et al, 2007). Other respiratory or gastrointestinal symptoms may be seen, and few patients may also experience a non-pruritic skin rash (Dumler et al, 2007; Weil et al, 2012). The disease is usually mild and self-limiting. Severe illness tends to occur in people of advanced age or who have concurrent immunosuppressive illness or drug therapy (Dumler et al, 2007). Laboratory findings include thrombocytopenia, leukopenia, increased hepatic transaminase activity (Dumler et al, 2007; Weil et al, 2012), elevated C reactive protein (Brouqui et al, 2004). Other less common findings are: anaemia, elevated serum creatinine and blood urea nitrogen levels (Brouqui et al, 2004). A difficulty for diagnosis is that subclinical infection is common (Weil et al, 2012).

Antibodies for HGA can persist in the body for weeks or years. There is a lack of evidence of *A. phagocytophilum* infection occurring more than once in the same individuals which could suggest some level of immunity, but further research is needed (Horowitz et al, 1998a).

Diagnosis of HGA relies on a high suspicion of infection. The disease must be considered when a febrile illness occurs in an endemic area during spring and summer. Furthermore, a history of outdoor activity and/or a recent tick bite should prompt investigation of HGA and

other tick-borne diseases (Dumler et al, 2007; Weil et al, 2012). If HGA is suspected, prompt doxycycline treatment should be initiated because the disease can progress rapidly and become fatal (Dumler et al, 2007).

Diagnostic confirmation by visualisation of parasitic morulae on a Giemsa or Diff Quick stained peripheral blood smear is rapid and inexpensive but has poor sensitivity. These are most reliably visible in acute infection prior to treatment. For leukocytotropic species (*A. phagocytophilum*), a buffy coat smear is preferred (Dumler et al, 2007; Silaghi et al, 2017). Serology (immunofluorescence antibody testing) is a sensitive diagnostic method but it is retrospective (Brouqui et al, 2004; Dumler et al, 2007; Weil et al, 2012). Most patients are seronegative in acute infection and cross reactions are observed with other *Anaplasma* species after seroconversion (Silaghi et al, 2017). A four-fold change in antibody titre during the convalescent phase is diagnostic but requires paired serum samples which are not always practical. Serology can also be complicated because IgG antibodies can persist months to years after infection, some areas have a high seroprevalence in the absence of infection and analysis of acute phase titres alone will only detect about 3% of patients (Dumler et al, 2007). PCR is both rapid and highly sensitive making it the diagnostic method of choice in acute illness (Brouqui et al, 2004; Dumler et al, 2007; Weil et al, 2012). However, further confirmation of positive qPCR results by sequencing is always advised (Silaghi et al, 2017). Isolation of *A. phagocytophilum* would be the most accurate method for diagnosis but there are only a small number of competent biohazard level 2 laboratories and it can take several weeks (Brouqui et al, 2004; Dumler et al, 2007; Silaghi et al, 2017). Prior treatment with doxycycline reduces the sensitivity of blood smear microscopy, *in vitro* culture and PCR (Dumler et al, 2007; Silaghi et al, 2017). A recent study using PCR on tick bite crust samples made an exciting discovery when they found that *A. phagocytophilum* could be detected in early diagnosis of patients who have already started antibiotic treatment (Kim et al, 2017).

As for treatment, in general all tetracycline antibiotics (doxycycline) are effective for treating TBF in ruminants (Woldehiwet and Scott, 1993) and HGA in humans (Maurin et al, 2003). The recommended therapy for adults is 100 mg doxycycline orally every twelve hours for seven to ten days. Response to treatment is fast (24-48 hours) and relapse or chronic infection has not been reported (Bakken & Dumler, 2000). Doxycycline is the drug of choice

in people because of better tolerance. In children under 8 years who are not seriously ill, three days of doxycycline treatment is recommended followed by 14 days of another antibiotic (amoxicillin) effective for Lyme borreliosis (Dumler et al, 2007).

4.2 Human Babesiosis

Babesia are protozoal parasites from the phylum Apicomplexa (Levine, 1971). The apicomplexa are a large and diverse group with over 5000 named species. Several species from this group are of interest in both veterinary and human medicine. *Babesia* and *Theileria* species from the order Piroplasmida are important causes of disease in cattle and other domestic animals. From the order Haemosporida the Plasmodium species, which cause malaria, have probably had the greatest impact on human health of all the protozoan pathogens. Another important order of apicomplexans is the Coccidea and genera include *Cryptosporidium*, *Toxoplasma*, *Sarcocystis* and *Neospora* (Wiser, 2011; Escalante & Lyala, 1995).

Members of the genus *Babesia* infect erythrocytes of many different vertebrate hosts including domestic and wild animals as well as humans (Lempereur et al, 2017). *Babesia* protozoa can be identified in the intra-erythrocytic merozoite phase. They measure between 1 μm and 3 μm in diameter and can be ring-shaped or take the form of a tear drop (also known as piroplasm) (Figure 2). Inside erythrocytes, they undergo binary fission and can be seen in pairs called the “paired form” or tetrads called “maltese crosses” (Mehlhorn & Shein, 1984).

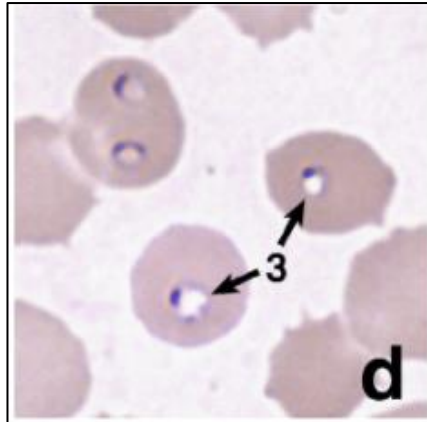


Figure 2: *Babesia microti* parasites within human erythrocytes seen on a Giemsa stained thin blood smear. Number 3 indicates the “ring form” (Gray et al, 2010)

Human babesiosis is an important emerging tick-borne disease and is caused by various *Babesia* species (Kjemtrup & Conrad, 2000). In Europe *Babesia bigemina*, a parasite of cattle, is the most common agent of human babesiosis and is often fatal especially in asplenic patients (Gorenflot et al, 1998). Other human babesial species have been identified in Europe namely *B. bovis*, *B. canis*, *B. microti* and some unknown species (Jacquemin et al, 1980). In Africa, sporadic cases of human babesiosis have been documented and were caused by uncharacterised *Babesia* species. These cases have been confirmed in Egypt (Michael et al, 1987), Mozambique (Rodriguez et al, 1984) and South Africa (Bush et al, 1990). The two South African cases were initially misdiagnosed as malaria and one patient died despite treatment (Bush et al, 1990). There is a recently discovered babesial parasite called WA1 which has now been named *Babesia duncani* (Conrad et al, 2006). This organism has caused seven human babesiosis cases in the western USA, but more research is needed regarding this new parasite (Herwaldt et al, 1997).

Babesia microti infection in humans was first reported in 1970 (Western et al, 1970).

Babesiosis is the second most common blood-borne parasite of mammals after trypanosomes (Telford et al, 1993). Increased awareness of human babesiosis had led to identification of new human cases all over the world. In Europe and the USA, cases are better documented but cases of human babesiosis have been found in Mexico, Mozambique, Sudan, South Africa and Taiwan. A patient in the Ivory Coast was even diagnosed with a coinfection of babesial and malarial agents (Kjemtrup & Conrad, 2000).

Because this literature review focusses mainly on tick-borne diseases from wild rodents which have risks for public health, only *B. microti* is discussed further. The first human case of *B. microti* reported in 1969, attracted a lot of attention as it was diagnosed in a spleen-intact individual (Western et al, 1970). As the following 13 cases to be diagnosed were all in Nantucket Island, the disease became known as “Nantucket fever” (Ruebush et al, 1977). In North America, the eastern seaboard represents an endemic region for *B. microti* which is the most common causative agent of human babesiosis in the USA (Spielman et al, 1979). It is interesting that there are not more cases of human *B. microti* in Europe despite it being well documented in England and other parts of Europe. It is unclear whether this could be because the tick vector is mainly nest dwelling (Randolph, 1975) or because the European strain is not as pathogenic (Telford & Spielman, 1997).

Epidemiology and reservoir hosts

Babesia microti mainly circulates amongst rodents and ticks in a sylvatic cycle but occasionally infects humans who infringe into the rodents’ natural habitats (Leiby, 2011). The primary reservoir of *B. microti* is believed to be white-footed mice (*Peromyscus*) which are persistent carriers of the parasite and possibly have life-long infection (Steketee et al, 1985). They are a major reservoir host in endemic areas of USA (Kjemtrup and Conrad, 2000). However other small mammals are also relatively competent reservoirs namely meadow voles (*Microtus*), cottontail rabbits (*Sylvilagus*), shrews (*Sorex*), chipmunks (*Tamias*) and raccoons (*Procyon*) (Hersh et al, 2012; Kjemtrup and Conrad, 2000). Using PCR, a study by Maamun et al (2011) found a *B. microti* prevalence of 22% (n = 27/125) in a population of wild-caught Kenyan baboons and African green monkeys (*Cercopithecus*). This is the first report of natural *B. microti* infection in primates in Africa and begs the question of whether infected non-human primates who share habitats with humans may be a potential risk for zoonotic outbreaks (Maamun et al, 2011).

Transmission and arthropod vectors

The route of transmission of *B. microti* to humans is typically from a tick-bite. However, accidental infections have been acquired through blood transfusion from infected individuals (McQuiston et al, 2000). This displays the subclinical nature of the disease as all the blood donors were healthy and asymptomatic at the time of donation. The other known

non-tick-borne route of infection is transplacental. Only two human cases have ever been documented (Krause et al, 1998; Esernio-Jensson et al, 1987).

The known vectors for *B. microti* are as follows: *Ixodes scapularis* (Spielman, 1979) in the USA, *I. ricinus* (Stańczak et al, 2004) and *I. trianguliceps* (Randolph, 1975) in Europe, *I. ovatus* in East Asia (Yano et al, 2005). The vectors in Africa have not yet been established. A small molecular survey of dogs (n = 141) and their ectoparasites (n = 146) collected in Mpumalanga Province, South Africa, detected *B. microti* DNA in pooled *R. simus* ticks (Kolo et al, 2016). Maamun et al (2011) also discovered *B. microti* DNA in semi-engorged *R. simus* ticks that were collected from five *Babesia* positive baboons in Kenya suggesting this tick as a potential vector of *B. microti* in Africa, but more research is needed.

Importance and incidence

In 2011, 847 confirmed cases of human babesiosis were reported to the CDC in the USA (Centers for disease Control and Prevention, 2012). Many cases of *B. microti* are subclinical and can therefore go undetected and unreported (Krause et al, 1998). The case fatality in hospitalised patients is approximately 5% but many cases are asymptomatic (White et al, 1998). Incidence reports for Africa and Europe are lacking.

Clinical signs, diagnosis and treatment

Infection with *B. microti* presents in a wide spectrum of clinical manifestations from asymptomatic to acute and fatal disease which is common in the elderly and immune compromised or asplenic people. In spleen-intact immunocompetent people, disease is mild and resembles transient flu-like illness that may go undiagnosed. Some infections become chronic and only manifest clinically if the patient becomes immunocompromised or splenectomised (Krause et al, 1998). The onset of illness usually starts 1-4 weeks after a tick bite and involves gradual malaise, myalgia, anorexia, fatigue and chills. These symptoms are often followed by fever, nausea and vomiting (Ruebush et al, 1977). In severe cases haemoglobinuria may occur (Pruthi et al, 1995). Other physical findings include: pulmonary oedema, splenomegaly, hepatomegaly and icterus (White et al, 1998). Laboratory abnormalities include: anaemia, thrombocytopenia, leukopenia and elevated liver enzymes (White et al, 1998; Lempereur et al, 2017).

It is recommended to use at least two independent methods to confirm a diagnosis of human babesiosis. Definitive diagnosis can be made by visualisation of the intraerythrocytic parasite on Giemsa stained thin blood smears under oil immersion. This is a cheap, easy and rapid method of diagnosis and is especially important where a quick diagnosis is necessary. However, the sensitivity and specificity rely completely on the examiner (Lempereur et al, 2017). Care must be taken in malaria endemic regions as it is possible to see both *Babesia* and *Plasmodium* species using this method and only an experienced microscope technician can differentiate between them. Due to the use of electronic cell counters for haematology the parasites can be missed easily unless the clinician requests a microscopist to review a slide (Kjemtrup and Conrad, 2000). Blood smears or organ smears (brain, spleen, liver or kidneys) may be used (Lempereur et al, 2017). Inoculation of susceptible animals (hamsters, gerbils or calves) with infected blood provides a retrospective definitive diagnosis but it is both time consuming, expensive and questionable for ethical reasons (Kjemtrup and Conrad, 2000; Lempereur et al, 2017). Currently, PCR is most valuable in conjunction with clinical signs and other diagnostic tools (Kjemtrup and Conrad, 2000). PCR is considered a more sensitive method of diagnosis. This also allows for species identification. Results are obtained within 2-3 days which is much faster than inoculation. Blood or spleen samples are used. There are several conventional and real-time PCR assays which are used, and they are usually more sensitive than microscopy (Wang et al, 2015). Alternative molecular methods are the Reverse Line Blot (RLB) hybridization assay (Gubbels et al, 1999) and modern high-throughput screening methods such as the microfluidic RT PCR system "Fluidigm" (Michelet et al, 2014) or Next Generation sequencing (NGS) (Bonnet et al, 2014). Serology by immunofluorescent antibody testing (IFAT) remains the test of choice and sensitivity and specificity for *B. microti* have been determined. However, results of this test outside of endemic areas must be interpreted with caution as the sensitivity and specificity changes with population prevalence (Kjemtrup and Conrad, 2000; Lempereur et al, 2017). For research, it is recommended to use at least two independent methods for detection wherever possible (Lempereur et al, 2017).

The recommended treatment for *B. microti* is combined oral quinine (650 mg every 8 hours) and oral clindamycin (1200 mg every 12 hours) for 7 days (Telford & Spielman, 1997). HIV positive patients may require other additional drugs such as doxycycline (200 mg per day)

and azithromycin (2000 mg per day) on top of the normal regimen for long-term treatment (Ong et al, 1990).

4.3 Lyme Disease

In recent years, Lyme disease has received more attention due to spreading awareness. In fact, the pathogen has existed for much longer and its presence was even detected in museum samples of *Ixodes* ticks and skins of white-footed mice from 100 years ago (Persing, 1995).

Lyme disease (LD) is a multisystemic disease caused by spirochete bacteria called *Borellia burgdorferi*. *Borrelia* (Figure 3) are thin, motile, elongated, cork screw shaped bacteria with a fluid outer membrane (Barbour & Hayes, 1986). They are very adaptable as they can move between arthropods with no antibody based immune response, they can infect vertebrates despite high immunological pressure and they can withstand large temperature changes of more than 20°C (Stanek & Strle, 2003).

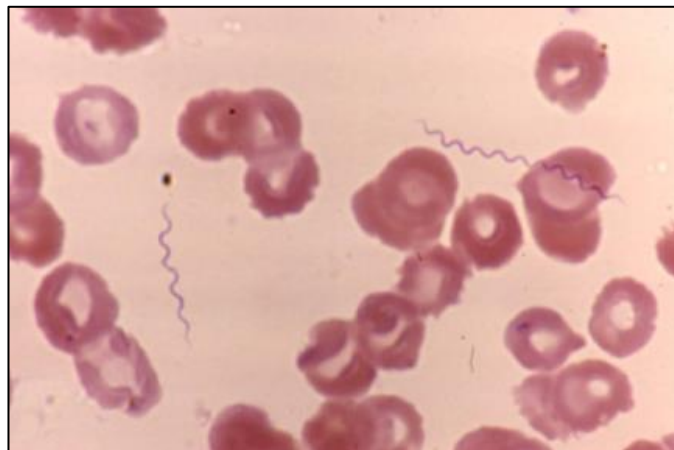


Figure 3: Peripheral blood smear demonstrating intercellular spiral-shaped Borrelia burgdorferi spirochetes which cause Lyme disease ([http://4.bp.blogspot.com/-kFKZFToLg18/UfETulsDQOI/AAAAAAAAAew/vsBzsR4M3kw/s400/Borrelia+burgdorferi+\(Lyme+Disease\)+Spirochaetaceae.png](http://4.bp.blogspot.com/-kFKZFToLg18/UfETulsDQOI/AAAAAAAAAew/vsBzsR4M3kw/s400/Borrelia+burgdorferi+(Lyme+Disease)+Spirochaetaceae.png)).

Lyme disease was first reported in Lyme, Connecticut in 1975 and has now been documented in more than 70 other countries on five continents (Wu et al, 2013). In North America, the causative agent is *B. burgdorferi* sensu stricto. In Europe, there are at least five

species of *Borrelia* which cause Lyme disease with *B. afzelii* and *B. garinii* being the most common but *B. burgdorferi*, *B. spielmanii* and *B. bavariensis* also occur (Strle et al, 1997). In Asia, *B. garinii* is the most predominant species (Strle and Stanek, 2009). As seen in Figure 4, the global geographic distribution of vectors which transmit Lyme disease is mainly in the northern hemisphere (Masuzawa, 2004; Hubalek, 2009; Hao et al, 2011; Stanek et al, 2012).

The disease is not endemic in Africa but there are anecdotal reports of suspected LD cases in South Africa. In 1982-1983, a horse riding school in Natal experienced a small outbreak of arthritis and subsequent recumbency in 6% (n= 7/115) of horses over a nine-month period. The symptoms of arthritis were preceded by a transient febrile illness in the previous summer months. The aetiology of this illness was never determined; unfortunately, the samples were destroyed and could not be tested in later years. Then in October 1988, the owner of the riding school was diagnosed with a recent infection of *B. burgdorferi* by IFA serology testing at the University of the Free State (Fivaz et al, 1990). This was confirmed by ELISA and western Blot testing at a reference centre, Hygiene-Institut der Universität Vienna, as a previous report had described a case of LD in a tourist who visited Natal (Stanek et al, 1986). Subsequently, the domestic animals on the property were tested and 61% (n= 71/117) of horses and 55% (n= 6/11) of domestic dogs had positive serum titres for *B. burgdorferi*. Additionally, three other people suffered from symptoms like LD and tested positive using IFAT. Only two of them reported a preceding tick bite. All four people were treated with high doses of penicillin and recovered fully. Actual confirmation of LD could not be made as the organism was never isolated and sera were not screened against other *Borrelia* that occur in South Africa. This tentative data strongly suggests that LD may in fact occur in South Africa and surveillance is recommended (Fivaz et al, 1990). However, subsequent serological and molecular research in the country has failed to reveal any presence of LD (Frean and Isaäcson, 1995).

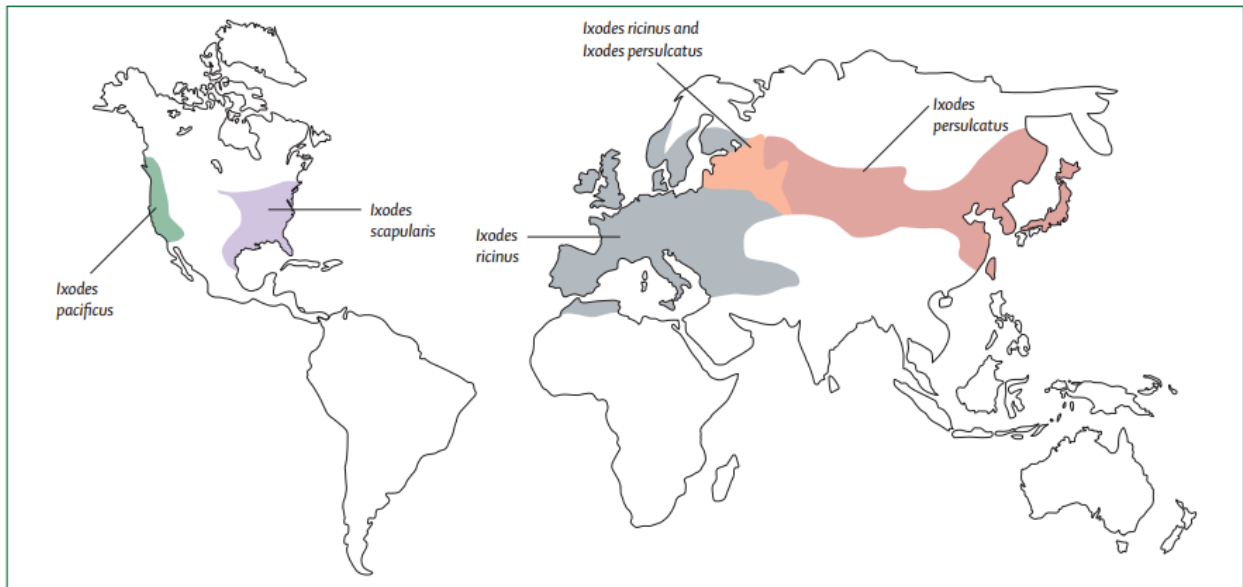


Figure 4: Global distribution of the vectors (*Ixodes ricinus* species complex) of *Borrelia* species

Epidemiology and reservoir hosts

In the USA, the vectors of LD are *Ixodes scapularis* and *I. pacificus* ticks; in Europe, it is *I. ricinus* and in Asia it is *I. persulcatus* (Meerburg et al, 2009). Although LD circulates mainly within rodents, humans and domestic dogs are incidental hosts that can suffer from clinical disease without playing a role in the transmission or epidemiology of infection (Radolf et al, 2012). Human infections usually occur in late spring, summer and early autumn which is the peak feeding periods for the ixodid ticks. Nymphal ticks are the main source of human infections (O’Connell, 2005). Endemic areas of LD in Europe are maintained by complex interactions between different tick species, various *B. burgdorferi* strains and many mammalian, avian and reptilian species on which the ticks feed (Gern, 1998; Piesman, 2002; Rudenko et al, 2011). The reservoir species differ in geographic regions but include various mice, voles and rats (Meerburg et al, 2009).

Transmission and arthropod vectors

People become infected after a bite from a tick which acquired the infection from an enzootic reservoir (Meerburg et al, 2009). For disease transmission to occur, a tick must remain attached for 48 hours or longer (Piesman et al, 1987) which is why ticks attached to people must be identified and removed as soon as possible. Unfortunately, patients who

have been treated for early LD are vulnerable to reinfection later as they do not develop an adequate immune response for protection (Stanek et al, 2012). In the last few decades the tick vector for LD in Europe has spread to higher latitudes and has become more abundant (Randolph, 2005). The WHO believes this will contribute to extended and more intense transmission seasons for LD and other tick-borne diseases (WHO, Geneva).

In South Africa, the *Ixodes persulcatus* tick complex is not present and thus far, LD has not been detected in the country. The absence of *Borrelia burgdorferi* in South Africa was also demonstrated by two survey studies in South Africa which were unable to detect any *B. burgdorferi* DNA by PCR in 1634 ticks collected from livestock (Mtshali et al, 2015) and 318 ticks collected from cats and dogs (Mtshali et al, 2017).

