

**THE OCCURRENCE OF TICK-BORNE PATHOGENS,
IN DOGS IN WELFARE ORGANISATIONS AND
TOWNSHIPS OF CAPE TOWN**

By

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DECLARATION

I declare that this dissertation, the occurrence of tick-borne pathogens, in dogs in welfare organisations and townships is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references. This dissertation which I submit for the Master of Science (Life Science) degree at the College of Agriculture and Environmental Sciences, at UNISA (University of South Africa), is my own work, and has not been submitted by me to any other university.

Rosalind Elizabeth Allan

Date

DEDICATION

To Vanessa Cunningham, for the excellent work she does in the townships.

*Saving one animal won't change the world,
but it will change the world for that one animal*

Anonymous

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SUMMARY

Ticks and tick-borne pathogens affect human and animal health worldwide resulting in significant economic loss. Previous studies have indicated that dogs are usually infested with ticks and also suffer from a variety of pathogens transmitted by different tick species. Therefore, the aim of this study was to identify ticks and to screen for the presence of tick-borne pathogens, with special focus on *Ehrlichia* and *Babesia* spp. from blood and tick samples collected from dogs in four rescue organisations and two townships in Cape Town, South Africa. PCR and the Reverse Line Blot hybridization assay were used to screen samples for pathogens and ticks were identified using a stereo microscope. Forty six (36.5%) of the blood samples tested positive for tick-borne pathogen DNA. Of the positive blood samples, 17 (13.5%) were infected with *Ehrlichia canis*; 16 (12.7%) with *Babesia rossi* and four (3.2%) samples were infected with *Babesia vogeli*. Incidental infections were also detected, these included *Ehrlichia ruminantium* (n=6, [4.7%]), *Theileria taurotragi* (n=2, [1.6%]) and *Anaplasma* sp. *Omatjenne* (n=1, [0.8%]) infections. DNA detected from 10 samples (7.94%) hybridized only to the *Ehrlichia/Anaplasma* genus-specific probes and four samples (3.17%) hybridized only to the *Theileria/Babesia* genus-specific probes. Tick-borne pathogen DNA could not be detected in four (26.6%) tick pools. Only two tick species were detected, with high occurrences of *Rhipicephalus sanguineus* ([n=457] 89%) associated with the transmission of *E. canis* and *B. vogeli* and *Haemaphysalis elliptica* ([n=52] 11%) the vector for *B. rossi*.

The results indicated that the sampled dogs harboured a wide variety of blood parasites that included *Babesia rossi* and *Ehrlichia canis*, the two most important tick-borne pathogens of dogs. Based on the results, it is evident that ticks and tick-borne diseases of dogs are a burden to dogs in the sampled localities. Establishing correct control and treatment measures of these pathogens will aid in welfare of affected dogs.

KEY TERMS

Dogs

Ticks

Babesia

Ehrlichia

Rhipicephalus sanguineus

Haemaphysalis elliptica

Townships

Welfare organisation

PCR (polymerase chain reaction)

Reverse line Blot hybridisation assay (RLB)

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degrees Celsius
μl	Microlitre
μM	Micromolar
μg	Microgram
CFT	Complement fixation test
CME	Canine monocytic Ehrlichiosis
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dUTP	Deoxyuridine triphosphate
E/A	<i>Ehrlichia/Anaplasma</i>
EDTA	Ethylenediaminetetraacetic-acid
ELISA	Enzyme linked immunosorbent assay
IFA	Immuno fluorescent assay
IgG	Immunoglobulin G (Gamma)
IgM	Immunoglobulin M (Mu)
ml	Millilitre
mM	Millimolar
OVAH	Onderstepoort Veterinary Academic Hospital
PCR	Polymerase chain reaction
Pmol	picomol
RCT	Rapid conglutination test
RLB	Reverse line blot
RMSF	Rocky Mountain spotted fever
RNA	Ribonucleic acid
Rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulphate
sp	Species
spp	Species
T/B	<i>Theileria/Babesia</i>
TBD	Tick-borne disease
TBEV	Tick-borne encephalitis virus
U	Units
UDG	Uracil DNA glycosylase