Importance and incidence

Lyme disease is the most commonly reported vector borne illness in the USA. Results of studies by the CDC estimate that about 300 000 people are diagnosed with LD annually in the USA (Centers for Disease Control and Prevention website, accessed 28 May 2017). In the UK, approximately 1 200 cases are serologically confirmed annually (O'Connell, 2014). No cases have ever been confirmed and reported in Africa (Ehounoud et al, 2017). A survey study of 427 patients in South Africa 1988-1989 found no positives for LD (Freaan and Isaäcson, 1995).

Lyme disease is often referred to as the "great imitator" because of the many ways it presents and the difficulty to confirm a diagnosis (Rudenko et al, 2011). There is a lot of inaccurate information about LD in the media and on the internet, which leads to misconceptions, over-diagnosis and over-treatment (Carrington et al, 1998). This carries the risk of increased adverse events and means that opportunities are missed for managing underlying conditions (O'Connell, 2005).

Clinical signs, diagnosis and treatment

Clinical presentations differ between continents. In Europe, patients may more likely develop skin manifestations - which are rare in USA (Meerburg et al, 2009). It appears that *B. afzelii* is mostly associated with skin manifestations, *B. garinii* is the most neurotropic and *B. burgdorferi* is the most arthritogenic (Strle & Stanek, 2009).

Stage I (localised) involves skin lesions called erythema migrans which are considered pathognomonic but other skin lesions can also develop. Polyarthralgia or true arthritis is seen in stage II (generalised). A large variety of neurological manifestations can occur, and cardiac involvement can be seen in the weeks after the onset of disease. In stage III (several years after stage I), acrodermatitis chronica atrophicans can be observed (Brouqui et al, 2004).

Antibodies are often not detectable in the primary stage of infection. For a diagnosis, the existing methods must be logically combined to achieve the highest diagnostic efficiency. Culture is time consuming, labour intensive and rarely used. PCR methods on their own cannot be recommended for routine laboratory diagnosis because a negative PCR result cannot be considered proof of the absence of borreliae (Brouqui et al, 2004). In cases where erythema migrans is present, serology is not necessary and antibiotic treatment should commence immediately. Diagnosis of LD is very challenging in the absence of erythema migrans. For a reliable diagnosis, there must be compatible clinical signs, serological results and history of tick exposure. Positive serology alone is not useful as it could indicate past infection and does not differentiate between current infection or re-infection. Recently serological tests have been optimised and are more reliable (Cutler et al, 2017). The poor sensitivity of direct diagnostic methods and the poor predictive value of indirect methods for the diagnosis of LD leave a lot to be desired. New technologies are being developed that detect the host response to infection by detecting a certain protein produced by the host. The use of spectrometry to detect certain Borrelia-specific proteins is also being investigated (Cutler et al, 2017). Generally screening tests have a poor reputation because of false positive reactions in the presence of other conditions like infectious mononucleosis, rheumatoid disease, autoimmune disease and other spirochaetal infections. Patients with a low likelihood of LD should not undergo screening tests (O'Connell, 2005).

Treatment is most effective in the early course of the illness (Strle, 1999). Effective oral antibiotics include: doxycycline, amoxicillin, phenoxymethylpenicillin, and cefuroxime axetil. Doxycycline is the only drug for which clinical trials have shown that just ten days of treatment is effective. Parenteral antibiotics are recommended for treating late Lyme neuroborreliosis and in the initial treatment of cardiac Lyme borreliosis in hospitalised patients. The first-choice drug is ceftriaxone and alternatives are cefotaxime and

intravenous penicillin (Stanek et al, 2012). Recommended dose, route and duration of treatment varies with the clinical picture. A 14-day course of oral amoxicillin or doxycycline is indicated for erythema migrans but more disseminated infections (without neurological involvement) may require two to four weeks of oral antibiotics. Neuroborreliosis should be treated with parenteral antibiotics for 14 to 28 days. Patients must be checked for co-infection and treated appropriately (O'Connell, 2005).

For prevention of LD, the most consistent recommendation is to remove attached ticks as soon as they are noticed (Kahl et al, 1998). Bathing within 2 hours of tick exposure significantly reduces risk of LD infection (Stanek et al, 2012). Tick and disease awareness, avoidance of tick-infested areas and use of insect repellents may also go a long way towards preventing the disease (O'Connell, 2014; Stanek et al, 2012).

4.4 Co-infection

Co-infection is an emerging problem as it may affect the severity and duration of symptoms in humans and makes diagnosis and treatment more challenging (Hersh et al, 2014). It has caused both confusion and enlightened the understanding of these diseases. Understanding the epidemiology of *B. microti* has helped the study of other tick-borne agents. It also revealed that *B. microti* is part of an important zoonotic tick-borne disease complex (Kjemtrup and Conrad, 2000). There is a condition called Post Treatment LD syndrome, where patients experience prolonged symptoms after appropriate therapy for LD. Persing (1995) suggested that this syndrome could be due to co-infection with pathogens from the piroplasm family (*B. microti*) or the genus *Anaplasma* (Persing, 1995). Because of the immunosuppressive nature of *Anaplasma* infections, co-infection may increase the severity of LD (Schouls et al, 1999). In fact, co-infection appears to aggravate both LD and babesiosis in humans (Krause et al, 1998). It is important to identify any co-infection as the clinical course of each of the diseases may be modified in the presence of the other and because it may affect the choice of antimicrobial therapy (Belongia et al, 2002; Krause et al, 2002; Steere et al, 2003; Lotric-Furlan et al, 2009; Rojas et al, 2014).

It is known that co-infection with HGA, human babesiosis, LD, other *Borrelia* and tick-borne viruses in the same patient is possible. These infectious agents share common vectors, namely the *Ixodes persulcatus* tick complex (Steere, 2001; Krause, 2002; Chapman et al,

2006; Raileanu et al, 2017), as well as common rodent reservoir hosts. Infections can be co-transmitted by the same tick bite or sequential bites from other ticks in an endemic area. The *Ixodes* tick complex are known as “bridge vectors” because their generalist feeding behaviours means they feed on diverse species of vertebrates and readily bite humans (Rudenko et al, 2011; Radolf et al, 2012). The tick nymphs are the most high-risk stage to public health because of their small size, their marked anthropophily, their abundance and their capacity to remain attached to hosts for long durations (Vassalo et al, 2000).

Research regarding co-infections of tick-borne illness in people in South Africa is behind that what is known in USA and Europe. Although these tick vectors are not present in South Africa, there is an abundance of other ticks and tick-borne illnesses and the principles of co-infection would apply to them too.

4.5 Tick-borne Rickettsial Diseases

Common tick-borne rickettsial diseases include “Mediterranean Spotted Fever” (MSF), the “Spotted Fever Group” (SFG) and “African Tick Bite Fever” (ATBF). They are caused by intracellular, gram-negative, obligate intracellular coccobacilli (Figure 5) from the family Rickettsiaceae (Parola, 2004; Portillo et al, 2017). Although there are many species causing tick-borne rickettsial disease, the syndromes are similar and are discussed as a group in this review. Only those *Rickettsia* that are found in Africa are described in this review.

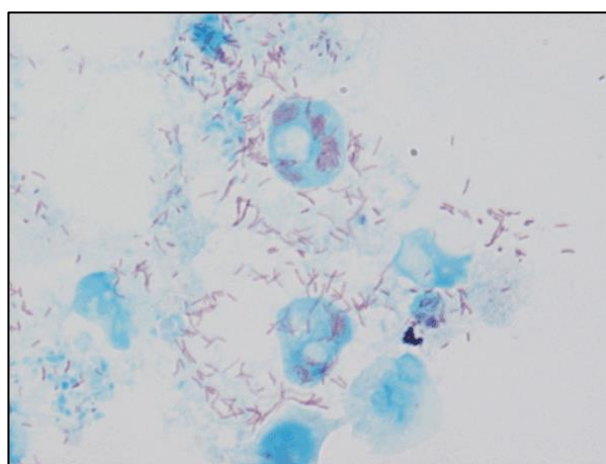


Figure 5: *Rickettsia conorii* observed in Vero cells (red rods; magnification $\times 1,000$) (Roverly et al, 2008)

Tick-borne rickettsiosis or tick-bite fever has been regularly reported in North and South Africa since 1910 (Bitam, 2012) but they are also known in Europe and North America. Rickettsial diseases occur with specific geographic distribution because each tick vector involved favours specific environmental conditions. Human infections usually occur in spring or summer when ticks are most active (Parola, 2004; Centers for Disease Control and Prevention, 2015).

African tick bite fever is probably the most prevalent and widespread of tick-transmitted rickettsiosis and was recognised as a human pathogen in 1922 (Raoult et al, 2001; Parola et al, 2005). In Africa, *Rickettsia africae* is widely distributed with most of the strains having been reported in South Africa (Mediannikov et al, 2010). *Rickettsia conorii* was first discovered in Tunisia in 1910 (Pijper, 1936) but since then it was also found in Kenya, Somalia, South Africa and Chad (Roverly & Raoult, 2008). *Rickettsia aeschlimannii* was first isolated in Morocco (Beati et al, 1997) but has since been found in Zimbabwe, Mali, Niger (Mediannikov et al, 2010) and Algeria (Bitam et al, 2006). *Rickettsia massilae* has been found in Central African Republic (Dupont et al, 1994), Mali (Parola, 2004), Algeria (Bitam et al, 2006), Morocco (Sarih et al, 2009) and Senegal (Mediannikov et al, 2010). *Rickettsia conorii* subspecies *israelensis* has been detected in Nigeria (Kamani et al, 2013a). *Rickettsia sibirica* subspecies *mongolitimona* has been shown in Senegal but no human cases have been reported (Mediannikov et al, 2010).

Epidemiology and reservoir hosts

It is difficult to determine the exact role of dogs and cats as reservoirs of rickettsioses because infected animals are often asymptomatic and are bacteraemic for short periods only (Vorou et al, 2007). However, it has been stated that dogs, rodents and ticks are sources of human infection and transmit *R. conorii* (via *R. sanguineus*) in peri-urban areas and *R. africae* (via *A. hebraeum*) in rural areas (Frean and Blumberg, 2007; Jensenius et al, 2003a). Larvae (also called “pepper ticks”) and nymphs typically transmit the pathogens (Frean et al, 2008). A study of 1634 ticks collected from livestock in South Africa found *R. africae* in ticks from all groups of animals including sheep, cattle, horses and goats. This suggests that these animals as well as ticks serve as reservoirs of *R. africae* (Mtshali et al, 2015).

Transmission and arthropod vectors

The following table gives a summary of the pathogenic *Rickettsia* species and their vectors which are found in Africa.

Table 1: Most known human tick-borne rickettsial diseases in Africa

Syndrome	Infectious agent	Vectors
“Mediterranean Spotted Fever”	<i>Rickettsia conorii</i>	<i>Rhipicephalus sanguineus</i> (Parola et al, 2005; Parola et al, 2013) <i>Rhipicephalus</i> species (Parola et al, 2013) <i>Haemophysalis</i> species (Kelly & Mason, 1990; Parola et al, 2013)
“African Tick-Bite Fever”	<i>Rickettsia africae</i>	<i>Amblyomma</i> species (Nlang et al, 1998) <i>Hyalomma</i> species (Mediannikov et al, 2010) <i>Rhipicephalus</i> species (Mediannikov et al, 2010)
“European rickettsiosis”	<i>Rickettsia aeschlimannii</i>	<i>Hyalomma</i> species as the main vector (Bitam, 2012) <i>Rhipicephalus appendiculatus</i> (Pretorius & Birtles, 2002) <i>Rhipicephalus evertsi eversti</i> & <i>Rh. annulatis</i> (Reye et al, 2012)
Un-named spotted fever	<i>Rickettsia massilae</i>	<i>Rhipicephalus sanguineus</i> (Parola et al, 2013)
“Israeli tick typhus”	<i>Rickettsia conorii</i> subspecies <i>israelensis</i>	<i>Rhipicephalus sanguineus</i> (Parola et al, 2013)
“Lymphangitis-associated rickettsiosis”	<i>Rickettsia sibirica</i> subspecies <i>mongolitimonae</i>	<i>Hyalomma truncatum</i> (Parola et al, 2013)

Although other arthropods like fleas, lice or mites can be involved, rickettsiosis is usually transmitted to humans through a tick bite (Portillo et al, 2017). Game hunting and travel in Southern Africa between November and April are known risk factors for contracting ATBF (Jensenius et al, 2003b). *Rhipicephalus sanguineus* is well adapted to human urban environments but tends to be host specific, preferring to feed on domestic dogs and rarely people. It will however parasitize small rodents and people if there are no dogs available (Parola, 2004). In contrast, *Amblyomma* ticks are very aggressive and often attack hosts. This explains why often grouped human cases are found. In more than 50% of cases, multiple inoculation eschars occur (Parola et al, 2001). Infection rates among *Amblyomma*

ticks are very high and up to 75% of those infected act as both reservoirs and vectors (Prabhu et al, 2011).

Mtshali et al (2017) found an overall prevalence of 37% for *Rickettsia* species in a survey of 318 ticks collected from dogs and cats in South Africa. They identified predominantly *R. africae* but also *R. conorii*. Although many of the positive ticks were unidentified, 35% of the positive ticks were *R. sanguineus*. In contrast to Mtshali et al (2017) whose study found *Rickettsia* species to be absent in Mpumalanga Province, *R. africae* was detected in ticks collected from domestic dogs in Mpumalanga by a different study (Kolo et al, 2016). In this other study, two of the *A. hebraeum* pools and one *Haemophysalis elliptica* pool tested positive. This new information suggests that domestic dogs may play an important role in the lifecycle of this organism (Kolo et al, 2016). When Halajian et al (2016) surveyed 205 ticks collected from wild animals in the Western Cape and Limpopo provinces, South Africa, they found two positive samples (one *A. sylvaticum* and one *R. simus*) with high identity (99.2-100%) with *R. massiliae* and eight samples with high identity (99.8-100%) with *R. africae*. This is the first report of *R. massiliae* being detected in South Africa and showed that these two vectors should be considered in the epidemiology of this pathogen. In Kenya's Masai Mara Nature Reserve, *R. africae* has been documented in *Amblyomma* ticks collected from wild animals as well as *A. variegatum* and *A. gemma* ticks from domestic animals (Macaluso et al, 2003). Also in Kenya, Mwamuye et al (2017) tested 270 pools of ticks with PCR and detected *R. africae* in two pools of adult *R. maculatus*, one pool of adult *A. eburneum* and four pools of nymphal *A. eburneum*. This places two new tick species as potential vectors for ATBF in East Africa. In Nigeria, a prevalence of between 4% and 8% was found for *R. africae* in a survey of 218 collected ticks (153 *A. variegatum*, 45 *R. (Boophilus) microplus*, 20 *R. sanguineus*). *Rickettsia africae* was detected in all three of the species of ticks (Ogo et al, 2012).

Importance and incidence

In South Africa, human cases of tick-bite fever are caused by two organisms: *Rickettsia conorii* and *Rickettsia africae* (Frean et al, 2008). Unfortunately, there are no actual case incidence reports for the country. It is a common disease in South Africa and the number of recognised cases are probably far outnumbered by subclinical cases (Frean et al, 2008). Up

to 11% of ATBF infections have been reported amongst international travellers returning from visiting game reserves in South Africa (Althaus et al, 2010).

Although a high seroprevalence is seen in South African populations, only 2 cases of clinical ATBF in black African patients have been documented. Most cases of ATBF are seen in Caucasian patients and usually in travellers to endemic areas (Cohen et al, 1996). In a survey of general health practitioners in Zimbabwe in 1991, clinical ATBF case incidence was estimated between 0 and 80 cases per 100 000 patients annually depending on the region (Kelly et al, 1991). There are several theories to explain the high seroprevalence but low incidence of clinical disease in black Africans. The disease may be milder, diagnosis may be more difficult as a rash is less obvious in dark-skinned individuals and it is also possible that people affected by the disease have limited access to health care workers and often go undiagnosed. Hoogstraal (1981) suggested that black African patients may develop immunity to tick bite fever at a young age which is why the disease is predominantly seen in Caucasians, tourists and temporary workers. Kelly & Mason (1991) obtained data to support this theory with high *R. conorii* antibody titres found more commonly in younger individuals.

Clinical signs, diagnosis and treatment

Depending on the tick involved, most cases experience an eschar at the tick bite site. Other symptoms include fever, flu-like symptoms, headache, lymphangitis, myalgia, fatigue and a maculopapular rash (Brouqui et al, 2004; Parola, 2004). The disease can become serious with neurological manifestations, organ failure and death. The risk factors for severe disease include delayed treatment, age, cirrhosis, diabetes, uraemia and chronic alcoholism (Parola, 2004). Tick bite fever can mimic meningococcal septicaemia, other gram-negative septicaemias or viral haemorrhagic fever (such as Crimean-Congo haemorrhagic fever, CCHF, which is also tick-transmitted). The incubation period can help in this regard with one to three days for CCHF and five to seven days for TBF (Jenseni et al, 2003a). Laboratory findings include: thrombocytopenia, leucocyte count abnormalities and elevated hepatic enzyme levels (Brouqui et al, 2004).

Identification of rickettsias by microscopy is usually done using immunohistochemical (IHC) assays on formalin-fixed tissue specimens. IHC assays use monoclonal or polyclonal antibodies. These techniques are limited to few research laboratories (Portillo et al, 2017).

Diagnosis is most often made by serology which is the easiest method but requires paired acute and convalescent (2-6 weeks apart) serum samples. The most accepted serological test is MIF, but ELISA and IPA assays are also available (Brouqui et al, 2004; Portillo et al, 2017). Unfortunately, seroconversion in ATBF is delayed and interpreting serological data can be confounded by cross-reactivity. Isolation by culture is the ultimate diagnostic goal but requires special techniques in a Biosafety Level 3 laboratory and are mainly for research purposes. R-T PCR methods are sensitive, specific and rapid (Brouqui et al, 2004; Portillo et al, 2017). Almost any sample (blood, buffy coat, eschar biopsies/swabs, organ biopsies, pleural fluid and CSF) can be used but must be taken in the acute course of infection. Culture is still used for study of the pathogen physiology, genetic descriptions and for improvement of diagnostic tools (Portillo et al, 2017).

In the past, skin biopsy of an eschar site is a sample that has been described as useful for diagnosing TBF. The problems with this procedure are that it may only be performed by a physician and in sterile circumstances with local anaesthesia. Unfortunately, it is not very practical if it cannot be collected by a general practitioner or a family doctor (Socolovschi et al, 2012). Recently, Bechah et al (2011) demonstrated that eschar swabbing could be used to identify rickettsial DNA for diagnosis. Eschar swabbing had a high acceptance rate by patients and clinicians and the sample can be used for molecular techniques. Socolovschi et al showed that eschar swabs offer a good alternative sample to skin biopsies for diagnosing rickettsial disease. Furthermore, these samples are easy to transport (stored at 4°C for up to three days) and may also be used to diagnose other diseases such as cat scratch disease, cutaneous leishmaniasis or human papilloma virus infection. In their study, they could confirm ATBF in three patients who still had negative serology (Socolovschi et al, 2012). The median times for ATBF seroconversion after the onset of symptoms are 28 days for IgG and 25 days for IgM (Fournier et al, 2002).

If rickettsiosis is suspected in a patient, early antimicrobial treatment must be started even if it is before confirming the diagnosis (Portillo et al, 2017). Rickettsias are all susceptible to the oxytetracycline antibiotic group, for example doxycycline (Parola, 2004). For adults, 100mg doxycycline twice daily for five to seven days is recommended (Raoult & Drancourt, 1991).

4.6 Tick-borne Relapsing Fever

Tick-borne relapsing fever (TBRF) is caused by various haematogenous spirochetes of the genus *Borrelia* (Figure 6). It is an under-recognized disease that occurs all over the world (Meerburg et al, 2009).

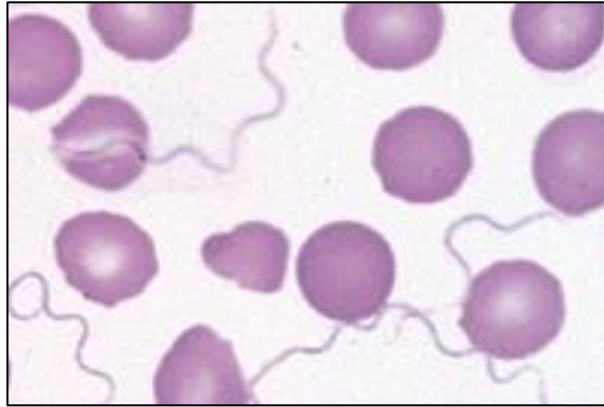


Figure 6: Peripheral blood smear demonstrating *Borrelia duttoni*

(https://www.indso.co.uk/wp-content/uploads/2015/09/MSBA0167_tn.jpg)

The disease in Africa was first described among prisoners in Constantine, Algeria, by a French army doctor in 1866 (Ministère de la Guerre, 1931). Two years later, borrelliae were observed microscopically on a blood smear (Obermeier, 1873). *Borrelia* species have been traditionally distinguished by geography and vector (Figure 7) but more specific diagnostic tests are being developed. The known human borrelliae are *B. hispanica*, *B. crocidurae*, *B. duttonii* and *B. recurrentis* (Elbir et al, 2013). The first three are tick-transmitted and the last one, *B. recurrentis*, is transmitted by lice.

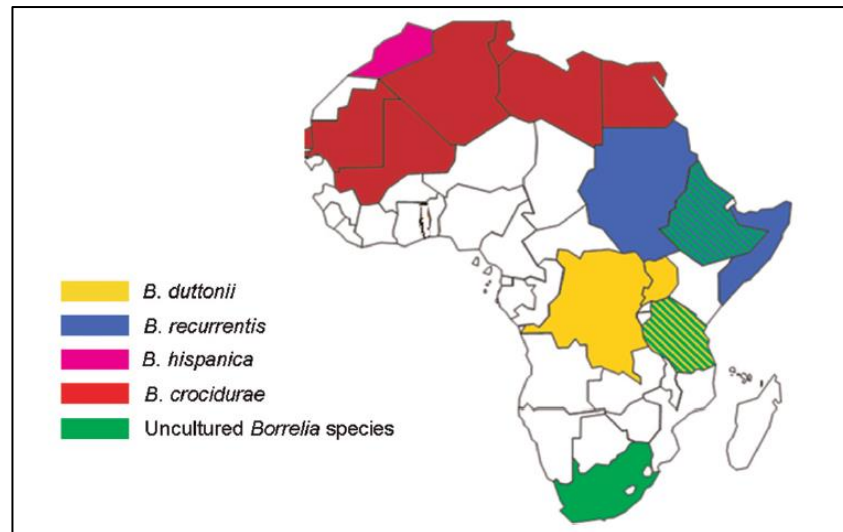


Figure 7: Geographic distribution of African relapsing fever of the *Borrelia* species (Elbir et al, 2013)

Epidemiology and reservoir hosts

These *Borrelia* are transmitted by various species of the soft body *Ornithodoros* ticks (Schwan and Hinnebusch, 1998). Many small mammals (often rodents) act as reservoir hosts for TBRF and these pathogens have been found in chipmunks, squirrels, rats and mice. *Ornithodoros* ticks encounter people as they live in the burrows or nests of rodents inside human dwellings (Meerburg et al, 2009). For *B. duttonii*, there is no known mammal reservoir and *O. moubata* sl. act as both vector and reservoir (Morel, 1965). *Borrelia hispanica* has various small mammal reservoir hosts (Sarih et al, 2009). Rodents and insectivores are the known reservoir hosts for *B. crocidurae* (Schwan et al, 2012).

Transmission and arthropod vectors

In Africa, TBRF occurs in three different vector/pathogen complexes involving soft ticks of the genus *Ornithodoros* (Schwan and Hinnebusch, 1998). In the savanna areas of eastern and southern Africa, TBRF is caused by *B. duttonii* with *O. moubata* sl (*O. moubata* ss and *O. porcinus*) as the vectors. In the wild, these ticks live in the burrows of large animals such as aardvarks (*Orycteropus*), warthogs (*Phacochoerus*) and porcupines (*Hystrix*). They have also adapted to reside in human dwellings and domestic animal shelters (Walton, 1962). In North Africa, TBRF is caused by *B. hispanica* and uses *O. erraticus* as the vector (Sarih et al, 2009). These ticks are found in large or small burrows and have also adapted to domestic animal shelters. Most people become infected in the summer when sleeping in fields or farm

buildings (De Buen, 1926). In West Africa, TBRF is caused by *B. crocidurae* using *O. sonrai* as the vector (Schwan et al, 2012). These ticks inhabit mainly rodent burrows and are quick feeders infecting people in their sleep if their burrows open into bedrooms (Trape et al, 1996).

People become infected via a bite from an infected tick which leaves the nest of its rodent host in search for an alternative host (Meerburg et al, 2009). Most cases occur in summer months when people are on holiday and staying in rodent-infested cabins but in the winter months a warm fire is enough to activate resting ticks in cabin walls (Centers for Disease Control and Prevention, 2015).

Importance and incidence

The disease occurs sporadically in Mediterranean countries, and in North and West Africa (Meerburg et al, 2009). Immunity after infection is short-lived and new infections can occur as soon as 6 months after recovery (Elbir et al, 2013). Relapsing fever has long been recognised as a significant cause of disease and death in several African regions (Goubau, 1984). Tick-borne relapsing fever is endemic in the DRC and Tanzania where it is listed in the top 10 killer diseases in children under 5 years of age (Cutler, 2010). In Senegal, the average incidence of TBRF at community levels is the highest described for any bacterial disease reaching 11 per 100 person-years (Vial et al, 2006) and in outpatient clinics it is the second most common cause of fever after malaria (Trape et al, 1991). In Morocco, it was found that 20.5% of patients with an unexplained fever had *B. hispanica* TBRF (Sarih et al, 2009). In Tanzania, *B. duttonii* is known to have a mortality rate of 2.3% (Mayegga et al, 2005). Incidence levels for South Africa were not available.

Clinical signs, diagnosis and treatment

People suffering from TBRF experience a characteristic syndrome of periodic fevers. The first attack is usually the most severe in height and duration of fever and succeeding attacks are milder (Barbour, 1999). The disease is characterised by fever, chills, headache, myalgia, arthralgia and coughing. Haemorrhage, iritis, hepatomegaly, splenomegaly may occur and abdominal pain, nausea, vomiting, diarrhoea and photophobia are common in African cases. Sometimes a rash may occur at the end of the first febrile episode. Neurological findings are common, and jaundice can be seen (Brouqui et al, 2004).

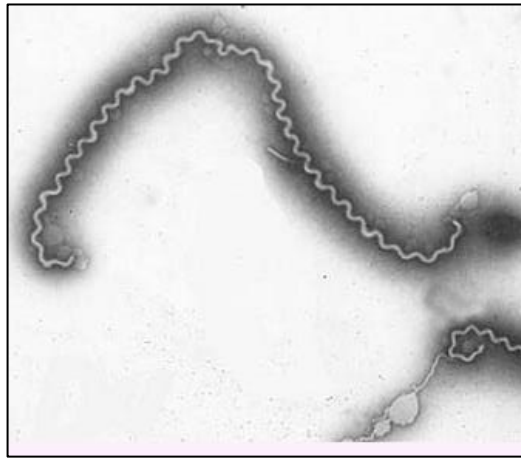
For the diagnosis of TBRF, *Borreliae* can be demonstrated on peripheral blood smears of febrile patients using dark-field microscopy or Giemsa staining (Figure 6). This method has 70% sensitivity and has value in conjunction with other tests (Brouqui et al, 2004). Culture is poorly suited for diagnostics because it is low-yield, time consuming and expensive. Serology is more useful for retrospective diagnosis as seroconversion has usually not yet occurred in acute infection. PCR is useful for supporting diagnosis, but assays must be properly standardised and performed (Cutler et al, 2017).

There are several antibiotic treatments that are effective for TBRF and they include tetracyclines, penicillin, ampicillin, erythromycin and chloramphenicol (Dupont et al, 1997). Doxycycline treatment is both safe and effective in preventing TBRF after exposure to ticks in a high-risk environment (Hasin et al, 2006). A potential serious side effect of antibiotic treatment for TBRF is a form of cardiovascular shock called the Jarisch-Herxheimer reaction. This reaction occurs after cytokines are released during the clearance of *borreliae* from the blood. This reaction has a mortality rate of 5% and is characterised by tachypnoea and hypotension (Warrell et al, 1970).

4.7 Leptospirosis

Leptospirosis is a globally important zoonotic disease caused by spirochaetal bacteria. It is an endemic disease that affects impoverished and developing communities and is often overshadowed in public and clinician awareness by high profile infections such as malaria and HIV. Additionally, the people affected by the disease usually lack early medical intervention (Meerberg et al, 2009). Unfortunately, many bacterial zoonoses including leptospirosis are under-diagnosed and under-reported in Africa and are therefore overlooked as public health priorities (World Health Organization, 2006). In 2011, the disease was recognised as a neglected disease by the WHO (World Health Organization, 2011). Despite this, the disease is often overlooked in research priorities for African countries (World Health Organization, 2006). More than 250 pathogenic *Leptospira* serovars have been identified and classified into 25 serogroups (Cerqueira et al, 2009; de Vries et al, 2014). Recent species determination by DNA sequence identity has identified 13 pathogenic *Leptospira* species and seven of these (*L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L. kirschneri* and *L. alexanderi*) are considered the foremost agents of

human and animals disease (Cerqueira et al, 2009). *Leptospira* bacteria are spiral shaped bacteria with hooked ends shown in Figure 8 (Levett, 2001).



*Figure 8: Scanning electron micrograph of *Leptospira* species (https://encrypted-tbn2.gstatic.com/images?q=tbn:ANd9GcTUJoF3ujn9fLGN9ulF7iTolLVvTrs_PVljmNchNm5Qno_KDh0XT).*

Leptospirosis is considered an emerging disease because of its epidemic proportions and increasing incidence worldwide. The geographic distribution of known Leptospirosis in Africa is shown in Figure 9 below. Leptospirosis is most common in tropical regions where people and animals live in close contact and the warm, humid conditions allow for survival and transmission of the bacteria (Adler & de la Pena Moctezuma, 2009). It is a direct zoonosis associated with exposure to water contaminated with urine from infected animals. The WHO reports that an increase in the incidence of Leptospirosis has been recorded in countries where there is surveillance but under reporting is a problem in developing countries and actual incidence may be much higher (World Health Organization, 2011). It is considered a serious public health threat as the disease can cause severe and fatal clinical syndromes (Vijayachari et al, 2008).

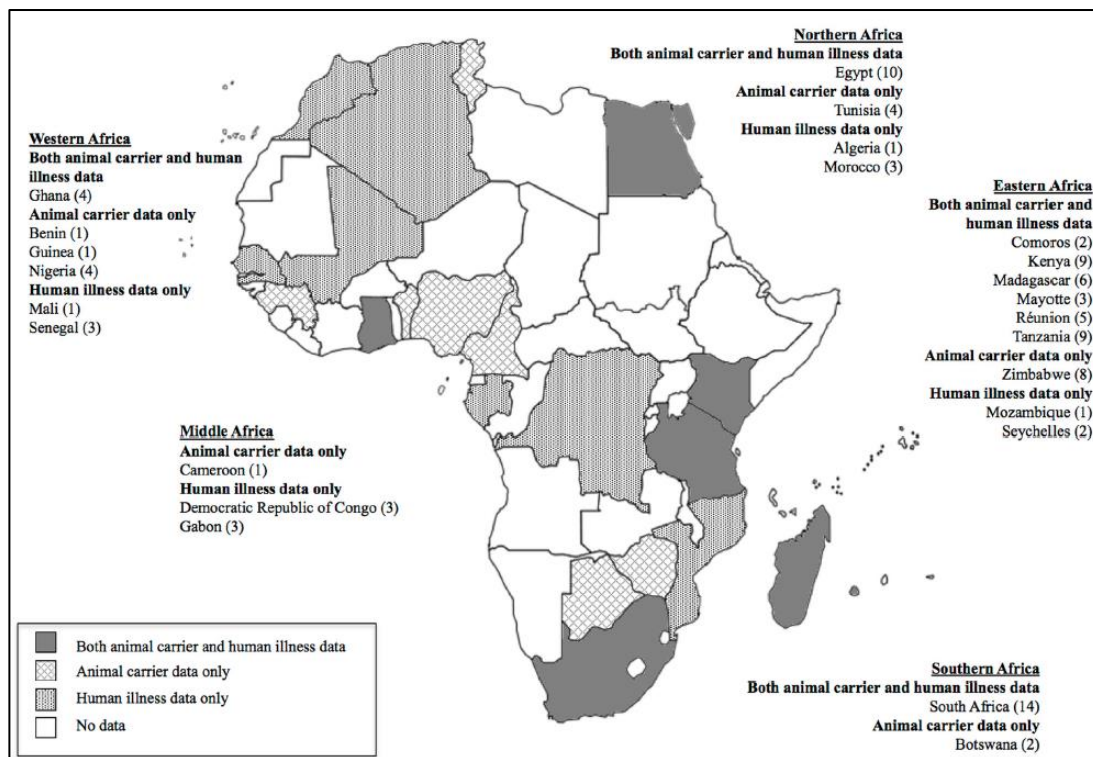


Figure 9: Geographic distribution of acute human leptospirosis and confirmed animal *Leptospira* species Infection in Africa. The numbers represent confirmed cases. (doi:10.1371/journal.pntd.0003899.g002)

Epidemiology and reservoir hosts

Leptospire reside in the renal tubules of their animal hosts and are excreted in urine. There are a large variety of both wild and domestic animal hosts. Rodents were the first recognised carriers, but many animals have been implicated as hosts including cattle, deer, pigs, goats, sheep, dogs, cats and wild animals. Examples of rodents implicated are house mice, rats, bandicoots and field rodents (Vijayachari et al, 2008; Allan et al, 2015). Animals may be asymptomatic carriers of infection (maintenance hosts) or may develop clinical disease (accidental hosts) depending on the serovar involved (Vijayachari et al, 2008; Cerqueira et al, 2009).

Katakweba et al (2012) performed a survey study looking for various haemoparasites in captured rodents from Tanzania, Namibia and Swaziland. In the Tanzanian group, they found *Leptospira interrogans* in the following serogroup proportions (n = 350): Icterohaemorrhagiae (10.29%), Pomona (2.86%), Hardjo (1.14%), Bullum (0.86%),

Grippytyphosa (1.43%) and Canicola (1.14%). The rodent species which tested positive (in descending seroprevalence order) were: *Mastomys natalensis*, *Rattus rattus*, *Crocidura hirta*, *Arvicanthis neumanni*, *Tatera vicinus* and *Graphiurus cf. murinus*. The rodents testing negative were *Acomys spinosissimus* and *Mus minutoides*.

Leptospirosis is widely prevalent in rodents, shrews, humans and livestock in parts of Tanzania (Machung'u et al, 1997). Interestingly, the disease was most present in the central part of Tanzania and was most prevalent in rodents captured in houses and peri domestic areas where they interact with humans (Katakweba et al, 2012). In urban environments in Durban, South Africa, Taylor et al (2008) reported that 10% of captured rodents (n = 262), all of which were Norway rats (*Rattus norvegicus*), were seropositive for leptospirosis.

Transmission

Leptospirosis is not a vector borne disease but is included in this review as it focusses on rodent borne diseases of public health importance in Africa. People can become infected through consumption of or skin or mucous membrane contact with food or water that is contaminated with infected urine. Consumption of raw milk products from infected livestock or the handling of dead infected hosts are other modes of transmission (Vijayachari et al, 2008; Meerbug et al, 2009; de Vries et al, 2014). In rural areas, risk factors for transmission are high rainfall, livestock holding and farming. In contrast, urban areas experience increased rodent-borne transmission which is associated with poor hygiene, inadequate waste disposal and overcrowding. All these conditions are found in most developing countries (de Vries et al, 2014). Brown and Prescott (2008) identified specific high-risk activities that are associated with leptospirosis infection: outdoor water sports (swimming, kayaking, fishing), occupational exposure (abattoir and sewer workers, farmers, veterinarians, animal husbandry and military personnel in temperate climates) and tropical exposures (sugar-cane harvesters, rice-paddy planters).

Importance and incidence

Disease data in Africa is very limited. A systematic literature review of available data in Africa showed that leptospirosis causes illness in 2.3% to 19.8% of hospital patients with a fever. When population level data were available, it was estimated that there are between three and 102 cases per 100 000 people per year (Allan et al, 2015). The greatest risk is to

the urban and rural poor people who experience a high rate of exposure and who have little available income to spend on early medical intervention. The WHO estimates 0.1-1 case per 100 000 people annually in temperate climates and 10 or more cases per 100 000 people in the humid tropics (WHO, Geneva).

In a serological survey of 219 people living in Cato Crest in Durban, South Africa, 23% were found positive for leptospirosis exposure (Taylor et al, 2008). In a serological survey of 530 domestic dogs in South Africa, *Leptospira* species were identified in 4.7% (n = 25) of the dogs. The two most frequent serovars identified were *L. interrogans* Canicola and *L. interrogans* Pyrogenes (Gatley, 2009).

Migration of refugees from countries with civil unrest poses an interesting challenge and opportunity. A case study in Italy described a young East African refugee who presented to their hospital intensive care unit with severe respiratory distress, hypotension and jaundice. She was diagnosed with leptospirosis and louse-borne relapsing fever (*Borrellia recurrentis*) and after appropriate treatment she recovered. However, ten days later she ran away from hospital for fear of repatriation and so the path of care could not be completed. This showed that even in a first world country with the most advanced technologies available for patient care, one cannot ignore psycho-social factors that affect patients suffering from neglected diseases (Cutuli et al, 2017).

Clinical signs, diagnosis and treatment

The symptoms can be mild and flu-like often leading to neglect and delayed diagnosis in poor areas until serious clinical illness occurs. Common clinical signs are acute fever, headaches, myalgia, vomiting and diarrhoea with or without any combination of: conjunctivitis, oliguria, jaundice, cough, haemorrhage or skin rash (de Vries et al, 2014). The mortality rate of severe leptospirosis is up to 15%. Complications include meningitis, hepatitis, renal failure and haemorrhage (Meerburg et al, 2009). Clinical syndromes that are associated with high morbidity and fatality are hepato-renal syndrome (“Weil’s disease”) and haemorrhagic pneumonitis. Early antibiotic treatment can reduce the severity of disease and number of complications (Vijayachari et al, 2008).

To make a diagnosis, a high index of suspicion is needed along with laboratory findings. Depending on the case, one or a combination of findings are used: Isolation of pathogenic

Leptospira species from a normally sterile site, high IgM titres (by ELISA or IFA) or a fourfold increase in antibody titre in acute and convalescent serum samples (MAT or IFA) support the diagnosis (de Vries et al, 2014). It is vital that the correct specimens are taken for accurate interpretations (Vijayachari et al, 2008).

Treatment of mild cases is achieved with oral doxycycline, azithromycin, ampicillin or amoxycillin. In severe cases, intravenous benzyl penicillin must be given immediately. For prevention, animal vaccination, rodent and wet area control and proper waste disposal go a long way (Vijayachari et al, 2008; de Vries et al, 2014).

Several animal vaccines already exist but even total herd immunisation was not able to result in eradication. Various human vaccines have been under development for many years and so far, none have been safe enough for registration and use. The possibility of newer vaccines is being explored and might be promising (Vijayachari et al, 2008).

4.8 Bartonellosis

Bartonellae are facultative intracellular gram-negative coccobacilli bacteria (Figure 10) with a worldwide distribution. Some cells are flagellated, and some are not (Saisongkorh et al, 2009).



Figure 10: Scanning electron micrograph of *Bartonella* species

(<http://www.publichealthalert.org/uploads/1/4/0/7/14079136/8114105.jpg>).

Bartonellae are adapted to cause long-lasting non-symptomatic intra-erythrocytic infections in their reservoir hosts and are transmitted by blood sucking arthropods such as sand flies, lice, fleas, biting flies and possibly ticks (Buffet et al, 2013; Gutiérrez et al, 2017). *Bartonellae*

have very high diversity and prevalence in their natural hosts. Clinical disease usually results when they are introduced into an incidental host or an immunosuppressed reservoir (Pulliainen & Dehio, 2012). In total, there is an overwhelming number of *Bartonella* species. Those that are adapted to rodents are shown in Table 2.

Table 2: Summary of rodent-adapted *Bartonella* species, their geographic distribution and potential as human pathogens (indicated in bold)

<i>Bartonella</i> species	Distribution	Human infection	Reference
<i>B. acomydis</i>	Japan	Not reported	Sato et al, 2012
<i>B. birtlesii</i>	Europe	Not reported	Bermond et al, 2000
<i>B. callosciuri</i>	Japan	Not reported	Sato et al, 2012
<i>B. coopersplainsensis</i>	Asia Australia	Not reported	Gundi et al, 2009
<i>B. doschiaae</i>	Europe North America	Not reported	Birtles et al, 1995
<i>B. elizabethae</i>	Worldwide	Yes	Daly et al, 1993
<i>B. grahamii</i>	Northern hemisphere	Yes	Birtles et al, 1995
<i>B. jaculi</i>	Japan Egypt	Not reported	Sato et al, 2012
<i>B. japonica</i>	Japan	Not reported	Inoue et al, 2010
<i>B. pachyuromydis</i>	Japan	Not reported	Sato et al, 2012
<i>B. phoceensis</i>	Asia Europe	Not reported	Gundi et al, 2004
<i>B. queenslandensis</i>	Asia North America Australia Africa	Not reported	Gundi et al, 2009
<i>B. rattaustraliani</i>	Australia	Not reported	Gundi et al, 2009
<i>B. rattimassiliensis</i>	Asia Europe	Not reported	Gundi et al, 2004
<i>B. rochalimae</i>	Northern hemisphere	Yes	Lin et al, 2008
<i>B. silvatica</i>	Japan	Not reported	Inoue et al, 2010
<i>B. tamiae</i>	Thailand	Not reported	Kosoy et al, 2008
<i>B. taylorii</i>	Europe Asia	Not reported	Birtles et al, 1995
<i>B. tribocorum</i>	Worldwide	Yes	Heller et al, 1998
<i>B. vinsonii</i> subspecies <i>arupensis</i>	North America	Yes	Welch et al, 1999
<i>B. vinsonii</i> subspecies <i>vinsonii</i>	North America	Not reported	Brenner et al, 1993
<i>B. washoensis</i>	Northern hemisphere	Yes	Kosoy et al, 2003

As seen in the table, there are six rodent-borne *bartonellae* with the potential to infect humans. They are *B. elizabethae*, *B. grahamii*, *B. rochalimae*, *B. tribocorum*, *B. vinsonii* subspecies *arupensis* and *B. washoensis* (Buffet et al, 2013).

Epidemiology and reservoir hosts

The disease occurs sporadically and in epidemics that affect humans, domestic animals, wild animals and marine animals (Saisongkorh et al, 2009). Rodents are known reservoirs for many *Bartonella* species and prevalence can be quite high between 11 and 62.2%. The rodents implicated vary globally but include rats, voles, shrews, gerbils, mice and ground squirrels (Meerburg et al, 2009; Saisongkorh et al, 2009). Pets and cattle are considered major reservoir hosts for *Bartonella* species, especially cats and dogs who are implicated in human infections (Saisongkorh et al, 2009). All *Bartonella* species identified in sick dogs are potentially pathogenic to humans and the clinical and pathological abnormalities seen in dogs are like those observed in human patients. This makes dogs an excellent sentinel and comparative model for human infections (Chomel et al, 2006). The role of domestic dogs as reservoirs is not yet clear though they have been infected with *B. v. berkhoffii*, *B. henselae*, *B. clarridgeiae*, *B. washoensis*, *B. elizabethae*, *B. quintana* and *B. bovis*.

Gundi et al (2012) reported on the detection of *Bartonella* DNA using PCR on spleen samples in 13.6% (n= 8/59) of small mammals captured in Democratic Republic of Congo (DRC) and 41% (n= 16/39) of rodents trapped in Tanzania. They concluded that such high prevalence rates could represent significant risk to humans (Gundi et al, 2012). Pretorius et al (2004) confirmed that bartonellae are widely distributed among endemic rodents in South Africa. They found putative bartonellae in 37.2% (n = 32/86) of small mammals sampled in the Free State province (Pretorius et al, 2004). In rats in Nigeria, bartonellae with 97-100% identity with *B. elizabethae*, 97-98% identity with *B. tribocorum* and 98% similarity to *B. grahamii* were isolated (Kamani et al, 2013b). Although bartonellae have been found in bats in South Africa and Swaziland, it appeared that the genotypes affecting South African rodents and bats were different (Dietrich et al, 2016).

Transmission and arthropod vectors

People become incidentally infected by arthropod transmission. Although *B. henselae* has been identified in ticks, their competence as vectors still needs to be established (Fritz, 2009). Fleas play a major role in transmission of feline *Bartonella* but other vectors such as ticks and biting flies have also been identified as harbouring *Bartonella* DNA (Chomel et al, 2006). Poverty, crowding, poor hygiene and living in close contact with rodents are all risk factors for human transmission. It is associated with homelessness and intravenous drug users too. The extent to which rodents contribute to human infections is still questionable. Bartonellosis is thought to be contracted via faeces deposited onto broken skin by rodent ectoparasites (Meerburg et al, 2009).