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ABSTRACT

In impoverished and resource limited communities such as townships, and welfare organizations, areas such as living and sleeping spaces are sometimes shared with animals, and occasionally humans. Dogs play an integral role in our lives and have become part of the family. Therefore, it is probable that ectoparasites, such as ticks, that feed on dogs also feed on other vertebrates, thereby, transmitting pathogens.

The primary aim of this study was to screen for the presence of tick-borne pathogens in dogs from welfare organisations and townships in Cape Town, with special focus on *Ehrlichia* and *Babesia* spp. The reason for this choice of subject is due to the fact that very few tick-borne infection studies have focused on resource limited communities. Furthermore, welfare organisations have continuously attracted awareness due to the amount of unrestricted work performed by veterinarians in communities with limited resources. Consequently, the topic was borne.

A total of 126 blood samples and 509 ticks (adults and nymphs) were collected directly from dogs from four welfare organisations and two townships in Cape Town. Samples were collected from April to July 2014. The four welfare organisations where samples were collected included the Animal Anti Cruelty League welfare organisations in Epping and Bellville, the Lucky Lucy Foundation in Joostenberg Vlake and The Emma Animal Rescue Society (TEARS), located in the Sunnydale area. Samples were also collected from the Asanda village and Nomzamo, two townships located just outside the Cape Town suburb, the Strand.

DNA was extracted from blood and ectoparasites and screened for the presence of *Ehrlichia*, *Anaplasma*, *Theileria* and *Babesia* species infections using touchdown PCR and RLB hybridization assays. Genus and species-specific probes were used during hybridization in order to identify specific parasite infections in the blood samples and the tick samples pooled according to geographical origin and species.

Forty six (36.5%) of the blood samples tested positive for tick-borne pathogen DNA. Of the positive blood samples, 17 (13.5%) were infected with *Ehrlichia canis*; 16 (12.7%) with *Babesia rossi* and four (3.2%) samples were infected with *Babesia vogeli*. Incidental infections were also detected, these included *Ehrlichia ruminantium* (n=6, [4.7%]), *Theileria taurotragi* (n=2, [1.6%]) and *Anaplasma* sp. *Omatjenne* (n=1, [0.8%]) infections. DNA detected from 10 samples (7.94%) hybridized only to the *Ehrlichia/Anaplasma* genus-specific probes and four samples (3.17%) hybridized only to the *Theileria/Babesia* genus-specific probes. None of these 14 samples hybridized to any of the species-specific probes.

Collected *Rhipicephalus sanguineus* (n=457) and *Haemaphysalis elliptica* (n=52) ticks were grouped into 15 pools, representing both tick species according to specific collection locations. Since only two *H. elliptica* from Asanda and one *R. sanguineus* from TEARS were collected, these ticks were mixed in pools of the dominant species as they were too few for DNA extraction. Ticks were collected from the Nomzamo Township (*R. sanguineus* n=400), Asanda village (*H. elliptica* n=2; *R. sanguineus* n=42), TEARS (*H. elliptica* n=21; *R. sanguineus* n=1), and the Animal Anti Cruelty League in both Epping (*R. sanguineus* n=14), and Bellville (*H. elliptica* n=29), in Cape Town. Analysis by the RLB assay showed that 11 (73.3%) of the 15 tick pools representing both tick species were positive for at least one parasite species. All positive samples hybridized with the *Ehrlichia/Anaplasma* genus-specific probe. Three (20%) tick pools containing both tick species tested positive for *Ehrlichia canis* infection, two (13.3%) tested positive for *Babesia rossi* and *Babesia vogeli* DNA was identified in one (6.6%) tick pool. The *Theileria/Babesia* genus-specific probe hybridised in three (20%) tick pools. These three pools were comprised of both *R. sanguineus* and *H. elliptica* tick species. These tick pools also tested positive for a specific *Babesia* tick-borne pathogen. Tick-borne pathogen DNA could not be detected in four (26.6%) tick pools.