Leulmi et al (2014) found *Bartonella* species in fleas from the DRC at a prevalence of 3.6% (n = 4/111). This is supported by a serological survey in human patients who tested positive for *B. henselae*, *B. quintana* or *B. clarridgeiae* (Laudisoit et al, 2011). Gundi et al (2012) also showed that *Bartonella* species are present in DRC and differ between hosts when they found that local rodents harboured *Bartonella* species closely related to *B. elizabethae* and *B. tribocorum*. Although the infection rates in bat blood were low (3.3%), a study of bats and bat flies collected in South Africa and Swaziland uncovered an incidence of 36% for *Bartonella* species in bat flies suggesting that they might be potential vectors (Dietrich et al, 2016). These flies are known to be very host specific but in Africa the increased human activity inside caves for religious practices (Anti et al, 2015) means that there could be a greater risk of human contact with bats and their flies.

Importance and incidence

The impact of rodent-borne *Bartonella* infections on public health is still unknown but with an increase in outdoor activities, it is possible that cases of human bartonellosis may rise (Buffet et al, 2013). Exact human case incidence rates in Africa do not exist. It is also likely that many cases go undiagnosed. A study performed in South Africa showed *Bartonella* prevalences of 9.5% in the blood of healthy individuals and 22.5% in HIV-positive human subjects (Trataris et al, 2012). A case of endocarditis caused by *B. quintana* was diagnosed in an immune compromised homeless patient from South Africa in 1996 and Bacillary angiomatosis has been reported in many HIV-positive patients in South Africa. In fact, much

of the current understanding of *Bartonella* associated disease has resulted from the AIDS epidemic (Freaan et al, 2002).

Clinical signs, diagnosis and treatment

Bartonella infections can manifest as symptomatic or asymptomatic syndromes. *Bartonella elizabethae* and other bartonellae have been associated with endocarditis. Other presentations of Bartonellosis (uncommon complications) include encephalopathy, encephalitis, radiculitis, myelitis, thrombocytopenic purpura, osteomyelitis and hepato-splenic disease (Saisongkorh et al, 2009). Bacillary angiomatosis is a known potentially severe vasculo-proliferative infection caused by *B. henselae* in AIDS patients. Another complication is that serology may be unreliable in patients with advanced HIV infection (Freaan et al, 2002). It is possible that the other zoonotic bartonellae could also cause unique disease syndromes in AIDS patients.

Molecular diagnostic tests are rapid, specific and sensitive. R-T PCR assays have higher sensitivity than isolation. It is recommended that attempt is made to culture and isolate DNA positive samples to allow for molecular characterisation and speciation. However, culture isolation requires specific conditions and is laborious, time-consuming and offers poor sensitivity to detect positives in wild animal samples (Gutiérrez et al, 2017). Serology by ELISA or IFA are available. Samples used are serum, plasma or CSF. Again, it does require paired serum sampling and does not distinguish between *Bartonella* species (Saisongkorh et al, 2009).

Bartonella infections are challenging to treat because they are persistent and often relapse. Infection involves an intraerythrocytic phase that provides a protective niche for the bartonellae. Treatment is dependent on the species (and syndrome) involved, the course of disease (acute or chronic) and the immune status of the patient (Rolain et al, 2004).

Symptoms of cat scratch disease typically do not respond to antibiotic therapy. It is thought that the manifestations of the disease are due to an immunological reaction in the lymph nodes and not caused by the actual bacterium. Mainly, analgesic and anti-inflammatory treatment is indicated. For severe ongoing cases, treatment with azithromycin or a combination of doxycycline and rifampin are described. For *B. quintana* infections, a 28-day course of doxycycline is recommended. In acute cases, this is combined with gentamicin.

The treatment of choice for bacillary angiomatosis is 3 months of erythromycin. The recommended treatment for endocarditis caused by *B. henselae* or *B. quintana* in immune suppressed individuals is a minimum of 14 days of gentamicin followed by 6 weeks of ceftriaxone with or without doxycycline (Rolain et al, 2004).

4.9 Q Fever

The febrile illness “Query fever” or “Q fever” was first described in 1935 when human cases in slaughterhouse workers were discovered in Australia (Derrick, 1983). The first cases in Europe were reported in soldiers in the Balkan region in 1940 (Imhauser, 1949) and subsequently in Germany (EFSA, 2010) and the Netherlands in 1956 (Westra et al, 1958). The disease is caused by an obligate intracellular gram-negative coccobacillus (Figure 11) called *Coxiella burnetii* (Fournier et al, 1998).

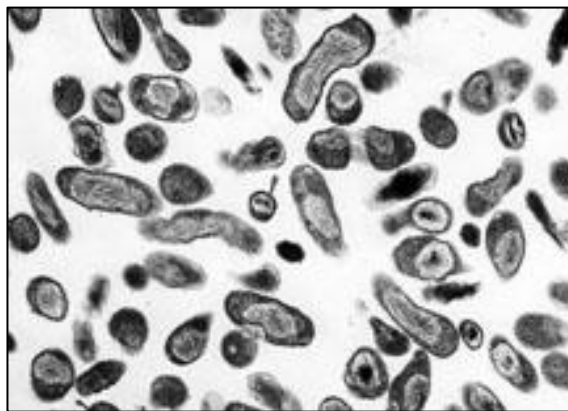


Figure 11: *Coxiella burnetii*, organism responsible for causing Q fever
(<http://www.boergoats.com/clean/articles/health/q-fever/Qfever.jpg>).

Coxiella burnetii are present worldwide and can exist in two forms. The small cell variant has “spore-like” characteristics and can survive various environmental extremes which allow it to spread far and wide. The large cell variant has evolved to persist within the phagolysosomes of monocytes and macrophages which allows the organism to persist and cause chronic disease. The highly infectious nature and stability in many environmental conditions has led to the inclusion of *C. burnetii* as a list B potential biological warfare agent (Cutler et al, 2007). Q fever infection in people is considered an occupational hazard with

the highest prevalence seen in veterinarians, farmers, farm workers, slaughterhouse workers, tannery workers and more cases in males than in females (Porter et al, 2011).

Epidemiology and reservoir hosts

Although ruminants (sheep, goats and cattle) are the commonly known animal reservoirs (Meerburg et al, 2009; Mori et al, 2017) these ubiquitous bacteria can infect a wide range of other hosts including people, cats, dogs, wildlife and non-mammalian species such as reptiles, fish, birds and arthropods (Cutler et al, 2007; Porter et al, 2011). Rodents can become infected and are suspected as a reservoir but their role in transmission to people is unsure. Norway rats, muskrats, rats, squirrels, wood mice and deer mice have all been implicated as potential reservoirs (Meerburg et al, 2009). *Coxiella burnetii* has a remarkable ability to survive in the environment and has even been known to contaminate soils for months to years and can even be spread by wind (Parker et al, 2006). *Coxiella burnetii* infections in South Africa have only been demonstrated serologically in cattle, goats and sheep. The seroprevalence in cattle is reported to be between 8% and 93% (Frean and Blumberg, 2007).

Transmission and arthropod vectors

Infected animals shed the organism in their milk, faeces, urine and birth products. Most human infections follow exposure to livestock and mankind serves as a sentinel for disease activity (Cutler et al, 2007). People become infected if they inhale contaminated dust or aerosol or if they have close contact with (or ingestion of) contaminated animal products such as milk, meat or wool and birthing products (Meerburg et al, 2009; Mori et al, 2017). Human to human transmission does not usually occur (Porter et al, 2011). Ticks can transmit the pathogen between animal hosts but not to humans (Meerburg et al, 2009) and therefore ticks are not recognised as vectors of Q fever in humans (Maurin & Raoult, 1999). Ticks appear to be more important in their role for pathogen amplification in animals than transmission (Mori et al, 2017). Ticks appear to play a significant role in the transmission of *C. burnetii* between wild vertebrates especially rodents, lagomorphs and birds (Porter et al, 2011).

A survey of 1634 ticks collected from animals in South Africa found that infection rate among cattle ticks was as low as 3% but among sheep, tick infection rates were as high as

32% (Mtshali et al, 2015). Another survey of 318 ticks collected from cats and dogs in South Africa found *C. burnetii* infection rates of 41%. Potential vectors recognised here were *R. sanguineus* and *H. elliptica* (Mtshali et al, 2017). In western Kenya, a study reported detection of *C. burnetii* in *H. leachi* ticks collected from domestic dogs (Knobel et al, 2013).

It is important to note that even though ticks can harbour pathogenic bacteria their natural bacterial microbiome includes bacterial endosymbionts which are important for tick physiology and survival, and they may also impact pathogen acquisition and transmission (Gall et al, 2016). Newer high-throughput next generation sequencing technologies have highlighted the complexity of the tick microbiome (Vayssier-Taussat et al, 2015). *Coxiella*-like endosymbionts are increasingly being detected in tick microbiome studies and should not be confused with *Coxiella burnetii*. The importance of these findings requires further research (Clay and Fuqua, 2011). Also, even though the rate of arthropod-borne Q fever transmission to people is low, more research is needed into potential vectors (Rolain et al, 2005).

Importance and incidence

Q fever is important because apart from the negative health effects infection causes in people, individuals also experience economic losses when their animals have widespread abortions and reproductive problems because of *Coxiella* infection (Meerburg et al, 2009).

In the USA in 2014, 140 acute cases of Q fever were reported to the CDC (Centers for Disease Control and Prevention website, accessed 28 May 2017). The largest community outbreak occurred in the Netherlands between 2007 and 2010 with 4026 human cases reported (van der Hoek, 2010).

In Africa, population level incidence estimates are severely lacking. In Fournier et al (1998), sero-epidemiological surveys showed that 18.3% of blood donors in Morocco, 26% in Tunisia, 37% in Zimbabwe, 44% in Nigeria, 10 to 37% in northeast Africa, and 14.6 to 36.6% in different areas of Canada had anti-*C. burnetii* antibodies which indicates exposure or sero-prevalence. Human Q fever disease incidence in Africa varies widely: 1-9% in Tunisia, 3% in Algeria, 5% in Burkina Faso, 0-9% in Cameroon and 5% in Tanzania with each study using different sampling methods and sampling numbers. As a result, it is believed that *C. burnetii* is an underappreciated threat to human and animal health throughout Africa

(Vanderburg et al, 2014). Even though Q fever is widespread in South Africa, the prevalence rate in humans is unknown and it causes far less human disease compared to *R. africae* (Frean and Blumberg, 2007).

Clinical signs, diagnosis and treatment

Animals infected with *C. burnetii* are usually sub clinical and therefore do not show symptoms of being sick. Signs of Q fever in animals are usually in the form of reproductive disorders such as abortions, stillbirths and poor fertility. Q fever is generally associated with transient outbreaks in animals and humans (EFSA, 2010). The disease in humans can be acute or chronic with potential long-term sequelae. The diagnosis is often missed as the symptoms can be non-specific.

Acute disease presents as an influenza-like illness (fever, headache, myalgia, arthralgia, anorexia, weight loss) with varying degrees of pneumonia (coughing) and hepatitis. In chronic disease, patients often develop endocarditis for months to years after acute illness. Chronic granulomatous hepatitis, chronic pulmonary disease, lymphadenopathy, chronic fatigue syndrome, meningitis and encephalitis are other potential long-term consequences. Endocarditis is usually fatal if not treated. People who develop this complication often have some underlying condition such as cardiac valve damage, prosthetic valves or are immune compromised (Meerburg et al, 2009; Parker et al, 2006; Cutler et al, 2007; Porter et al, 2011). Q fever during pregnancy can cause abortions, neonatal deaths or premature births. Children can develop osteomyelitis and/or endocarditis (Parker et al, 2006). However, a striking absence of clinical signs in children under 15 years of age has been noticed (Cutler et al, 2007). Interestingly, no specific genotype is associated with acute or chronic infection and host factors seem to be more important than genomic variation in determining the outcome of infection (Porter et al, 2011).

Direct diagnosis of *C. burnetii* is made by visualisation, bacterial culture or PCR. The bacteria can be visualised in tissue samples preferably using Gimenez stain or Mac and Giemsa stains can be used. Sensitivity and specificity of visualisation is poor. In chronic cases, Immunohistochemistry has been used too. Culture requires a Biosafety Laboratory level 3 because of the high infectivity of *C. burnetii*. Although it is laborious, time-consuming and success depends on sample quality, it is the gold standard for diagnosis and further research

(Porter et al, 2011). R-T PCR is used for molecular diagnosis. The best samples are those taken during acute infection before antibiotic treatment. Samples include blood, serum, urine, throat swabs, valve material with endocarditis, bone biopsies with osteomyelitis, aborted material, milk, vaginal swabs and faeces. Consecutive samples are recommended because the organisms shed intermittently (Mori et al, 2017). Indirect diagnosis is made using serology. Early diagnosis by serology is difficult because specific antibodies are often absent for the first 2 to 3 weeks of infection. Bacteriological analyses are still recommended to confirm any suspicion of Q fever. Complement Fixation Test (CFT), Enzyme-Linked Immunosorbent Assay (ELISA) and Immunofluorescent Assay (IFA) are the commonly used techniques (Porter et al, 2011). Serum samples contain fewer of the intracellular organisms and tissue samples usually yield better results for PCR or culture. Serology by IFA is commonly used in human medicine as it is more sensitive and specific but requires paired serum samples. Often patients are reluctant to re-test themselves once they feel better especially in rural areas where it requires considerable travel (Parker, et al, 2006).





The recommended treatment for acute disease is twice daily 100mg doxycycline for 14 days. In pregnant women and children under 8 years of age co-trimoxazole is used. For endocarditis, in addition to life-long doxycycline therapy, hydroxychloroquine is recommended for a minimum of 18 months (Parker et al, 2006; Cutler et al, 2007; Porter et al, 2011).





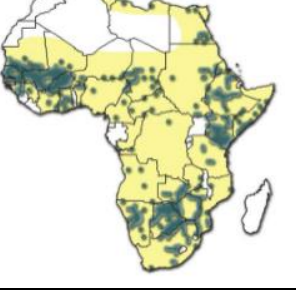
5. Common tick vectors and rodent hosts in Africa




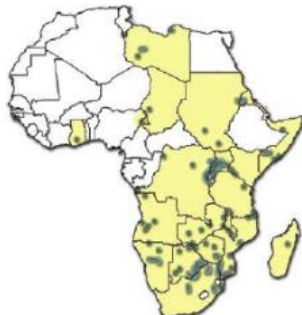
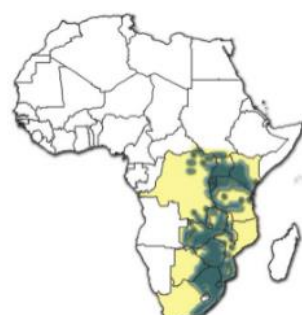
It is difficult to assess the threat of some of the diseases discussed above in Africa because there is little data available. The main vector for HGA, human babesiosis, LD and some other *Borrelia* species - *Ixodes persulcatus* tick complex - is widespread in the northern hemisphere. Although other *Ixodes* genera are more common in Africa, the *Ixodes persulcatus* tick complex is absent in Africa (Chapman et al, 2006; Krause, 2002; Steere, 2001; Raileanu et al, 2017). More research is needed to determine what alternative vectors exist. This also applies to rodent hosts.




A summary of the geographic distribution of the main tick species in Africa which are known to bite and infect humans with infectious diseases is provided in Table 3. Those which have rodents as hosts are highlighted in yellow.

Table 3: Summary of common ticks that transmit infectious diseases to humans in Africa
 (Walton, 1962; Pretorius and Birtles, 2002; Walker et al, 2003; Sarih et al, 2009; Robbins and Carpenter, 2011; Schwan et al, 2012; Madder et al, 2013; Parola et al, 2013)

Common name	Scientific name	Human Pathogens	Hosts for adult tick	Hosts for immatures	Distribution
South African bont tick	<i>Amblyomma hebraeum</i>	<i>R. africae</i> , <i>R. conorii</i> *Tick most responsible for human tick bites*	Cattle Large wild ruminants Small ruminants	Small antelopes Scrub hares Guineafowls Tortoises *rarely infest rodents*	
Ornate sheep tick	<i>Dermacentor marginatus</i>	<i>R. conorii</i>	Cattle Sheep Goats Dogs	Rabbits Small mammals Birds Humans	
Yellow dog tick	<i>Haemaphysalis elliptica</i> (former <i>H. leachi</i>)	<i>R. conorii</i>	Dogs Wild carnivores	Dogs Wild carnivores Murid rodents	
Anatolian hyalomma tick	<i>Hyalomma anatolicum</i>	CCHF virus	Cattle Sheep Goats Camels Horses Donkeys	Cattle Hares Rodents Larger animals Humans	

Shiny hyalomma tick	<i>Hyalomma scupense</i>	<i>Coxiella burnetii</i> CCHF virus	Cattle (mainly) Horses Sheep Goats Camels	Cattle (mainly) Horses Sheep Goats Camels	
Somali hyalomma tick	<i>Hyalomma impeltatum</i>	CCHF virus	Cattle Camels Large domestic animals	Rodents Hares Ground birds	
Lusitanian hyalomma tick	<i>Hyalomma lusitanicum</i>	CCHF virus	Cattle Ruminants	Small mammals such as rabbits	
Mediterranean hyalomma tick	<i>Hyalomma marginatum</i>	CCHF virus *main CCHF virus vector in Europe*	Cattle Horses Sheep Goats Camels	Hares Rabbits	
Course-legged hyalomma tick	<i>Hyalomma rufipes</i>	<i>R. conorii</i> CCHF virus *main CCHF virus vector in Southern Africa*	Cattle Sheep Goats Horses Wild ungulates	Hares Birds	

Shiny/smooth hyalomma tick	<i>Hyalomma truncatum</i>	<i>R. conorii</i> CCHF virus <i>R. sibirica</i> subspecies <i>mongolitimonae</i>	Cattle Sheep Goats Camels Horses Wild herbivores Domestic dogs	Hares Rodents (gerbils)	
Pale-legged hyalomma tick	<i>Hyalomma turanicum</i>	CCHF virus	Cattle Sheep Goats Horses Large wild herbivores	Scrub hares Ground birds	
Sheep tick	<i>Ixodes Ricinus</i>	<i>Borrelia lusitaniae</i> <i>B. garinii</i>	Cattle Sheep	Lizards	
Eyeless tampan	<i>Ornithodoros moubata</i> sl - <i>O. porcinus</i> - <i>O. moubata</i> ss	<i>B. duttoni</i> <i>C. burnetii</i>	Domestic pigs Poultry Humans	Domestic pigs Poultry Humans	
Brown ear tick	<i>Rhipicephalus appendiculatus</i>	<i>R. conorii</i> <i>R. aeschlimannii</i>	Cattle Goats Buffaloes Elands Waterbucks Nyalas Greater kudu Sable antelopes Dogs Sheep	Smaller antelopes Scrub hares	

Zebra tick	<i>Rhipicephalus pulchellus</i>	<i>R. conorii</i> CCHF virus	Cattle Camels Sheep Goats Zebras Black rhinoceroses Elands Gemsboks	Same as adults Hares	
Kennel tick	<i>Rhipicephalus sanguineus</i>	<i>R. conorii</i> <i>R. massilae</i> <i>R. conorii</i> subspecies <i>israeliensis</i>	Dogs Cattle	Dogs Humans	
Glossy tick	<i>Rhipicephalus simus</i>	<i>R. africae</i>	Cattle (mainly) Sheep Goats Horses Dogs Large wild carnivores Zebras Warthogs Rhinoceroses	Murid rodents	

Africa has an abundance of approximately 463 rodent species (Monadjem et al, 2015). Rodents belonging to the family Muridae are well recognised for their role as wildlife reservoirs of zoonotic infectious diseases which are transmitted directly or indirectly (Bastos et al, 2005). South Africa is known to have approximately 50 murid rodent species (Skinner & Chimimba, 2005) and some of these, such as members of the genera *Rattus*, *Mastomys* and *Tatera*, have been implicated in human disease transmission and causing damage to crops and stored grain (Venturi et al, 2004). Table 4 summarises those rodents in Africa which potentially have close contact with humans, either due to damaging crops and stored grain or by living near human settlements. The table shows which of the rodents carry any of the diseases described in the literature review. All the rodent species identified in this Mnisi study are included in the list. Rodents which are unlikely to contact people are not discussed.

Table 4: List of common rodents in Africa and the diseases they carry

Common name	Scientific name	Indirect disease transmission (tick vector)					Direct disease transmission			Reference
		Human granulocytic anaplasmosis	Human babesiosis	Tick-borne rickettsial disease	Lyme disease	Tick-borne relapsing fever	Leptospirosis	<i>Bartonella</i>	Q fever	
Multi-mammate mice - Natal multi-mammate mouse - Southern multi-mammate mouse	<i>Mastomys</i> species - <i>M. natalensis</i> - <i>M. coucha</i>			X		X	X	X X	X	Fiedler, 1994 Du Plessis et al, 2016 Pretorius et al, 2004
African grass rat	<i>Arvicanthus niloticus</i>						X		X	Fiedler, 1994
Four striped mouse	<i>Rhabdomys pumilio</i>			X				X	X	Fiedler, 1994 Pretorius et al, 2004
Striped grass mouse	<i>Lemniscomys striatus</i>			X					X	Fiedler, 1994
Single striped grass mouse	<i>Lemniscomys rosalia</i>									Monadjem et al, 2016
Gerbils - Bushveld gerbil	<i>Tatera</i> species - <i>T. leucogaster</i>			X				X	X	Fiedler, 1994 Pretorius et al, 2004 Kingdon et al, 2013
Crested porcupine	<i>Hystrix cristata</i>		X							Fiedler, 1994
House mouse	<i>Mus musculus</i>						X			Fiedler, 1994
Rats - Black rat - Brown / Norway rat - Asian house rat	<i>Rattus</i> species - <i>R. rattus</i> - <i>R. norvegicus</i> - <i>R. tanezumi</i>			X			X	X	X	Fiedler, 1994 Daly et al, 1993 Taylor et al, 2008 Mostert, 2010
African pouched rat	<i>Saccostomus campestris</i>							X		MacFadyen et al, 2016 Pretorius et al, 2004
Tete veld rat	<i>Aethomys ineptus</i>									Chimimba & Linzey, 2008
Namaqua rock rat	<i>Micaelamys namaquensis</i>							X		Pretorius et al, 2004 Bastos, 2007

6. Prevention of tick-borne diseases

Luckily, because the mode of transmission of tick-borne diseases is known to be via tick bites, targeted methods of prevention of tick bites may be put in place. It is always advised that an integrated control program is used instead of relying on a single method (Fritz, 2009).

People living in endemic regions must follow more holistic and practical approaches. Especially in Africa, where the people who are affected generally live in poverty. Domestic environmental modification methods include having an area free of long grass, shrubs and bushes bordering residential properties and holding pens for livestock (potential tick hosts). Reduction of reservoir hosts (rodents) is another environmental method which could reduce human disease (Fritz, 2009). This can be achieved by either killing rodents, for example using predators such as dogs and cats or using traps or rodenticides, or by keeping them out using barriers to block their entry into homes or by improving hygiene and food storage in the home to avoid attracting rodents. Reduction of ticks on animals is achieved by regular dipping or pour-on applications of synthetic pyrethroids (Brodie et al, 1986). Such acaricides are often a luxury that cannot be afforded, and rural people usually rely on dip tanks provided by state veterinary services in South Africa. Contact with ticks can be avoided by wearing protective clothing and insect repellants and thorough daily examination for attached ticks and their immediate removal can prevent disease transmission (Meerburg et al, 2009). Home-made insect repellants containing white vinegar can be cheap and effective but must be applied regularly – every 4 hours. Other components that can be added to the repellent mixture include lemon juice or citrus oil, eucalyptus, lavender and citronella (<http://wtaq.com/blogs/the-great-outdoors/158/home-made-tick-repellent-so-cheap-and-so-easy/>). There are also plants that naturally deter ticks. These include lavender, garlic, pennyroyal (mint family), pyrethrum (type of chrysanthemum), sage, eucalyptus, and mint (<http://www.christiansonnursery.com/using-plants-as-natural-pest-repellents/>).

For foreign visitors entering tick endemic areas, the advice is simple and mainly involves avoidance of ticks. Visitors should limit contact with long grass, bushes and shrubs and stay in the centre of paths and roads. People who are going camping or hiking should wear light-coloured, long-sleeved protective clothing to prevent tick bites and assist early tick removal.

They may also use insect repellants on their bodies to keep ticks away. For pets, topical acaricides and acaricide-impregnated collars may be put onto dogs and cats (Fritz, 2009). For short term visits to endemic Lyme disease areas, prophylactic use of doxycycline can be recommended (Wormser, 2005).

Tick vaccines provide an effective and environmentally sound approach for control of tick-borne diseases (de la Fuente, 2007). A vaccine containing both vector and pathogen-derived antigens could protect vertebrate hosts against tick infestations and pathogen infection. It would also decrease pathogen transmission and reduce the risk of disease in humans and animals (de la Fuente et al, 2016b). More research in this area is needed.

7. Further Research

In Africa, there is a serious lack of thorough human population-level incidence or prevalence estimates which makes it difficult to understand the importance and relevance of the febrile diseases described in this review. Reporting and recording of such results needs to be more regular to be of any use. Additionally, as these are zoonotic diseases, a more “One Health” approach to their research is needed. Human and animal research in the same disease should be integrated to demonstrate their value and to identify transmission routes and knowledge of hosts which will aid prevention and control strategies (Allan et al, 2015). Improved clinician awareness and development of treatment guidelines to manage febrile patients should be a priority in resource poor settings (Crump et al, 2011). Although more research studies for detecting zoonotic tick-borne pathogens in both ticks and animal hosts are being published, the actual risks of infection to humans and livestock requires investigation. This is done through seroprevalence studies, by assessing vector competence and by establishing the pathogenicity of these organisms (Mwamuye et al, 2017). Further research is needed to shed more light on the epidemiology, species characterisations, pathogen distributions, level of pathogenicity in domestic animals and livestock and the vectorial capacity of the tick species throughout Africa. Additionally, better methods to control ticks would go a long way to improve animal welfare and public health (Mtshali et al, 2017).

Another issue that has been mentioned several times is the under reporting of rodent-borne zoonoses and the insufficient attention given to the diagnosis of these diseases. There is a

need for field studies of rodent populations in urban, peri urban and rural settings to determine the roles of rodent species and improve understanding of rodent-human interactions in ecosystems (Meerburg et al, 2009). For example, researchers believe that Leptospirosis is underdiagnosed in Sub-Saharan Africa due to a combination of lack of awareness, lack of accessibility, poor facilities and surveillance systems. It is considered a neglected tropical disease and is further neglected because many African countries are already confronted with large scale epidemics of HIV, malaria and tuberculosis which already consume their limited resources (de Vries et al, 2014).

Prompt detection and diagnosis of the diseases in this review are vital for a good outcome for the patient. Many of the haemoparasites are not easily diagnosed using conventional techniques, such as microscopy, and require a combination of diagnostic techniques as well as various molecular methods. These are often not available within an African setting and research is needed to develop simpler, faster and more available methods for diagnosis. For example, Boston hospitals make use of a tick-associated pathogen panel (TAPP) which includes a PCR assay for detection of organisms causing HGA, other human ehrlichioses, LD and babesiosis (Weil et al, 2012). Use of PCR for diagnostics in non-endemic regions must be done with caution but these types of test panels could be used as models to develop similar tests for parasitic and bacterial diseases that are endemic in Africa. This kind of development could change the future for patients with febrile disease in Africa. Another promising field is called next generation sequencing. This offers huge potential and data have only recently been forthcoming (Cutler et al, 2017). This new application in genetic sequencing is very promising, like when PCR was first discovered, and its potential is only limited by our imaginations (Metzker, 2010). The hope is to develop a huge genetic database that is shared with the scientific community to support more rapid advances.

In a review on the subject, Kelesidis and Falagas (2015) showed that the large-scale manufacture of substandard and counterfeit antimicrobial drugs from Southeast Asia and west Africa represents a huge problem. The most common antibiotics produced include beta-lactams and among the antimalarial drugs, chloroquine and artemisin derivatives are common. Consequences of these substandard and counterfeit drugs being widely available are that people are less likely to seek medical attention and receive a diagnosis, these medications can be harmful to them, and the treatments often fail, resulting in increased

morbidity and mortality. Indiscriminate use of antimicrobials and antiparasitic treatments is a major driver of increased antimicrobial resistance which has major global public health implications. Local, national, and international initiatives are required to combat this issue (Kelesidis and Falagas, 2015). In developing countries, irrational, wasteful and sometimes dangerous use of medicines by people is a common problem. Strengthening of drug regulatory systems and better education of health care providers is needed to prevent this. Better education of children in appropriate use of medicines is needed to improve medicine use throughout communities and for future generations (Bush and Hardon, 1990).

Control and eradication will never be achieved unless research is targeted towards prevention. Development of new chemotherapeutics, vaccines, insecticides, repellents and biological products is crucial. Responsible use and combination or rotational use of therapies is important to prevent drug resistance. Safe and environmentally friendly insecticides and repellents are needed. International organisation involvement (UN, WHO) plays an important role in the funding and coordinating of projects and should be encouraged. The approach to vector-borne disease prevention should be a coordinated global approach rather than relying on separate governments (Harrus and Baneth, 2005).

Many of the vector-borne pathogens in this review have demonstrated huge genetic diversity at the species and subspecies level. Many have both pathogenic and non-pathogenic strains that are constantly adapting and changing with their circumstances. Colwell et al (2011) highlighted the importance of new advances in molecular biology for diagnosis of these diseases. It seems unrealistic that eradication will ever be possible and better control strategies are needed. With the rapid advances in molecular and genomic data development, soon effective vaccines might be created (Colwell et al, 2011). Future studies on rickettsioses, bartonellosis and other vector-borne diseases should be performed to better understand their epidemiological and clinical relevance in tropical and subtropical areas, to estimate the real prevalence and to allow the establishment of antivectorial control plans (Leulmi et al, 2014).

Cutler et al (2010) discussed the threat of new, re-emerging and neglected zoonoses in the industrialised world and described the different sources of zoonotic diseases. They made a sound recommendation that human disease surveillance should be enhanced with

longitudinal veterinary surveillance of food-producing animals and wildlife. This would allow prompt detection and implementation of control measures and vaccination where possible to prevent disease spread (Cutler et al, 2010). Additionally, although rodents are the focus of control for many tick-borne diseases, the other animal hosts should not be ignored. For example, with leptospirosis, livestock may be as important as rodents in their role as hosts. Controlling leptospirosis in affected livestock may directly and indirectly improve human health and well-being in Africa by reducing transmission of leptospirosis and increasing livestock productivity (Hartskeerl et al, 2011). Survey studies to determine the serovars prevalent in animal populations are needed. By knowing which serovars are in circulation, vaccines can be produced to control the disease in the animal hosts and then reduce transmission spill over into humans who interact with these animals (Gatley, 2009).

Active surveillance of rodent populations is helpful to predict future disease prevalence and identify novel rodent-borne diseases. There is a need for models of rodent population density and dispersion rate changes with climate, vector and pathogen abundance. These models could be used to predict expansions of rodent-borne diseases and allow planning to improve public health. Rodent control measures should be put in place in areas where humans, livestock and rodents are living close together. Visitors to rodent-dense locations should be educated so that they are aware of the risks of rodents and take precautions against arthropod vectors (Meerburg et al, 2009).

It is important to monitor environmental and climatic changes as these factors affect arthropod vectors (Parola et al, 2008), rodents and pathogens (Meerburg et al, 2009). It is recommended that national public health surveillance includes systematic vector entomological surveys especially in areas at risk of introduction of new vectors. Also, medical professionals in non-endemic areas should be informed about diagnosis and treatment of vector-borne parasitic diseases that travellers can acquire while visiting the tropics (Zamarron Fuertes et al, 2010).

Where possible and available, the public should be protected by vaccination of high risk groups of people and/or the animals that the public have close contact with. At present, there are no registered, effective and safe vaccines for leptospirosis in humans but there are several available for livestock and domestic dogs. However, protection is serovar or

serogroup specific and immunity is short lived requiring at least annual boosters (Brown & Prescott, 2008). Some promising reports from leptospiral vaccines have been written but the long-term efficacy studies have not yet been published and unwanted side effects in humans limit its use (Bharti et al, 2003). Vaccines for *L. interrogans* are mainly used in livestock but do not stop disease transmission to humans. More research into the development of new vaccines (DNA vaccines) must be supported (Vijayachari et al, 2008). Q fever vaccines have been effective in protecting animals against aerosol challenges, but humans were found to develop severe reactions to vaccination. Further research into new recombinant vaccines is needed. Animal vaccination together with vaccination of high risk people would significantly reduce the zoonotic risk of *C. burnetii* (Porter et al, 2011).

Arthropod vectors and their microorganisms (bacteria, viruses and protozoa) have co-evolved over millions of years to a point where they appear to co-exist with little impact on the vector (de la Fuente et al, 2015). Tick pathogens have developed different strategies of manipulating the vector's immune response which facilitates multiplication and transmission. These strategies may apply to infection of the tick vector or the mammalian host (de la Fuente et al, 2016a). Recent evidence has shown that the tick microbiome affects the tick competence as a vector for pathogen infection (Gall et al, 2016). More research in this area is ongoing. Incorporating certain tick-derived antigens into vaccines could lead to reduced vector infestation and pathogen infection in ticks feeding on immunised animals (de la Fuente & Contreras, 2015). Overall, the most effective and environmentally friendly programs to control tick populations should include: effective and early diagnosis, tick vaccines, improving the genetics of livestock breeds for tick resistance, rational strategic application of acaricides, antivirals and other therapeutic interventions (de la Fuente et al, 2017).

The epidemiology of *A. phagocytophilum* differs between the USA and Europe. In the USA, HGA is an increasing public health problem (Centers for Disease Control and Prevention website, accessed 28 May 2017) whereas domestic ruminants, other than llamas, do not appear susceptible to infection (Pusterla et al, 2001). In comparison, by 2010 there had been no documented cases of HGA in the UK despite some serological evidence (Sumption et al, 1995; Thomas et al, 1998) although increasing numbers of cases are being described in eastern Asia especially China, South Korea and Japan (Bakken & Dumler, 2015). Whereas,

TBF is a common disease of ruminants in Europe and UK (Woldehiwet and Scott, 1993). These differences between continents for the same organism, *A. phagocytophilum*, suggest there is variability between and within host species and geographic regions. There is also great genetic variation between strains of *A. phagocytophilum* and further research is needed to better understand how this contributes to altered pathogenicity. Ongoing investigation into the extent to which *A. phagocytophilum* can persist in tissues and pose a risk for chronic disease in human and canine patients is needed (Carrade et al, 2009). Persistent infections in humans infected with HGA variants must be explored (Schouls et al, 1999; Woldehiwet, 2010).

Kjemtrup and Conrad (2000) identified several knowledge gaps regarding human babesiosis. They recommend improving diagnostic tests, characterising new babesial agents, assessing the taxonomy of piroplasms, improving and standardising serological and molecular diagnostic tests and developing accurate and rapid ELISA for rapid screening of donor blood. Hersh et al (2012) demonstrated significant host specialisation by *B. microti* where genetic differentiation between samples from different hosts was found. A thorough assessment of genetic diversity of *B. microti* infections in humans is critical to determine the public health implications and the role of different hosts in human disease. On the public health side, better clinician awareness of babesiosis as a differential diagnosis in febrile patients is needed. Additionally, clinicians must be made aware of the details and pitfalls of diagnosis and treatment as well as prevention and control of human babesiosis. The public must also be educated in the risk of infection from tick bites especially in endemic areas (Gray et al, 2010).

Better understanding of co-infection with malaria and other tick-borne pathogens and knowledge of alternative diagnoses to malaria is needed to improve diagnosis and case management in febrile illness. Development of better guidelines for treatment and management of febrile patients is needed. A positive test for malaria should not exclude further investigation. Clinicians working in resource poor settings urgently need improved diagnostics for detection of invasive bacterial disease (Nadjm et al, 2012).

Borrelia burgdorferi has had an extraordinary zoonotic success due to its remarkable ability to adapt to divergent host environments and evade the defences of its mammalian

reservoir. It co-evolves with its arthropod vector and mammalian hosts and can take advantage of host physiological processes (Radolf et al, 2012). Future studies of the ecology of Lyme disease seeking to identify the *Borrelia* species in both ticks and reservoir hosts in various endemic areas is recommended. These studies would help to understand the complete spectrum of *Borrelia* species involved in human Lyme disease worldwide and its unknown, rare, unusual clinical manifestations (Rudenko et al, 2011).

In Africa, greater efforts are needed to diagnose and treat human *Bartonella* infections especially in HIV-positive patients. The inter-relationships between the stages of HIV disease, exposure to cats and their fleas and risk of *Bartonella* infection requires further investigation. Better understanding is needed of the modes of transmission and vectors involved in dog bartonellosis (Freaan et al, 2002). Further research into the presence and diversity of bartonellae in Africa is needed and whether these agents might be responsible for human cases of febrile illness of unknown etiology (Gundi et al, 2012). The rodent-adapted *Bartonella* species have been shown to have a high number of host-adaptability genes. Whole genome comparisons might provide new insights into these species and their host-vector associations (Buffet et al, 2013).

Much is still unknown about the life cycle and immunology of *C. burnetii*. There is also regional variation in disease presentation that warrants further research. Vaccine development still leaves a lot to be desired and a test that detects Q fever early in the disease is needed (Parker et al, 2006). Better understanding of the role of humoral and cell mediated immunity in control of infection and clinical presentation are needed for devising vaccination strategies in target populations (Cutler et al, 2007). Thus far, evidence supports that vaccination is effective in preventing abortion storms and reducing the shedding of the organism by small ruminants but that vaccinations must be sustained for several years (Hogerwerf et al, 2011). The survival times of *C. burnetii* in manure and the general environment or the period in which surviving bacteria are a threat to public health are not known and further research is needed in this area. The outbreaks of Q fever in Europe between 1982 and 2010 showed a need for better cooperation and flow of information between veterinary and medical professionals and that initiatives to build stronger links between authorities would go a long way to control the disease (Georgiev et al, 2013).

MATERIALS AND METHODS

1. Sample collection

Rodent trapping took place using Sherman traps across three habitat types in Bushbuckridge East, Mpumalanga Province, namely (i) urban/periurban (Gottenburg / Hlalagagle), (ii) communal rangelands (Thlavekisa), and (iii) adjacent protected rangeland areas (Manyeleti) in August 2014, January 2015 and September 2015. This formed part of a project entitled “Land use and host community characteristics as predictors of risk for rodent-borne zoonotic diseases”. The necessary permits have been obtained from the Department of Agriculture, Forestry and Fisheries (DAFF), permission under regulation 20 (1) (a) (vi) and (vii) of the Animal Disease Act 35 of 1984 for the capturing and sampling of the wild rodents, and for the movement of samples from the collection sites to the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, Pretoria. Ethical clearance has also been obtained from the Animal Ethic Committee (AEC) of the University of Pretoria (protocol no. V046-14, V030-14, V105-15).

Only adult animals were selected for this study and were identified by means of Stuarts' Field Guide to Mammals of Southern Africa (Stuart & Stuart, 2001). Any non-target species were released at the trap site. Target animals were euthanized using intra-peritoneal injection with sodium pentobarbitone (200 mg/kg) and blood samples were collected after the last heartbeat, from the heart onto FTA filter paper (Merck, South Africa). Forty of the blood samples collected were available for use in this study and are listed in Table 5.

Table 5: Rodent species, locality information and sample sizes of the animals included in the study.

Rodent species	Locality collected				TOTAL
	Manyeleti (protected area)	Gottenberg (urban/periurban)	Hlalagagle (urban/periurban)	Thlavekisa (communal rangelands)	
<i>Aethomys ineptus</i>	4				4
<i>Tatera leucogaster</i>	3		3		6
<i>Lemniscomys rosalia</i>		2			2
<i>Mastomys coucha</i>	1				1
<i>Mastomys natalensis</i>	1	7	3	5	16
<i>Mus minutoides</i>			1		1
<i>Rattus rattus</i>				3	3
<i>Rattus tanezumi</i>		1		2	3
<i>Saccostomus campestris</i>	1		3		4
TOTAL	10	10	10	10	40

2. DNA extraction

Genomic DNA was extracted from punched out circles of dried blood spots on the FTA filter paper cards using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

In short, three punched out circles of 3 mm diameter each from the dried blood spots on FTA filter paper were placed in a 1.5 ml microcentrifuge and 180 µl of Buffer ATL was added. After 10 minutes incubation at 85°C, it was quickly centrifuged to insure all drops came down from the lid. This was done between all steps to prevent losses. Then 20 µl proteinase K stock solution at a concentration of 20mg/ml was added and mixed by vortexing. After incubation at 56°C for 1 hour, 200 µl Buffer AL was added to the sample and mixed by vortexing. Following incubation at 70°C for 10 minutes, 200 µl ethanol (96-100%) was added to the sample and thoroughly mixed by vortexing. This mixture was then applied to the QIAamp Mini spin column (in a 2 ml collection tube) and centrifuged at 6 000 x g (8 000 rpm) for 1 minute. The filtrate was discarded, and the spin column placed into a clean 2 ml collection tube. At this point, 500 µl Buffer AW1 was added to the QIAamp Mini spin column

and centrifuged at 6 000 x g (8 000 rpm) for 1 minute. Again, the filtrate was discarded, and the spin column placed into a clean 2 ml collection tube. Another 500 µl Buffer AW2 was then added to the QIAamp Mini spin column and centrifuged at 20 000 x g (14 000 rpm, full speed) for 3 minutes. The filtrate was discarded again and the QIAamp Mini spin column was placed into a new 2 ml collection tube and centrifuged at full speed for 1 minute to eliminate any Buffer AW2 carryover. Finally, the filtrate was discarded again and the QIAamp Mini spin column was placed into a clean 1.5 ml microcentrifuge tube. Next, 100 µl of elution buffer AE was added to the QIAamp Mini spin column, it was incubated at room temperature (15–25°C) for 1 minute and then centrifuged at 6 000 x g (8 000 rpm) for 1 minute. The samples were then stored at -20°C until further use. Three punched-out circles (3mm diameter) usually yield 150 ng and 75 ng of DNA from anticoagulated and untreated blood respectively.

3. Reverse Line Blot (RLB) Hybridization Assay

3.1 Polymerase Chain Reaction (PCR)

Theileria and *Babesia* genus-specific primers RLB F2 (5'-GAC ACA GGG AGG TAG TGA CAA G-3') and biotin-labelled RLB R2 (5'-biotin-CTA AGA ATT TCA CCT CTG ACA GT-3') (Nijhof et al, 2005) were used to amplify a 460–520 bp fragment of the V4 hypervariable region of the parasite 18S rRNA gene as previously described (Nijhof et al, 2005). For the simultaneous detection of *Anaplasma* and *Ehrlichia* spp. a 492–498 bp fragment of the hypervariable V1 region of the parasite 16S rRNA gene was amplified using the *Anaplasma* and *Ehrlichia* genus-specific forward primer Ehr-F (5'-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3') (Schouls et al, 1999) and biotin labelled reverse primer Ehr-R (5'-biotin-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3') (Bekker et al, 2002). The PCR reaction mixture consisted of 12.5 µl of Platinum® Quantitative PCR SuperMix-UDG (containing 60 U/ml Platinum® *Taq* DNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 6 mM MgCl₂, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 800 µM dUTP, and 40 U/ml uracil DNA glycosylase (UDG) (Invitrogen™ by Life technologies™, South africa), 8 pmol (0.25 µl) of each primer, 5 µl of DNA and 7 µl PCR grade water to a final volume of 25 µl. Separate PCR master mix reactions were prepared for the amplification of *Theileria* and *Babesia* species using RLB-F2 and RLB-R2 primers (18S rRNA) and the Ehr-F and Ehr-R primers (16S rRNA) for the amplification of *Ehrlichia* and *Anaplasma* species. PCR master mix with no DNA template served as negative

control, while DNA extracted from *Babesia bovis* and *Anaplasma centrale* vaccines (purchased from Onderstepoort Biological Products, South Africa) was included as positive control DNA.

A touchdown PCR thermocycler program (Table 6) was applied for amplification using a GeneAmp PCR System 9700 (Applied Biosystems).

Table 6: Thermocycling programme used for Theileria/Babesia and Ehrlichia/Anaplasma touchdown PCR

Number of cycles	Time	Temperature	Purpose
1 cycle	3 min	37°C	Activate UDG
1 cycle	10 min	4°C	Inactivate UDG and activate <i>Taq</i> polymerase
2 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	67°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
2 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	65°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
2 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	63°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
2 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	61°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
2 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	59°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
40 cycles	20 sec	94°C	Denature double stranded DNA template
	30 sec	57°C	Anneal primers
	30 sec	72°C	Extension of PCR products by <i>Taq</i> polymerase
1 cycle	7 min	72°C	Final extension

3.2 Membrane preparation

A Biotin[®] C membrane (Separations, South Africa) with genus and species-specific probes for *Anaplasma*, *Ehrlichia*, *Theileria* and *Babesia* attached was used for the RLB. The details of the probes can be found in Table 6. In short, the membrane was prepared as follows. The size of the piece of membrane used corresponded to the size of the support cushion of a MN45 miniblotted apparatus (Immunogenetics, Cambridge, UK). For activation, the membrane was incubated in 10 ml of freshly prepared 16% EDAC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) for 10 minutes. A volume of 150 µl of each probe at a concentration of 5 pmol/µl was loaded onto the membrane. The probes (listed in Table 7) were prepared by dilution in 0.5 M NaHCO₃ (pH 8.4) until the 5 pmol/µl concentration was achieved. After 2 minutes of incubation at room temperature, the membrane was inactivated with 100 ml of freshly made 100 mM NaOH (sodium hydroxide) for 8 minutes at room temperature on a shaker. Finally, the membrane was washed in 100 ml 2X SSPE/0.1% SDS at 60°C for 5 minutes and then stored in a refrigerator.