The fore-mentioned tick-borne pathogen DNA detected in the dog blood samples, and the ectoparasites collected from the same dogs during this study, suggests that dogs play a large role in the endemicity of these pathogens.

Chapter 1

INTRODUCTION

Ticks parasitize a wide variety of animals for blood-meals (hematophagy), and transfer many tick-borne pathogens to vertebrates, including humans, causing tick-borne diseases in the process (Schroder and Reilly 2013). These pathogens can be viral, e.g. tick-borne encephalitis virus (TBEV); bacterial, e.g. Lyme disease or ehrlichiosis; rickettsial, e.g. Rocky Mountain spotted fever; or even protozoal, e.g. babesiosis.

Ticks are ectoparasites, classified in the subclass Acari, and in the order Parasitiformes. The feeding period of an adult tick varies amongst the different species, and the engorged females usually drop off before clinical signs can be observed (Waner 2008). The incubation periods of tick-borne diseases vary from 8-21 days, depending on the disease (Schoeman 2009).

Ticks and tick-borne pathogens affect human and animal health worldwide resulting in significant economic loss. Costs due to morbidity and mortality, abortion, loss of milk and meat production are one of the examples where livestock is affected (Jongejan and Uilenberg 2004). Among many other industries, the equine industry is an additional example of animals also greatly affected by tick-borne diseases, namely equine piroplasmiasis, due to restrictions imposed on affected animals destined for international trade or participation in racing or other sporting events (United States Department of Agriculture-Animal and Plant Health Inspection Service 2009).

Beside the financial implications attached to hospitalization and treatment for sick dogs, the impact of tick-bite associated diseases in dogs goes beyond the affected animal, as pet owners are usually emotionally attached to their animals.

Tick-borne diseases are clinically significant, but are feared to be an even greater threat in communities that cannot afford the correct care and treatment for the infected animal (Jongejan and Uilenberg 2004). As a matter of fact, Young et al., (1988) reported that the

most important health and management problem in Africa is the control of ticks and tick-borne pathogens.

1.1 Supportive studies

There have been many useful ectoparasite, ehrlichiosis and babesiosis studies performed in Africa. For example, a community-based parasitology study was conducted by Ugbomoiko et al., (2008) in Ilorin, Nigeria, between 2006 and 2007. This is an intense rainfall region with extremely hot temperatures. The urban area of Ilorin is surrounded by rural village settlements, with poor living conditions. Most people in the village keep dogs, but have no access to a veterinarian. The ectoparasites studied included: ticks, mites, lice and fleas collected from 396 dogs from both the urban and rural areas.

The authors noted that in general, in resource poor communities, treatment to eliminate parasites, where treatment is carried out at all, is only applied in the advanced stages of the disease. The findings of this study showed that 60.4% of the dogs presented with ectoparasites. Dogs from the villages were more frequently infested than those from the urban environment (77.9% vs 41.7%).

Additionally, dogs from the villages were more commonly infected with two or more ectoparasite species. The most prevalent ectoparasite found was the common dog flea *Ctenocephalides canis*, with *Rhipicephalus sanguineus* being the second most prevalent (Ugbomoiko et al. 2008).

Another study was performed in the North-West Province of South Africa, where 344 dogs belonging to people in resource-poor areas were examined for ectoparasites. In contrast with the previous study, the most common ectoparasite identified was *R. sanguineus* (96.6%) (Bryson et al. 2000). Other ticks identified included *Haemaphysalis leachi* (2.85%), and *Amblyomma hebraeum*, *R. appendiculatus*, *R. evertsi evertsi* and *R. simus*, which accounted for 0.53% of ectoparasites. The authors concluded that 'the predominance of *R. sanguineus* accounts for the high occurrence of canine ehrlichiosis (*Ehrlichia canis*) within the survey region, compared to canine babesiosis (*Babesia rossi*,

referred to as *Babesia canis* in the manuscript), which is transmitted by *H. leachi*, and is a much rarer disease'.