Table 7: List of probes used in the RLB Hybridization assay. Symbols for degenerate positions: R = A/G and W = A/T.

Oligonucleotide probe identification	Probe Sequence (5'-----3')	Reference
<i>Anaplasma/Ehrlichia</i> genus-specific	GGG GGA AAG ATT TAT CGC TA	Schouls et al (1999)
<i>A. bovis</i>	GTA GCT TGC TAT GRG AAC A	Bekker et al (2002)
<i>A. centrale</i>	TCG AAC GGA CCA TAC GC	Bekker et al (2002)
<i>A. marginale</i>	GAC CGT ATA CGC AGC TTG	Bekker et al (2002)
<i>A. phagocytophilum</i>	TTG CTA TAA AGA ATA ATT AGT GG	Bekker et al (2002)
<i>Anaplasma</i> sp. Omatjenne	CGG ATT TTT ATC ATA GCT TGC	Bekker et al (2002)
<i>E. canis</i>	TCT GGC TAT AGG AAA TTG TTA	Schouls et al (1999)
<i>E. chaffiense</i>	ACC TTT TGG TTA TAA ATA ATT GTT	Schouls et al (1999)
<i>E. ruminantium</i>	AGT ATC TGT TAG TGG CAG	Bekker et al (2002)
<i>Theileria/Babesia</i> genus-specific	TAA TGG TTA ATA GGA RCR GTT G	Gubbels et al (1999)
<i>Babesia</i> genus-specific-1	ATT AGA GTG TTT CAA GCA GAC	* Nijhof (unpublished)
<i>Babesia</i> genus-specific-2	ACT AGA GTG TTT CAA ACA GGC	* Nijhof (unpublished)
<i>B. bicornis</i>	TTG GTA AAT CGC CTT GGT C	Nijhof et al (2003)

<i>B. bigemina</i>	CGT TTT TTC CCT TTT GTT GG	Gubbels et al (1999)
<i>B. bovis</i>	CAG GTT TCG CCT GTA TAA TTG AG	Gubbels et al (1999)
<i>B. caballi</i>	GTG TTT ATC GCA GAC TTT TGT	Butler et al (2008)
<i>B. canis</i>	TGC GTT GAC CGT TTG AC	Matjila et al (2004)
<i>B. divergens</i>	ACT RAT GTC GAG ATT GCA C	Nijhof et al (2003)
<i>B. felis</i>	TTA TGC GTT TTC CGA CTG GC	Bosman et al (2007)
<i>B. gibsoni</i>	CAT CCC TCT GGT TAA TTT G	Nijhof et al (2003)
<i>B. leo</i>	ATC TTG TTG CTT GCA GCT T	Bosman et al (2007)
<i>B. major</i>	TCC GAC TTT GGT TGG TGT	Georges et al (2001)
<i>B. microti</i>	GRC TTG GCA TCW TCT GGA	Nijhof et al (2003)
<i>B. occultans</i>	CCT CTT TTG GCC CAT CTC GTC	He et al (2012)
<i>B. rossi</i>	CGG TTT GTT GCC TTT GTG	Matjila et al (2004)
<i>B. vogeli</i>	AGC GTG TTC GAG TTT GCC	Matjila et al (2004)
<i>Babesia</i> sp. (sable)	GCT GCA TTG CCT TTT CTC C	Oosthuizen et al (2008)
<i>Theileria</i> genus-specific	ATT AGA GTG CTC AAA GCA GGC	* Nijhof (unpublished)
<i>T. annae</i>	CCG AAC GTA ATT TTA TTG ATT TG	Yisaschar-Mekuzas et al (2013)
<i>T. annulata</i>	CCT CTG GGG TCT GTG CA	Georges et al (2001)
<i>T. bicornis</i>	GCG TTG TGG CTT TTT TCT G	Nijhof et al (2003)
<i>T. buffeli</i>	GGC TTA TTT CGG WTT GAT TTT	Gubbels et al (2000)
<i>T. equi</i>	TTC GTT GAC TGC GYT TGG	Butler et al (2008)
<i>T. lestoquardi</i>	CTT GTG TCC CTC CGG G	Schnittger et al (2004)
<i>T. mutans</i>	CTT GCG TCT CCG AAT GTT	Gubbels et al (1999)
<i>T. ovis</i>	TTG CTT TG CTC CTT TAC GAG	Bekker et al (2002)
<i>T. parva</i>	GGA CGG AGT TCG CTT TG	Nijhof et al (2003)
<i>T. separata</i>	GGT CGT GGT TTT CCT CGT	Schnittger et al (2004)
<i>T. taurotragi</i>	TCT TGG CAC GTG GCT TTT	Gubbels et al (1999)
<i>T. velifera</i>	CCT ATT CTC CTT TAC GAG T	Gubbels et al (1999)
<i>Theileria</i> sp. (buffalo)	CAG ACG GAG TTT ACT TTG T	Oura et al (2004)
<i>Theileria</i> sp. (kudu)	CTG CAT TGT TTG TTT CCT TTG	Nijhof et al (2005)
<i>Theileria</i> sp. (sable)	GCT GCA TTG CCT TTT CTC C	Nijhof et al (2005)

* Dr. Ard M. Nijhof (Institut für Parasitologie und Tropenveterinärmedizin, Freie Universität, Berlin, Germany)

3.3 Reverse Line Blot (RLB) Hybridization

The RLB hybridization was performed according to Nijhof et al (2005) and was modified by mixing the PCR products of each primer set (25 µl) before the preparation of the hybridization step.

The Biotodyne[®] C membrane was activated at room temperature for 5 minutes under gentle shaking in 50 ml 2 X SSPE/0.1% SDS in a plastic container. The two separate PCR mixtures were mixed together and then diluted with 130 µl of 2 X SSPE/0.1% SDS to make a total volume of approximately 150 µl. To denature the PCR products adequately, they were placed in a thermal PCR cycler machine at 99.9°C for 10 minutes and then immediately placed on ice to maintain the state of denaturation. The membrane was placed into a forty-five lane Mini-blotter (Immunitics Cambridge) with the 45 sample slots aligned perpendicularly to the line of the previously applied probes. All residual fluid was aspirated using a vacuum with an unfiltered tip. Forty of the sample slots were filled with the diluted biotin-labelled PCR products according to the sample list. The first lane was filled with a *Theileria/Babesia* and *Ehrlichia/Anaplasma* positive control and the second lane was a negative control. Hybridization took place in an incubator at 42°C for 60 minutes on a horizontal surface. Samples were subsequently removed by aspiration and the membrane was removed from the blotter. The membrane was placed into a plastic container and washed twice in preheated 2 X SSPE/0.5% SDS for 10 minutes at 50°C on the shaking platform. After washing, the membrane was incubated with 10 ml pre-heated 2 X SSPE/0.5% SDS and 12.5 µl (1.25 U) streptavidin-POD (peroxidase labelled) conjugate (Roche Diagnostics, South Africa) for 30 minutes at 42°C under gentle shaking. The excess conjugate was washed off by washing the membrane twice in preheated 2 X SSPE/0.5% SDS for 10 minutes at 42°C under gentle shaking. In the next step, the membrane was washed twice for 5 minutes in 2 X SSPE at room temperature under gentle shaking. After washing, 10 ml of enhanced chemiluminescence (ECL) (DNA Thunder[™], Separation Scientific, South Africa) (5 ml ECL1 and 5 ml ECL2) were added to the membrane and mixed gently for 1 minute at room temperature. To prevent movement while developing the x-ray film, the membrane was placed in between 2 transparent sheets within an X-ray cassette. In a dark room, an X-ray film (X-OMAT[™] Blue XB-1, Kodak, Separation Scientific, South Africa) was placed on top of the membrane and the cassette was closed for about 1 second and then removed in a

smooth movement. The X-ray did not need to be exposed to the fluorescent reaction for as long as previously described (20 minutes) as this causes overexposure and difficulty in reading the results. The X-ray film was developed in a developer solution for 1 minute and fixed in a fixer solution for at least 30 seconds. Dark spots appeared where hybridization had occurred (Gubbels et al 1999) because of a chemiluminescence reaction.

Before the membrane was re-used it was stripped of all hybridized PCR products by two washes in pre-heated 1% SDS at 80°C for 30 minutes with gentle shaking in a water bath. It was then rinsed in 20 mM EDTA (pH 8.0) for 15 minutes at room temperature under gentle shaking and stored at 4°C for future use.

4. Real-Time PCR

4.1 *Babesia microti* SYBR Green real-time PCR assay

The qPCR assay for the detection of *B. microti* was performed according to Herch et al (2012). Primers smba_JF (5'- GCG TTC ATA AAA CGC AAG GAA GTG T -3') and smba_KR (5'- TGT AAG ATT ACC CGG ACC CGA CG-3') were used in a real-time PCR reaction to amplify a 133 bp fragment of the 18S rDNA region in the *B. microti* species complex (Hersh et al, 2012). The real-time PCR master mix consisted of a final concentration of 1X iTaq™ Universal SYBR® Green Supermix (Bio-Rad, South Africa) (containing unknown concentrations of iTaq DNA Polymerase, dNTP's, MgCl₂, SYBR® Green I, enhancers, stabilizers, fluorescein, and ROX normalization dyes), 0.5 µM of the forward and reverse primer, 1 µl of DNA template and 7 µl of PCR grade water to make up a 20 µl reaction mix. Amplification was done using an Applied Biosystems ABI StepOnePlus™ Real-Time PCR System (Life Technologies, South Africa). PCR master mix with no DNA template served as negative control, while *B. microti* plasmid DNA (Zivkovic et al, 1984) was included as positive control DNA. Reaction conditions were 10 minutes at 95°C, followed by 40 cycles for 15 seconds at 95°C and 30 seconds at 55°C. This was followed by a melting curve analysis to distinguish true-positive samples from false-positive samples or mis-priming. PCR products were heated from 60°C to 95°C; temperature was increased by 0.3°C every 30 s. The qPCR results were analysed using the StepOne Plus software v2.2.

4.2 *Anaplasma phagocytophilum* Taqman real-time PCR assay

The Taqman real-time PCR (qPCR) assay was used to screen samples for *A. phagocytophilum* according to Courtney et al (2004). Primers ApMSP2 forward (5'-ATG GAA GGT AGT GTT

GGT TAT GGT ATT-3') and ApMSP2 reverse (5'-TTG GTC TTG AAGCGC TCG TA-3') were used to amplify a 77 bp fragment of the *msp2* gene of *A. phagocytophilum*. The TaqMan probe ApMSP2p-HEX (5'-TGG TGC CAG GGTGA GCT TGA GAT TG-3') labelled at the 5' end with hexachloro-6-carboxy-fluorescein (HEX) and 3' ends with TAMRA was used. The Taqman® Universal PCR master mix (Applied Biosystems, South Africa) consisted of a final concentration of 1X iTaq Universal probes Supermix (Bio-Rad, South Africa), 0.28 µM of the forward and reverse primer, 2 µM Taqman probe, 2.5 µl DNA template and 3.65 µl PCR grade water to make up a 20 µl reaction mix. PCR master mix with nuclease free water served as negative control, while *A. phagocytophilum* positive control DNA was obtained from Dr Erich Zwegarth, Freie Universität Berlin, Berlin, Germany. Cycling conditions included an initial activation of *Taq* polymerase at 95°C for 10 minutes, followed by 40 cycles of a 15 second denaturation at 95°C followed by a minute annealing-extension step at 60°C (Courtney et al, 2004). The reaction was run on an Applied Biosystems ABI StepOne Plus™ Real-Time PCR System and results were analyzed using the StepOne Plus software v2.2.

4.3 Sanger sequencing of the 16S rRNA and *msp2* genes

Selected samples that tested positive for *A. phagocytophilum* on the qPCR assay were characterized using the universal 16SrRNA gene and the *msp2* gene of *A. phagocytophilum*. The full-length 16S rRNA gene was amplified using the universal primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and Rp2 (5'-ACG GCT ACC TTG TTA CGA CTT-3'), as previously described (Parola et al, 2000). Primers *msp2*-3f (5'-CCA GCG TTT AGC AAG ATA AGA G-3') and *msp2*-3r (5'-GMC CAG TAA CAA CAT CAT AAG C-3') were used to amplify a 334 bp fragment of the *msp2* gene of *A. phagocytophilum* as previously described (Massung & Slater, 2003), respectively. Primers were used at a final concentration of 0.5 µM with Phusion Flash® High Fidelity PCR Master Mix (Thermofisher Scientific, South Africa) according to the manufacturer's instructions. PCR products were then cloned using the Clone Jet® PCR Cloning Kit (Thermofisher Scientific, South Africa) according to the manufacturer's instructions. Recombinants were selected and sent for sequencing on the Sanger platform which was performed by INQABA Biotechnologies (South Africa). The obtained 16S rRNA and *msp2* gene sequences were trimmed, assembled, edited and consensus sequences generated using the CLC Main workbench 7 (Qiagen). Sequence

identity searches were performed using BLASTn on the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/>) (Altschul et al., 1990).

A multiple sequence alignment was performed for the deduced amino acid *mSP2* sequence, along with sequences of related genera available in GenBank, using ClustalX (version 1.81 for Windows) (Thompson et al., 1997). The alignment was truncated to the size of the shortest sequence using BioEdit v7 (Hall, 1999). The evolutionary history was inferred by using the Maximum Likelihood method based on the Poisson correction model (Zuckerkandl & Pauling, 1965) in combination with the bootstrap method (Felsenstein, 1985) using 1000 replicates/tree using the Molecular Evolutionary Genetics Analysis version 7.0 (MEGA7) software package (Kumar et al., 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated. There was a total of 108 positions in the final dataset. The genetic distances between the sequences were estimated by determining the number of amino acid differences between sequences using MEGA7 (Kumar et al., 2016). All consensus trees were edited using MEGA7.

RESULTS

1. The Reverse Line Blot (RLB) hybridization assay

A total of 40 blood samples from rodents collected across three habitat types in Bushbuckridge East, Mpumalanga, South Africa were simultaneously screened for the presence of *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* species using the RLB hybridization assay. A representation of the RLB membrane is illustrated in Figure 12.

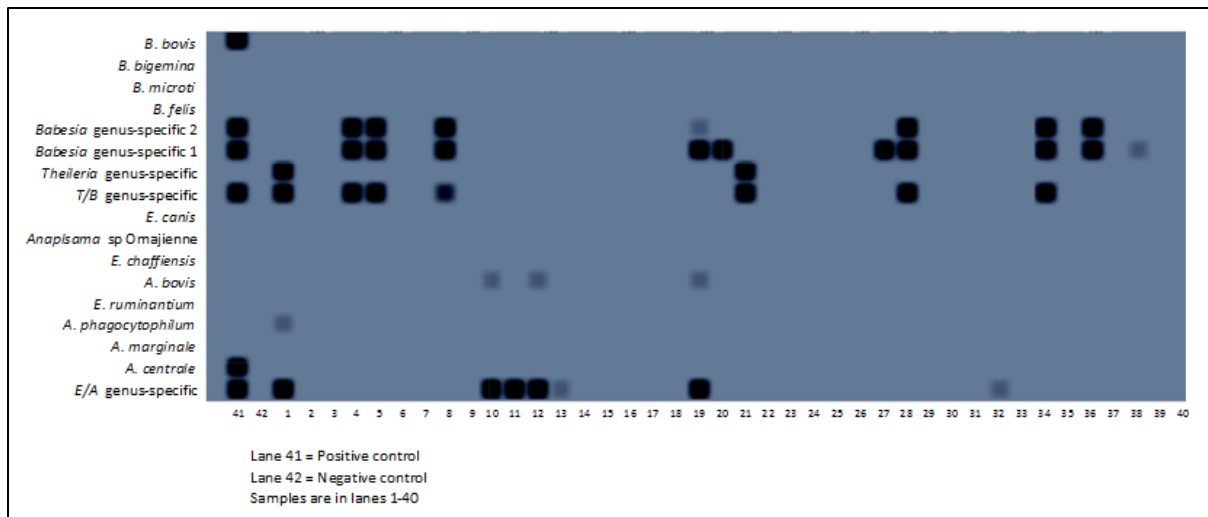


Figure 12: Representative RLB hybridization assay results for the simultaneous detection of *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* species. The genus and species-specific oligonucleotides probes were applied in horizontal lanes and the PCR products in vertical lanes. Positive and negative control, respectively are labelled 41 and 42 in the diagram. The sample DNA is labelled numbers 1 to 40 in the diagram.

The RLB results, shown in Table 8 and Figures 12 & 13, indicated the presence of a variety of tick-borne blood parasites either as single or mixed infections (at genus level only). For 17.5% of the samples (n=7), PCR products hybridized with the *Theileria/Babesia* group-specific probes, 5% (n=2) with the *Theileria* group-specific probe, 17.5% (n=7) with the *Anaplasma/Ehrlichia* group-specific probes, 17.5% (n = 7) with both the *Babesia* 1 and 2 genus specific probes and 7.5% (n = 3) with the *Babesia* 1 genus specific probe only. One (2.5%) Tete veld rat, *Aethomys ineptus*, tested positive for the presence of *A. phagocytophilum* DNA, two (5%) single-striped grass mice, *Lemniscomys rosalia*, and one (2.5%) Natal multi-mammate mouse, *Mastomys natalensis*, tested positive for *A. bovis* DNA.

None of the samples tested positive for the presence of *B. microti*. The remaining 57.55% (n=23) of samples tested negative (or below the detection limit of the test).

Table 8: Summary of the RLB results shown according to locality

	MANYELETI (n = 10)	GOTTENBERG (n = 10)	HLALAGAGLE (n = 10)	THLAVEKISA (n = 10)	TOTAL (n = 40)
Single infections	1 (2.5%)	3 (7.5%)	0	0	4 (10%)
<i>A. bovis</i>		3			
<i>A. phagocytophilum</i>	1				
<i>B. microti</i>					
Mixed infections:	0	0	0	0	0
<i>A. bovis</i>					
<i>A. phagocytophilum</i>					
<i>B. microti</i>					
<i>Theileria/Babesia</i> genus-specific only	4 (10%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	7 (17.5%)
<i>Anaplasma/Ehrlichia</i> genus-specific only	1 (2.5%)	3 (7.5%)	2 (5%)	1 (2.5%)	7 (17.5%)
<i>Babesia 1</i>	3 (7.5%)	2 (5%)	2 (5%)	3 (7.5%)	10 (25%)
<i>Babesia 2</i>	2 (5%)	2 (5%)	1 (2.5%)	2 (5%)	7 (17.5%)
<i>Theileria</i> genus-specific	2 (5%)	0	0	0	2 (5%)
Negative/Below detection limit	5 (12.5%)	6 (15%)	6 (15%)	6 (15%)	23 (57.5%)

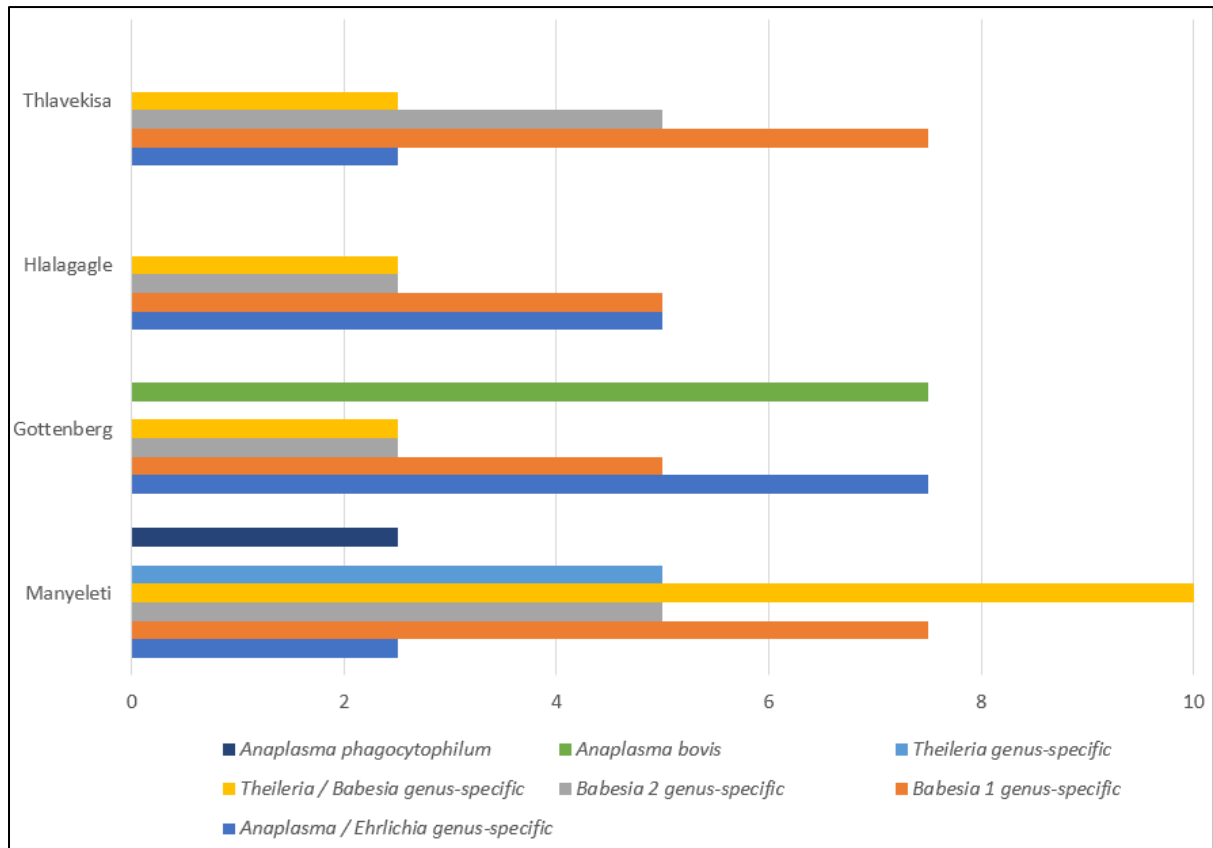


Figure 13: Positive Reverse Line Blot hybridization assay results as a percentage of the total number of samples (n = 40) showed according to locations of sample collection.

2. *Babesia microti* real-time PCR (qPCR)

Rodent DNA samples were tested for the presence of *B. microti* DNA using a SYBR Green qPCR assay and a melting curve of each sample was analysed. The average melting temperatures of the positive and negative controls were 82.30°C and zero, respectively. Figure 14 illustrates the obtained melting curves for the controls. Nine of the rodent samples (22.5%) produced melting temperatures within 4°C of the positive control and these are shown in Figure 15.

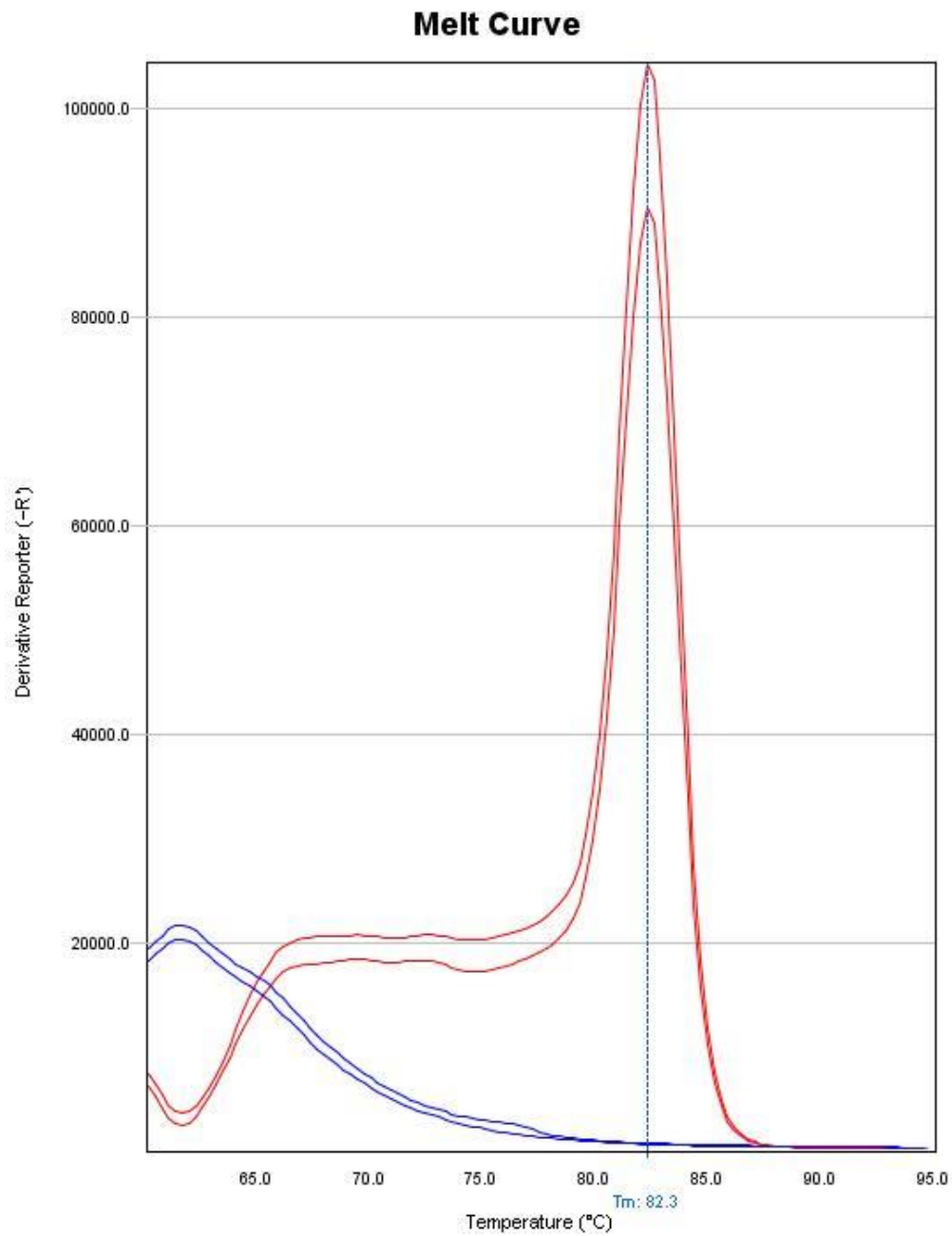


Figure 14: Melt temperature of *Babesia microti* plasmid DNA in real-time PCR assay. The red lines indicate the positive control melting curves, whereas the blue lines indicate the negative control melting curves.

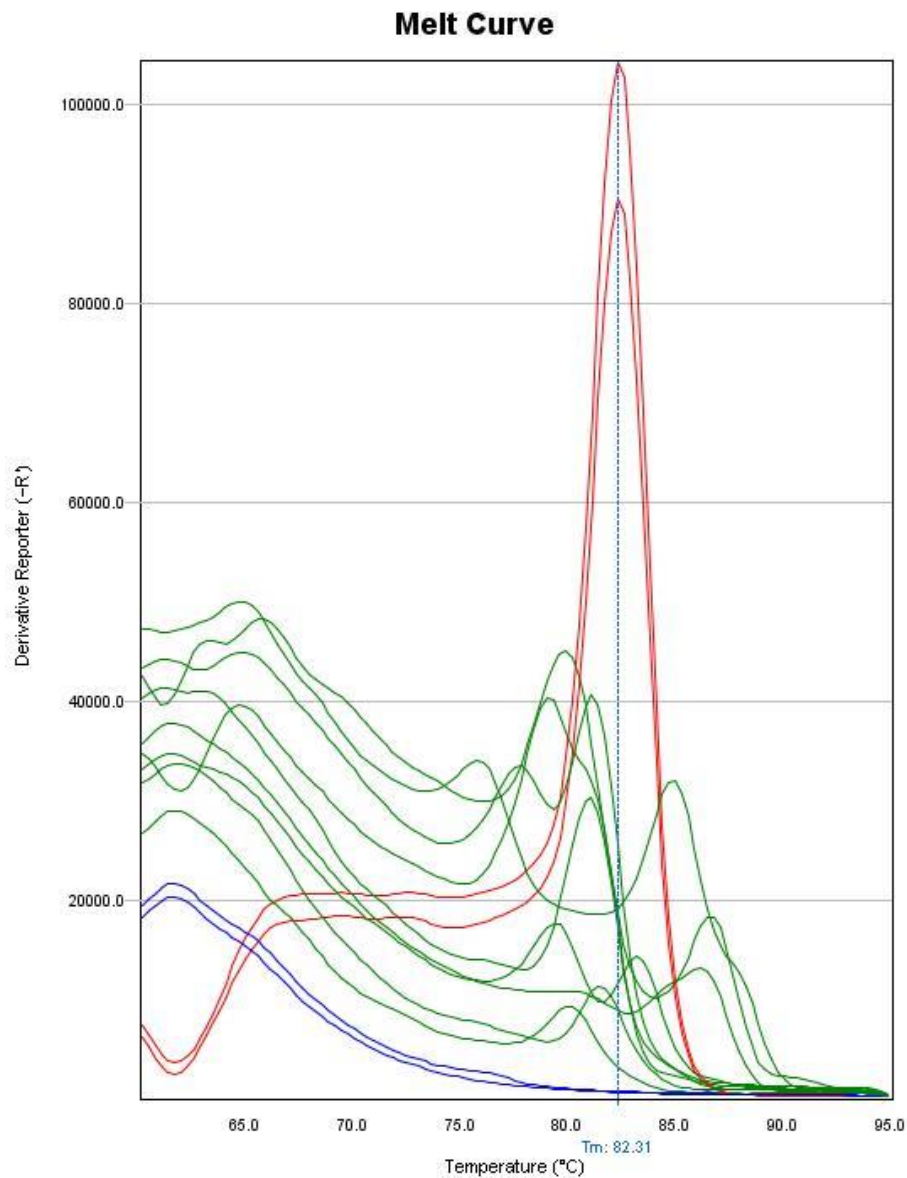


Figure 15: This graph shows the melting curves for the controls (red lines) and nine samples (22.5%) which had melting temperatures within 4°C of *Babesia microti* (green lines). The dark blue lines represent the negative controls.

The real-time PCR results and the resulting melting temperatures of nine of the rodent blood samples ranged between 79.16°C and 86.17°C (Table 9). The melting temperature of the *B. microti* positive control that was confirmed by gene sequence analysis in a separate study (Troskie, 2017) was 82.30°C.

Table 9: Real-time PCR results for the detection of *Babesia microti*: samples with melting temperatures within 4°C of that of the *B. microti* positive control with Tm = 82.30°C

Locality	Sample ID number	Rodent species	Melting temperature (°C)
Manyeleti (protected area)	24	<i>Tatera leucogaster</i>	80.05
Gottenburg (urban/periurban)	29	<i>Mastomys natalensis</i>	79.91
	40	<i>Mastomys natalensis</i>	86.17
Hlalagagle (urban/periurban)	65	<i>Tatera leucogaster</i>	80.95
	71	<i>Mastomys natalensis</i>	81.10
	33	<i>Saccostomus campestris</i>	81.54
Thlavekisa (communal rangelands)	83	<i>Mastomys natalensis</i>	79.16
	93	<i>Rattus tanezumi</i>	79.47
	85	<i>Rattus tanezumi</i>	84.83

3. *Anaplasma phagocytophilum* real-time PCR (qPCR)

Blood samples were tested for the presence of *A. phagocytophilum* DNA using an *A. phagocytophilum*-specific qPCR assay (Courtney et al, 2004). Amplification curves were obtained for the positive control and for positive field samples. No fluorescence was observed in the negative control and in negative field samples (Figure 16).

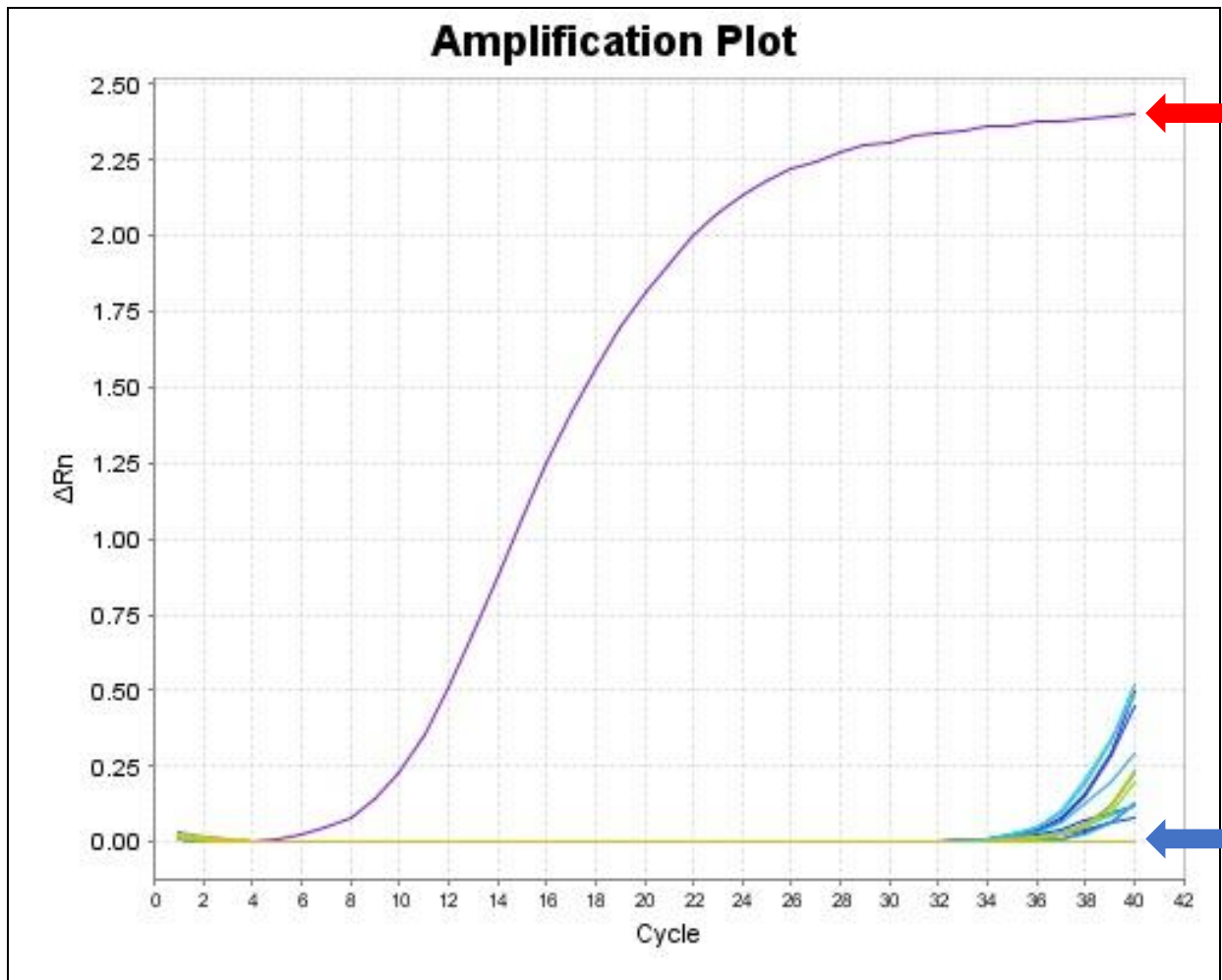


Figure 16: Amplification plot of the *A. phagocytophilum* qPCR run showing the fluorescence (Rn) vs. the cycle number. The positive control (red arrow) has a CT value of 6.1 while the negative control (blue arrow) remained undetermined.

The results demonstrated that 14 samples (35.0%) tested positive for *A. phagocytophilum* DNA (Table 10). Of the 14 positive samples, six were from *Mastomys natalensis*, four from *Rattus* species (two *Rattus rattus* and two *Rattus tanezumi*), two from *Tatera leucogaster* and one each from *Saccostomus campetris* and *Lemniscomys rosalia*. Additionally, eight of the samples (20%) originated from Thlavekisa which is a communal rangeland, and four of the samples (10%) were from Hlalagagle which is considered an urban or peri-urban area.

Table 10: Real-time PCR results for the detection of *Anaplasma phagocytophilum*

Locality	Sample ID number	Rodent species	CT value
	Positive control		6.1
Manyeleti (protected area)	25	<i>Tatera leucogaster</i>	36
Gottenburg (urban/periurban)	32	<i>Lemniscomys rosalia</i>	36
Hlalagagle (urban/periurban)	65	<i>Tatera leucogaster</i>	35
	73	<i>Mastomys natalensis</i>	35
	71	<i>Mastomys natalensis</i>	36
	33	<i>Saccostomus campestris</i>	37
Thlavekisa (communal rangelands)	83	<i>Mastomys natalensis</i>	35
	87	<i>Rattus rattus</i>	35
	93	<i>Rattus tanezumi</i>	35
	94	<i>Mastomys natalensis</i>	35
	85	<i>Rattus tanezumi</i>	36
	86	<i>Mastomys natalensis</i>	36
	84	<i>Mastomys natalensis</i>	37
	90	<i>Rattus rattus</i>	37

All the sample results for the RLB and the two qPCR assays are combined in Table 11. For *B. microti*, the melting temperatures are given in the table; these samples will have to be sequenced in the future to confirm if they are in fact negative or positive.

Table 11: Summary of results of RLB, qPCR and sequencing with rodent samples grouped into locations

Locality	Sample ID number	Rodent species	RLB result	<i>B. microti</i> qPCR result	<i>A. phagocytophilum</i> qPCR result	16S rRNA phylogenetic characterization	<i>Msp2</i> phylogenetic characterization
Manyeleti (protected area)	23	<i>Aethomys ineptus</i>	T/B & A/E catch all; A. phagocytophilum	-	-	ND	ND
	24	<i>Aethomys ineptus</i>	-	Tm 80.05	-	ND	ND
	25	<i>Tatera leucogaster</i>	-	-	+	<i>Achromobacter spanius</i>	<i>A. phagocytophilum</i>
	26	<i>Mastomys natalensis</i>	T/B, B1 & B2 catch all	-	-	ND	ND
	27	<i>Aethomys ineptus</i>	T/B, B1 & B2 catch all	-	-	ND	ND
	28	<i>Tatera leucogaster</i>	-	-	-	ND	ND
	48	<i>Saccostomus campestris</i>	B1 catch all	-	-	ND	ND
	49	<i>Aethomys ineptus</i>	T/B catch all	-	-	ND	ND
	50	<i>Mastomys coucha</i>	-	-	-	ND	ND
	51	<i>Tatera leucogaster</i>	-	-	-	ND	ND
Gottenburg (urban/periurban)	29	<i>Mastomys natalensis</i>	-	Tm 79.91	-	ND	ND
	30	<i>Mastomys natalensis</i>	T/B, B1 & B2 catch all	-	-	ND	ND
	31	<i>Mastomys natalensis</i>	-	-	-	ND	ND
	32	<i>Lemniscomys rosalia</i>	A/E catch all; A. bovis	-	+	ND	ND
	34	<i>Mastomys natalensis</i>	A/E catch all; A. bovis	-	-	ND	ND
	37	<i>Mastomys natalensis</i>	-	-	-	ND	ND
	38	<i>Mastomys natalensis</i>	-	-	-	ND	ND

	39	<i>Rattus tanezumi</i>	-	-	-	ND	ND
	40	<i>Mastomys natalensis</i>	-	Tm 86.17	-	ND	ND
	41	<i>Lemniscomys rosalia</i>	T/B, B1 & B2 catch all;	-	-	ND	ND
A. bovis							
Hlalagagle (urban/periurban)	33	<i>Saccostomus campestris</i>	A/E catch all	Tm 81.54	+	<i>Achromobacter spanius;</i> <i>Bartonella</i> sp. 099	ND
	35	<i>Saccostomus campestris</i>	A/E catch all	-	-	ND	ND
	36	<i>Tatera leucogaster</i>	-	-	-	ND	ND
	63	<i>Tatera leucogaster</i>	-	-	-	ND	ND
	64	<i>Mus minutoides</i>	-	-	-	ND	ND
	65	<i>Tatera leucogaster</i>	-	Tm 80.95	+	ND	ND
	66	<i>Saccostomus campestris</i>	B1 catch all	-	-	<i>Achromobacter spanius;</i> <i>Bartonella</i> sp. 099	<i>A. phagocytophilum</i>
	71	<i>Mastomys natalensis</i>	T/B, B1 & B2 catch all	Tm 81.1	+	ND	<i>A. phagocytophilum</i>
	72	<i>Mastomys natalensis</i>	-	-	-	ND	ND
	73	<i>Mastomys natalensis</i>	-	-	+	ND	<i>A. phagocytophilum</i>
	83	<i>Mastomys natalensis</i>	-	Tm 79.16	+	ND	ND
Thlavekisa (communal rangeland)	84	<i>Mastomys natalensis</i>	A/E catch all	-	+	ND	ND
	85	<i>Rattus tanezumi</i>	-	Tm 84.83	+	ND	ND
	86	<i>Mastomys natalensis</i>	T/B, B1 & B2 catch all	-	+	ND	ND
	87	<i>Rattus rattus</i>	-	-	+	ND	ND
	88	<i>Mastomys natalensis</i>	B1 & B2 catch all	-	-	ND	ND
	89	<i>Rattus rattus</i>	-	-	-	ND	ND
	90	<i>Rattus rattus</i>	B1 catch all	-	+	ND	ND

93	<i>Rattus tanezumi</i>	-	Tm 79.47	+	ND	ND
94	<i>Mastomys natalensis</i>	-	-	+	ND	<i>A. phagocytophilum</i>

Footnote (see Table 4): - = Below the detection limit of the assay

A/E = *Anaplasma/Ehrlichia* genus-specific probe

B1 catch all = *Babesia* genus-specific probe 1

B2 catch all = *Babesia* genus-specific probe 2

T/B = *Theileria/Babesia* genus-specific probe

ND = Not done

4. Sequencing and phylogenetic analysis

The near full-length 16S rRNA (~ 1 390 bp) parasite gene of three samples (two that tested positive for *A. phagocytophilum* on the qPCR assay [25, 33], and one that only had a positive signal on RLB for the *Babesia* genus-specific probe 1 [66]) were amplified using universal primers, cloned and the recombinants sequenced. Seven recombinant sequences were obtained from the three samples. BLASTn searches showed that five of the obtained sequences had 99% sequence identity to *Achromobacter spanius* (Accession nr LN890048), while two sequences had 99% identity to *Bartonella* species 099 (Accession nr KF792113.1). No *A. phagocytophilum* 16S rDNA sequences were obtained. No further 16S rDNA phylogenetic analysis was performed.

The partial *msp2* (~ 334 bp) parasite gene of four of the samples (25, 71, 73 and 94) that tested positive for *A. phagocytophilum* on the qPCR assay, as well as one sample (66) that only had a positive signal on RLB for the *Babesia* genus-specific probe 1, were amplified, cloned and the recombinants sequenced. Twelve recombinant sequences were obtained from the five samples. Two Msp2 sequence types (111 amino acids in length) were identified (designated Ap1 and Ap2); Ap1 differed by one amino acid from Ap2. Ap1 (represented by eight sequences obtained from three samples: 25-2, 25-5, 25-7, 25-8, 66-1, 66-3, 73-1, 73-4) was identical to *A. phagocytophilum* human strain Webster (Accession nr AAO30100) and *A. phagocytophilum* strain Dog2 (Accession nr CP006618). Ap2 (represented by four sequences obtained from three samples: 25-1, 71-1, 71-5, 94-3) was identical to *A. phagocytophilum* human strain HZ (Accession nrs AY319265, AY763477) as well as *A. phagocytophilum* strains previously described from various hosts (including deer, ticks and mite) (Accession nrs EF143800, KP768190, DQ105672, DQ097228, KC954740, AY642115).

The observed sequence similarities were subsequently confirmed by phylogenetic analyses. The Maximum likelihood tree based on the Msp2 deduced amino acid sequences is shown in Figure 17. The obtained sequences types Ap1 and Ap2, as well as representative *A. phagocytophilum* sequences obtained from GenBank, formed a monophyletic group.