An ectoparasite study performed in Nigeria by Omudu et al., (2010), demonstrated that stray dogs left to roam the streets and scavenge for food, had a higher parasite infestation, compared to those living within restricted urban areas. This finding corroborates Bryson et al., (2000) findings in the North West Province of South Africa. The authors concluded that this was probably due to these animals having greater interaction with other animals, including livestock (Omudu et al. 2010; Bryson et al. 2000).

A similar study was performed in Jos, Plateau State, Nigeria (Adamu et al. 2014). The main objective of this study was to detect and characterise tick-borne pathogens in dogs presented to a veterinary hospital, using molecular techniques. One hundred blood specimens were collected in 2010 from domestic dogs presented at an animal hospital in Jos, Plateau State. The blood samples were screened for the presence of *Theileria/Babesia* and *Ehrlichia/Anaplasma* genomic DNA using PCR and Reverse Line Blot (RLB) hybridization assays. Additionally ticks were also collected. Seventy two (72%) percent of the blood specimens were positive for one or more tick-borne pathogen. *Babesia rossi* was the most predominant pathogen detected in 38 of the positive blood samples. *Theileria* sp., *Ehrlichia canis* or *Anaplasma* sp. *Omatjenne* and *Theileria equi* were also identified. One hundred and forty six ticks belonging to eight species were collected and identified. *Rhipicephalus sanguineus* was the most predominantly occurring tick-vector identified (n=107) (Adamu et al. 2014).

A survey conducted between 1999 and 2000, in the North-Eastern Kwazulu-Natal region of South Africa showed that *Haemaphysalis leachi* was the most prevalent tick-vector found on dogs residing in rural communities (Horak et al. 2001). Other tick species identified during this study include *Amblyomma Hebraeum* and *R. simus*. Dogs belonging to more affluent citizens tend to have a higher occurrence of *H. leachi*. Alternatively, dogs belonging to people in townships have a higher occurrence of *R. sanguineus* (Horak et al. 2001).

In South Africa, there are many rural areas consisting of communities that are unable to control ticks and the pathogens they transmit due to lack of access to veterinary services and knowledge.

1.2 Literature review

1.2.1 Tick-borne diseases

Tick-borne pathogens cause diseases affecting both humans and animals alike. Ticks become infected with disease when feeding on a diseased vertebrate host. Consequently, ticks may act as pathogen vectors, but also as pathogen reservoirs (Parola and Raoult 2001).

The occurrence of ticks, tick-bites and tick-borne disease cases is becoming increasingly common in veterinary practices. There are some severe canine diseases caused by tick-borne pathogens, the most important being ehrlichiosis, anaplasmosis, babesiosis, and, in the United States, Rocky Mountain Spotted Fever (RMSF) and hepatozoonosis (Shaw et al. 2001). Most dog owners are unaware of these diseases and only learn about them when their animal is diagnosed with a tick-borne disease by a veterinarian. Unfortunately, an increasing number of dogs are being misdiagnosed or not diagnosed at all, resulting in an increase in morbidity and mortality rates. Although a veterinarian may be able to recognize the presence of disease, it may be difficult to identify the specific pathogen responsible. The clinical signs often present as non-specific, sometimes resulting in an incorrect treatment plan being implemented. Correct identification of the pathogen is essential for effective case management and it therefore becomes imperative that pathogenic infections are accurately identified in order to implement the correct treatment plan. The remedy to prevent or limit misdiagnosis and thus the implementation of an incorrect treatment plan is to record a full history of the dog, incorporating risk factors in conjunction with a full blood count which includes a white cell and a platelet count. This will enable the veterinarian to make the correct decision for a specific treatment. A history should be noted of travel into any tick-endemic area or of any recent dog fights. When clinical signs and symptoms are suggestive of infection by a tick-borne pathogen further investigation should be done.