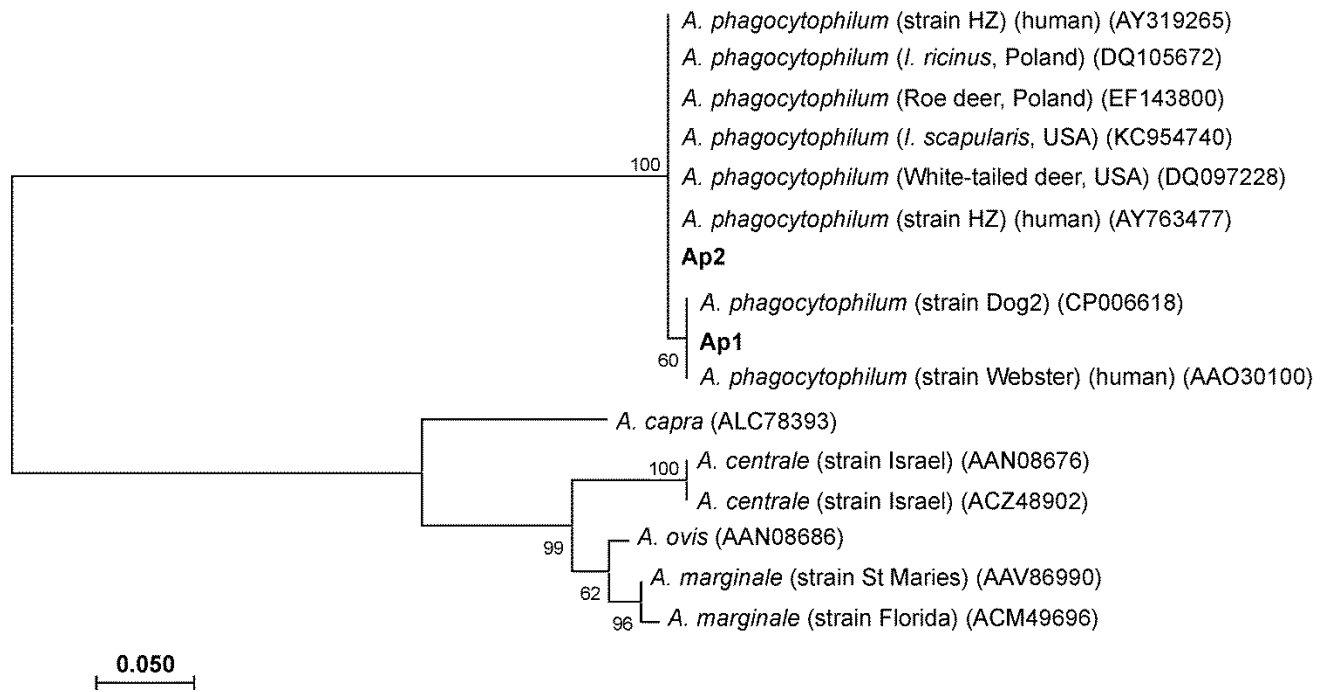


Figure 17: Maximum likelihood tree based on Msp2 deduced amino acid sequences showing the phylogenetic relationships between the *Anaplasma* spp. The evolutionary history was inferred by using the Maximum Likelihood method based on the Poisson correction model. The tree with the highest log likelihood (-730.3527) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 16 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 108 positions in the final dataset.

DISCUSSION

It is known that human febrile illness is a common presenting sign in patients seeking medical care in Africa and that these illnesses are frequently misdiagnosed (Amos et al, 2012). The understanding of aetiologies is often limited which leads to their misclassification as malaria and subsequent delays in appropriate treatment (Amos et al, 2012; Amexo et al, 2004). The potential contribution of rodent pathogen transmission to human febrile illnesses is a public health concern in Africa.

Mnisi, the area where this study was done, is a rural resource-poor community with high poverty where inhabitants live livestock-dependant lifestyles. It shares 75% of it's boundary with wildlife conservation reserves and is considered a livestock-wildlife-human interface. A recent community survey (Berrian et al, 2016) showed a close level of contact between the people, their animals and rodents. Quan et al (2013, 2017) demonstrated significant levels of exposure by people to several zoonotic infections including tick bite fever, Q fever, leptospirosis, bartonellosis, brucellosis and arboviruses all of which cause febrile illness.

In our study, the Reverse Line Blot (RLB) hybridization assay results revealed that most (32.5%) of the PCR products hybridized only with the *Anaplasma/Ehrlichia* and/or *Theileria/Babesia* genus-specific probes, suggesting the presence of a novel species or variants of a species. One *Aethomys ineptus* (2.5%) sample tested positive for the presence of *A. phagocytophilum* DNA, while two *Lemniscomys rosalia* (5%) and one *Mastomys natalensis* (2.5%) tested positive for *A. bovis* DNA. Interestingly, the RLB showed that six samples (samples 26, 27, 30, 41, 71, 86) tested positive for both *Ehrlichia/Anaplasma* and *Babesia* genus-specific probes indicating possible co-infection. It is known that rodents act as reservoirs for multiple infectious disease agents of humans and they can be carriers of more than one disease agent at a time. These results could indicate the presence of co-infection, but species identification is needed to confirm.

Results of the *B. microti* SYBR green real-time PCR (qPCR) proved difficult to interpret due to the wide melting temperature range observed. In the paper by Herch et al (2012), the melting point range of the assay was between 83.5 –84.5°C. In our study, the average melting temperature of the positive *B. microti* control was 82.30°C. Results of nine of the

rodent samples (22.5%) produced melting temperatures within 4°C of that of the positive control (i.e. 79.16 - 86.17°C). Furthermore, Herch et al (2012) did not determine the efficiency or the limit of detection of this assay and indicated that the rate of false positives and negatives was less than 5%. In the study done by Troskie (2017) under South African conditions, the efficiency of the assay was determined to be 98.8% and the limit of detection 8.37 gene copies/ μ l with a 95% confidence level between 4.085 and 27.356. They also found it difficult to determine the melting temperature range to declare samples positive for *B. microti* and speculated that this might be due to insufficient variation in the target area of the 18S rRNA gene investigated or due to nonspecific binding of other double-stranded DNA. The assay in the Troskie (2017) study also had a wider melting temperature range between 80.76-83.76°C.

Going forward we propose to sequence the parasite 18S rDNA gene of the nine rodent samples that produced melting temperatures within 4°C of that of the positive control to determine whether they indeed are South African variants of *B. microti* and/or a novel species. Each of the geographic locations is represented in the nine samples with three samples each from Thlavekisa and Hlalagagle, two samples from Gottenburg and one sample from Manyeleti. In this group, *M. natalensis* (n=4), *T. leucogaster* (n=2) and *R. tanezumi* (n=2) are the most represented rodent species. If DNA sequencing confirms these as positive for *B. microti*, these rodent species should be investigated for their competence as reservoir hosts of *B. microti*.

The Taqman real-time qPCR for *A. phagocytophilum* (Courtney et al 2004) resulted in a significant incidence of DNA detection. Fourteen of the rodent samples (35%) tested positive for the presence of *A. phagocytophilum* DNA which is higher than expected. Reports of human granulocytic anaplasmosis (HGA) occurring in Africa have been few (M'ghirbi et al, 2009, 2012). In South Africa there has been no official diagnoses of *A. phagocytophilum* in humans. The first report of the molecular detection of a bacterium closely related to *A. phagocytophilum* in South Africa was in whole blood specimens from three dogs (Inokuma et al, 2005). In a recent study, the same *Anaplasma* sp. (SA dog strain) as well as a partial 16S rRNA gene sequence closely related (99%) to *A. phagocytophilum* in several dog samples collected in the Mnisi community were detected (Kolo et al, 2016). Mtshali et al (2015) reported *A. phagocytophilum* infection rates of 6%, 17% and 1.25% in

ticks collected from cattle, goats and sheep, respectively. In a recent study, Mtshali et al (2017) reported *A. phagocytophilum* DNA in 18% of the 318 ticks collected from cats and dogs. The tick infection rates in some areas were particularly high and the potential vectors identified were *R. sanguineus* and *H. elliptica* but the actual incidences and identity of the true hosts in South Africa remain unknown (Mtshali et al, 2017).

Of the 14 *A. phagocytophilum* DNA positive samples, six were from *M. natalensis*, four from *Rattus* species (two *R. rattus* and two *R. tanezumi*), two from *T. leucogaster* and one each from *S. campestris* and *L. rosalia*. As for geographic location, one rodent originated from Manyeleti, one from Gottenburg, four (28.57%) from Hlalagagle and the remaining eight (57.14%) from Thlavekisa. Although Thlavekisa held a significant number of positive samples for *A. phagocytophilum*, as a communal rangeland, the level of direct exposure of people to rodents is probably not that high. Hlalagagle is, however, urban or peri-urban; the level and closeness of contact between people and rodents in this area should be investigated so that measures can be taken to reduce contact and potential transmission of *A. phagocytophilum*. If the rodent species carrying *A. phagocytophilum* are more dispersed in rangelands and countryside than near human settlements, it could be one of the reasons why no cases of HGA have been reported in South Africa. However, these results provide motivation for active surveillance of the rodents (and ticks they carry) in this community.

To confirm the *A. phagocytophilum* qPCR results, the near full-length 16S rRNA parasite gene of three samples and the partial *msp2* parasite gene of five of the samples were amplified, cloned and the recombinants sequenced. Although no *A. phagocytophilum* 16S rDNA sequences were obtained from the limited number of samples investigated, two *Bartonella* spp. 16S rDNA sequences were obtained from two *S. campestris* trapped in Hlalagagle. This was not an unexpected finding as the National Institute for Communicable Diseases (NICD) has previously, through their routine diagnostic zoonosis survey of adult patients with acute febrile illness (AFI) (fever >37.5°C) at the Hluvukani community clinic from October 2012 to June 2013 (n=119) (Quan et al., 2013), reported 9.5% patients that had acute bartonellosis. There are six rodent-borne bartonellae with the potential to infect humans: *B. elizabethae*, *B. grahamii*, *B. rochalimae*, *B. tribocorum*, *B. vinsonii* subspecies *arupensis* and *B. washoensis* (Buffet et al, 2013). In South Africa, the study by Pretorius et al (2004) confirmed that bartonellae are widely distributed among endemic rodents and the

authors indicated an incidence of 44.2% in their study. We propose that future studies should be done to establish which *Bartonella* spp. are prevalent in rodents in the Mnisi communal area by using gene sequence analysis.

From the 12 partial *msp2* gene sequences obtained, two Msp2 sequence types were identified (designated Ap1 and Ap2); Ap1 differed by one amino acid from Ap2. Ap1 was identical to *A. phagocytophilum* human strain Webster and *A. phagocytophilum* strain Dog2, while Ap2 was identical to *A. phagocytophilum* human strain HZ as well as *A. phagocytophilum* strains previously described from various hosts (including deer, ticks and mite).

Msp2 is an *A. phagocytophilum* surface protein, belonging to the MSP2/P44 superfamily. *msp2* comprises two conserved sequences flanking a hypervariable region, which should be sequenced for *A. phagocytophilum* phylogenetic analysis. In a study by Silaghi et al (2011), the authors used this hypervariable region to distinguish European from American *A. phagocytophilum* variants. However, horses were the only animal hosts common to these two continents. For this reason, the observed clustering could be linked to the animal host species, rather than to the geographical origin, as suggested by de La Fuente et al. (2005). In our study, we could only obtain partial *msp2* gene sequences (334 bp) spanning a conserved region of the gene. Although valuable data, as we could confirm the presence of *A. phagocytophilum* DNA in our samples, full-length gene sequences will have to be obtained if we want to draw any clear conclusions on the genetic variability of the *A. phagocytophilum* strains circulating in the Mnisi communal area. Studies in other parts of the world suggest that multiple strains of *A. phagocytophilum* may be circulating in wild and domestic animals, and these strains may have differential host tropisms and pathogenicity. Also, the degree to which genetic variation contributes to altered pathogenicity of different strains of *A. phagocytophilum* is poorly understood. This should be investigated in the South African context.

As for the use of the molecular diagnostic assay used in our study, we found significant differences between the findings of the RLB assay and the qPCR for *B. microti* and *A. phagocytophilum*. Since five of the samples which were positive for *A. phagocytophilum* DNA on qPCR were confirmed by sequencing, it shows that in this study the RLB assay was

not very sensitive. It is possible that RLB is limited by the amount of DNA present in samples. In qPCR, the amount of PCR product is measured at each cycle by monitoring the reaction during the exponential amplification phase of the reaction. In this way, it is possible to estimate the original quantity of target DNA (de Waal, 2012). In comparison to the positive control, the *A. phagocytophilum* PCR positive samples had high CT numbers which indicates very low initial levels of DNA. Of the 14 qPCR positive samples for *A. phagocytophilum*, the RLB was only able to identify three samples (sample 32, 33 and 84) by the *Ehrlichia/Anaplasma* genus probe. It is important to understand limitations of diagnostic tests to analyse results correctly. A separate study would have to be performed to determine the minimum amount of DNA that is detectable by the RLB assay.

It is true that a positive PCR result demonstrates only the presence of target (pathogen) derived DNA. This was sufficient for this project which aimed simply to identify the presence of the pathogens in wild rodent samples. However, PCR results must be interpreted with care when making inferences in terms of clinical disease. The ability for these haemoparasites to induce acute or latent infection as well as induce carrier status makes PCR result interpretation challenging. One must consider the surrounding circumstances (the patient history and symptoms, serological evidence, geographic location and presence of ticks).

A limitation of this study is that only 40 wild rodent blood samples were available. For more accurate determination of prevalence of *B. microti* and *A. phagocytophilum* in this population, larger numbers of rodent samples are recommended. Additionally, findings of a survey such as this would be more useful when done in parallel to testing of human febrile patients and other mammalian hosts which are present in the area. Also, although *Ixodes scapularis* ticks are not present in South Africa, there is an abundance of other tick species which may be capable of being vectors for *A. phagocytophilum*. It is reasonable that survey studies should be performed on the ticks in the area to test them for *A. phagocytophilum*. Further surveillance of this kind is, thus, strongly recommended.

Although sample selection aimed to be representative in both rodent species and location, some rodent species were more represented than others. Of the 40 samples, 16 (40%) were *Mastomys natalensis*, six (15%) were *Tatera leucogaster*, six (15%) were *Rattus species*

(three each of *R. rattus* and *R. tanezumi*), four (10%) were *Aethomys ineptus*, four (10%) were *Saccostomus campestris*, two (5%) were *Lemniscomys rosalia*, one (2.5%) was *Mastomys coucha* and one (2.5%) was *Mus minutoides*. It is known that a variety of small mammals can act as reservoir hosts for *A. phagocytophilum* and that the distribution of these hosts is determined by geographic location (Carrade et al, 2009). The natal multi-mammate mouse (*M. natalensis*) was by far the most abundant rodent species caught in this study and the species with the most positive results for *A. phagocytophilum*. This species can achieve very high abundances in nature because of its social systems and space-use patterns (Borresmans et al, 2014). In the past, *M. natalensis* has been the focus of many studies of flea-borne pathogens and not usually in connection with tick-borne pathogens (Laudisoit et al, 2009).

The sample collection locations would have influenced the variety of rodents caught which could affect our findings for *A. phagocytophilum*. Further rodent sampling and testing is planned in the Mnisi community and this will shed more light on the subject. In terms of location, exactly 10 (25%) samples came from each location. But within each location the rodent species numbers varied a lot, and this could influence the results for the location in question.

We furthermore recommend that the rodent reservoirs should be ranked for their risk level of close human contact and disease transmission. There is certainly need for further research on this in Africa. Rodent species could be ranked in terms of risk by looking at their distribution, preferred habitats and behaviour. For example, *Mastomys* species are known for their abundance and wide distribution across sub-Saharan Africa (Monadjem et al, 2015). They are very successful breeders and are commonly seen in homes where they scavenge for stored grain to eat (Leirs, 2013). Rats are also commonly found in and around human settlements. Both black rats (*R. rattus*) and Asian rats (*R. tanezumi*) are well-known reservoirs of human diseases (Fiedler et al, 1994). Rats are strong competitors for food and become very aggressive and territorial if food supply is limited. They show more tolerance for other rats if food is abundant (Bridgman et al, 2013; Lore et al, 1986). Interestingly, mice and rats never co-exist in an area because rats often display muricide – the killing of mice. Just the smell of a rat will cause a mouse to flee (Karli, 1956). In comparison, the African pouched rat (*S. campestris*) is mainly found in burrows around riverbeds, rocky habitats and

bushveld. Even though they are not that closely associated to human settlements, they are often caught in traps because of their slowness and mild temper (MacFadyen et al, 2016). Members of the gerbil genus (*T. leucogaster*) are well-known for their role in transmission of human diseases such as the plague, but they do not normally enter dwellings and prefer their complex burrow systems (Fiedler et al, 1994). Even though they might not often contact people, they could still be important in their role of transmission of human pathogens to other rodents by contact or sharing of vectors such as fleas or ticks. Although *Lemniscomys* species play a lesser role in transmission of human diseases compared to other rodents, they can be abundant in and around villages (Fiedler et al, 1994).

Finally, the significant level of incidence of *A. phagocytophilum* DNA found in this study demonstrates that the threat of tick-borne pathogens from rodents should not be underestimated in Southern Africa. Hopefully in the future more will be learned about this potential threat.

CONCLUSION

The main purpose of this project was two-fold. The first was to provide a narrative literature review which would provide information on rodent-borne bacterial and parasitic pathogens of potential zoonotic importance present in Africa. The second was to screen wild rodent blood samples (n=40) collected in the Mnisi community for the presence of haemoparasites using the RLB hybridization assay and then to specifically test for *A. phagocytophilum* and *B. microti* infections using previously described qPCR assays.

The results of this study, which found an incidence of 35% for *A. phagocytophilum*-DNA in the Mnisi rodent population, were enlightening. Until recently, it was thought that the pathogen was only present in the USA and Europe. But after partial *mosp2* gene sequencing was performed, these findings of *A. phagocytophilum* DNA in wild rodents have been confirmed. This is supported by other studies which detected gene sequences closely related to *A. phagocytophilum* (Inokuma et al, 2005; Kolo et al, 2016). Together this demonstrates the presence of the pathogen in South Africa. A study by Quan et al (2013) showed an unexpectedly high incidence of *Bartonella* in adult febrile patients in the Mnisi area. This indicates a significant exposure of the people to ectoparasites and since they are mainly agro-pastoralists, their risk of vector-transmitted disease is high.

Going forward, more data is needed to decide if active surveillance for *A. phagocytophilum* and *B. microti* is necessary in Southern Africa. Integrated studies of ticks, rodents, mammals and people in this community must commence. It is important to determine the pathogenicity of the variants identified, if the parasitaemia levels are high enough to present a threat, if competent vectors and hosts are present and if there is enough contact between people and the tick vectors to cause significant disease.

Human deaths due to mysterious febrile illness occur in Sub-Saharan Africa (Rajaratnam et al, 2010). The important question is whether they could be caused by or complicated by human babesiosis or human granulocytic anaplasmosis. These diseases should be seriously considered in the case of febrile human patients. The challenge faced in African settings is that most of the hospitals and clinics are also lacking in the most basic diagnostic resources. Increased awareness and training should be provided to medically trained personnel who

work with febrile human patients especially in areas where a high level of exposure to rodents and their ectoparasites is known. Because of global travel, training and awareness are also important for medical personnel outside of endemic areas especially where there is a history of the patient travelling to a tropical region.

It is true that tick-borne infectious diseases in people rarely presents as sudden epidemics with the consequent large-scale humanitarian and economic shocks. However, they pose significant direct threats to the public in terms of morbidity and in severe cases, mortality. In the African context, large households often rely on a few productive members of the family. Should a key family member become too ill to work or simply become less productive at work, many others suffer consequently. Also in rural Africa, a large proportion of people are small scale farmers or agro-pastoralists and their exposure to ectoparasites is high. At a large-scale production level, effects of these chronic diseases could be felt in terms of a reduced work force (increased sick leave days being taken by workers) or generally reduced productivity. The chronic drain on the people, the public healthcare system and government funds can have a worse eroding effect on economy of a country in the long-term than occasional massive disease epidemics and their sudden unexpected costs. Active surveillance and prevention is therefore much more cost-effective method than disease disaster management.

All the diseases described in the literature review have significant economic impacts on animal reproduction, animal trade and the production of animal products. Their zoonotic potential makes them more important as they also threaten human health. With advances in human medicine, the number of immunosuppressed, premature, elderly and chronically ill individuals in the global population have increased. Therefore, the susceptible proportion of the global population to chronic infection is larger than ever.

Poor governance and poor capacity to recognize and respond to infectious disease problems are major issues for Sub-Saharan Africa. The drivers of disease emergence are complex making predictions of future risks very difficult. However, there are many new technological advances that offer hope for improved disease detection capabilities and vaccine developments. In the future, hopefully there will be more initiatives like the Foresight project which emphasized the importance of fostering inter-disciplinary approaches to

infectious disease research in order to transcend traditional intellectual boundaries (King et al, 2006).

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- 3) <http://blast.ncbi.nlm.nih.gov/> (National Centre for Biotechnology Information website)
- 4) [http://4.bp.blogspot.com/-kFKZFTolG18/UfETulsDQOI/AAAAAAAAAew/vsBzsR4M3kw/s400/Borrelia+burgdorferi+\(Lyme+Disease\)+Spirochaetaceae.png](http://4.bp.blogspot.com/-kFKZFTolG18/UfETulsDQOI/AAAAAAAAAew/vsBzsR4M3kw/s400/Borrelia+burgdorferi+(Lyme+Disease)+Spirochaetaceae.png) (Lyme disease image)
- 5) <http://www.boergoats.com/clean/articles/health/q-fever/Qfever.jpg> (Q fever image)
- 6) <http://www.publichealthalert.org/uploads/1/4/0/7/14079136/8114105.jpg> (Bartonellosis image)
- 7) https://encrypted-tbn2.gstatic.com/images?q=tbn:ANd9GcTUJoF3ujn9fLGN9ulF7iToILVvTrs_PVljmNchNm5QnoKDh0XT (Leptospirosis image)
- 8) https://www.inds.co.uk/wp-content/uploads/2015/09/MSBA0167_tn.jpg (*Borrelia duttoni* image)
- 9) <https://www.who.int> (WHO, Geneva)

APPENDICES

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APPENDIX 1



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	The occurrence of wild rodent tick-borne diseases and their risks for public health: A review of the African context
PROJECT NUMBER	V119-15
RESEARCHER/PRINCIPAL INVESTIGATOR	L de Boni

STUDENT NUMBER (where applicable)	UP_02619229
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	Rodent	
NUMBER OF ANIMALS	50	
Approval period to use animals for research/testing purposes		November 2015-November 2016
SUPERVISOR	Prof. M Oosthuizen	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	30 November 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	



agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6; 12/11/1/1

Prof Marinda Oosthuizen
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
University of Pretoria

Dear Prof Oosthuizen,

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your fax / memo / letter/ Email dated 19 October 2015, requesting an amendment to permission granted on 23 December 2015 under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following amended research/study, with the following conditions :

Conditions:

1. All conditions stipulated within the approval dated 2014-12-23 must still be complied with;
2. The only allowable deviation from the research protocol for which Section 20 approval was granted on 2014-12-23 is the inclusion of the following postgraduate students to part take in the study:
 - (i) Dr Agatha Kolo
 - (ii) Dr Liesl De Boni
 - (iii) Ms Samantha Wills

Title of research/study: Discovering emerging tick-borne pathogens that could impact on human health and livestock production in South Africa: Developing real-time PCR assays for the specific and sensitive detection of these pathogens

Researcher (s): Prof Marinda Oosthuizen, Dr Agatha Kolo, Dr Liesl De Boni, Ms Samantha Wills

Institution: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, UP

Your Ref./ Project Number:

Our ref Number: 12/11/1/1; 12/11/1/1/6

Kind regards,

DR. MPHOMAJA
DIRECTOR OF ANIMAL HEALTH

Date: 2015 -11- 13