Early diagnosis of any tick-borne disease is crucial to the survival of the animal. With correct diagnosis and early treatment, the clinical signs of the disease may be significantly alleviated. However, veterinarian consultations, diagnostic tests, as well as treatment are expensive, and therefore predominantly only available to owners who can afford their pet such necessity. As a result, impoverished and resource limited communities such as townships, and welfare organizations benefit enormously from free services provided by veterinarians, veterinary nurses and other volunteers, rendering their services in outreach programs such as free spay days or free vaccination programs, where tick-borne diseases may be identified during the consultation.

1.2.2 Tick-vectors

Ticks are ubiquitous, obligate hematophagous arthropods and are important disease causing vectors of all classes of vertebrates (Nava et al. 2009). These ectoparasites can be divided into three families. The Ixodidae ticks (692 species) are referred to as the 'hard ticks' which possess a hard dorsal scutum. The Argasidae ticks (186 species) are the 'soft ticks' or 'tampans' which lack a scutum. The third tick family, the Nuttalliellidae family, is only found in Southern Africa and is represented only by one tick species: *Nuttalliella namaqua* (Parola and Raoult 2001; Nava et al. 2009).

Several genera of Ixodid ticks include *Ixodes*, *Rhipicephalus*, *Dermacentor*, *Hyalomma*, *Boophilus* and *Haemaphysalis*. Genera of the Argasidae family include *Argas*, *Ornithodoros*, *otobius* and *Antricola* (Parola and Raoult 2001; Nava et al. 2009). A few of the well-known ticks parasitizing a mainly dogs are shown in Table 1.

Table 1 Distribution and known hosts of various tick species affecting dogs worldwide

Tick species	Distribution	Known hosts
<i>Dermacentor reticulatus</i>	Europe; Asia	Mammals; cattle; dogs; horses; deer
<i>Dermacentor variabilis</i> (American dog tick)	USA; Canada, Mexico	Mammals; mice; vole; dogs.
<i>Haemaphysalis bispinosa</i>	Asia, North America, Australia and Europe	Dogs and other mammals
<i>Haemaphysalis elliptica</i>	Southern Africa	Murid rodents; dogs
<i>Haemaphysalis leachi</i>	Southern Africa, North Africa	Cattle; sheep; dogs; cats; horses
<i>Haemaphysalis longicornis</i>	Japan, former USSR, China, Korea, Australia	Rodents; birds; large mammals - cattle; sheep, dogs; horses
<i>Rhipicephalus sanguineus</i> (Brown dog tick)	Tropical/semiotropical worldwide	Mostly dogs and other mammals
<i>Rhipicephalus simus</i>	Southern Africa	Dogs; cats; ruminants; large carnivores; rodents

1.2.2.1 Vectors of tick-borne diseases of humans

Ticks, being competent vectors, are able to transmit a wide variety of pathogens to different vertebrate species. *Babesia* spp. *Borrelia* spp. *Rickettsia* spp. and *Ehrlichia* spp. are tick-borne pathogens of dogs that can also be transmitted to other vertebrates such as humans. *Ehrlichia chaffeensis*, *E. canis* and human granulocytic agent (HGE) have also been identified in humans (Sambri et al. 2004).

African tick-bite fever in humans is caused by *Rickettsia africae*, which is transmitted by *Amblyomma variegatum* and *A. hebraeum* ticks and is primarily found in Africa and the Caribbean Islands (Jongejan and Uilenberg 2004).

Boutonneuse fever, sometimes called fièvre Boutonneuse, or African tick-bite fever is caused by *Rickettsia conorii* infections. This zoonosis usually resembles the classical Mediterranean spotted fever and is transmitted by *Amblyomma* spp. ticks in peri-urban or urban areas (Frean et al. 2008). *Rickettsia conorii* infections normally produce subclinical infections and the linking of this pathogen with clinical disease is difficult to gauge (Shaw et al. 2001).

One of the first zoonotic diseases identified in humans was babesiosis in the United States. It was common amongst many animals, and was not classified as a risk to human health. In the 1960's, however, a series of *Babesia microti* incidents were isolated in residents of Nantucket Island (With Nantucket fever). Ever since, *Babesial* infections have become a relatively common zoonotic tick-transmitted disease (Homer et al. 2000). Human babesiosis is caused by several *Babesial* species, and geographical distribution is based on the availability of competent hosts. *Babesia microti* is a rodent-borne piroplasm and causes human babesiosis in North America. In Europe, babesiosis in humans is caused by *Babesia divergens*, the bovine *Babesial* pathogen (Homer et al. 2000; Kjemtrup and Conrad 2000). There has also been some reports of *B. bovis* and *B. canis* infections in humans, but these have not been well documented (Homer et al. 2000).

The first case of human monocytic ehrlichiosis was identified in the United States in 1986 (Popov et al. 1998). The disease was caused by *Ehrlichia chaffeensis*, which was isolated and identified in 1991 (Popov et al. 1998). The aetiological agent of human granulocytic ehrlichiosis (HGE) was identified in 1994 (Popov et al. 1998). Human granulocytic ehrlichiosis is very closely related to *Ehrlichia equi* and *Ehrlichia phagocytophila* (Dumler et al. 1995).

1.2.2.2 Vectors of tick-borne diseases of dogs

The escalating topographical distribution of many tick-vector species, the increase in number of new tick-transmitted organisms, and the organism's ability to cause disease, make tick-borne diseases the most important of vector-borne infections in dogs. The most commonly recorded tick-vectors found on dogs world-wide is *Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *Haemaphysalis elliptica*. *Amblyomma hebraeum* has also been collected from dogs. Tick-vectors transmitting *Babesia* and *Ehrlichia* pathogens are from the *Ixodidae* tick family. The tick-vectors for each species of canine *Babesia* are different; *Babesia vogeli* is transmitted by *R. sanguineus*, while *Babesia canis* is transmitted by *Dermacentor reticulatus* and *B. rossi* by *Haemaphysalis elliptica* (Matjila et al. 2004; Irwin 2009). *Ehrlichia canis* is transmitted by *R. sanguineus* (Tsachev et al. 2008; Waner 2008). *Babesia gibsoni* is transmitted by *R. sanguineus*,

Haemaphysalis longicornis and *Haemaphysalis bispinosa*. The latter two tick species are non-endemic to South Africa (Matjila et al. 2008a).

Rhipicephalus sanguineus is a three-host tick of dogs and all stages feed on the same host species, but each life stage requires a new host. These ticks are highly adapted to living amongst humans. They are active all year in all regions, especially in tropical or temperate climates (Dantas-Torres 2010).

Haemaphysalis elliptica is a three-host tick species of which the immature stages use murid rodents as hosts and are only unusually found on the same carnivore hosts as the adults. The adults of this tick usually parasitize domestic and wild carnivores including dogs and cats (Apanaskevich et al. 2007; Horak et al. 2010). These ticks have a variable seasonality. According to Fourie et al., (2010), 'They are most abundant on dogs in the Eastern Cape Province from June to February, during the period May to September in the Western Cape Province, during the period October to February in Free State Province and from January to April in north-eastern KwaZulu-Natal Province, South Africa'.

With dogs living in close contact with humans, they can serve as parasitic reservoirs to humans, as well as other types of infections. One study that demonstrated this was conducted in a canine kennel in North Carolina, North America where 27 dogs and their 23 owners were investigated (Kordick et al. 1999). Eight of the 23 people examined were seroreactive to *Bartonella henselae*, one to *Ehrlichia chaffeensis*, and one to *Rickettsia rickettsii*.

1.3 Increases in zoogeographical range of tick-borne pathogens

It is considered that the zoogeographical range for tick-borne pathogens and their host ticks is increasing due to a number of factors that include climate change; increased interaction of wildlife reservoirs with humans, livestock and pets; grazing systems and increased mobility of pets and their owners. Tick species are finding new niches and becoming established in non-endemic regions.

Over the past 30 years, the global temperature has increased by 0.2°C per decade (Hansen et al. 2006). Generally, most ticks thrive in warm, humid, tropical environments (Estrada-Peña et al. 2012). *Rhipicephalus sanguineus* in particular has successfully adapted to these warmer climates and has become extremely aggressive (Gray et al. 2009; Parola et al. 2008).

Other examples of how climate change has affected tick distribution include *Ixodes ricinus* which now has spread to more northern and western areas of Sweden. Also, *Dermacentor variabilis* has now been reported in the north eastern parts of USA (Shaw et al. 2001).

Tick-borne pathogen occurrence increase has been associated with increased contact between pets and their owners with the pathogenic reservoirs in wildlife (Shaw et al. 2001). Ticks and tick-borne pathogens have co-evolved with various wildlife species which may constitute host reservoirs for livestock, pets and humans. Wildlife hosts only become a threat when they have contact with the non-infected animals. This usually happens when non-infected livestock is moved into infested areas with infected wildlife (Jongejan and Uilenberg 2004).

Grazing systems employed by farmers may also result in a wider distribution area of ticks, and thus a further distribution pattern of their pathogens (Rubaire-Akiiki et al. 2004). According to Jongejan and Uilenberg (2004), there is a clear correlation in the USA between the increase in tick density, and the ever increasing abundance of deer. This is due to the conversion of agricultural, tick-infested land, into enclosures suitable for the maintenance of herds of deer. Further encroachment into peri-urban and urban areas results in an increase in accessibility of pets and their owners by animals like ruminants and other livestock which increases the likelihood of pets becoming infected and distributing tick-borne pathogens.

The zoogeographical range for tick-borne pathogens and their host ticks has also increased with the increased mobility of pets and their owners (Shaw et al. 2001). International travel of pet owners and their pets, besides export and import of animals,

has contributed to the increased distribution area of ticks (Gaff and Gross 2007; Toth and Roberts 2011). Pets travelling to warm climatic regions are at risk of contracting tropical or subtropical tick-borne diseases, which are usually only noticed by their owner after returning home. With particular reference to Ehrlichiosis and Babesiosis, dogs from Europe acquire these diseases when travelling to the Mediterranean region (Jongejan and Uilenberg 2004).

Canine Babesiosis and Ehrlichiosis are endemic in South Africa. It is known that veterinary services, vector and disease control strategies are limited in resource poor communities, thus leading to an increase in the occurrence of ticks and tick-borne pathogens. With the Western Cape being a popular traveller destination with a Mediterranean weather pattern, a study focusing solely on the investigation of tick-borne diseases of dogs, particularly *Babesia* and *Ehrlichia* spp. is very appropriate.

The increase in tick infestations due to the above mentioned factors makes the occurrence of ticks more difficult to manage, thereby increasing the spread of the parasites they transmit.

1.4 Tick-borne diseases of dogs

1.4.1 Canine babesiosis

Babesiosis is caused by infection with intra-erythrocytic parasites (sporozoites) of the *Babesia* genus. Babesiosis, caused by the *Babesia* organism, was originally recognised in cattle in 1888 by Romanian biologist Victor Babes, and later, in sheep (Babes 1888; Schoeman 2009; Uilenberg 2006).

Parasites causing babesiosis are some of the most ubiquitous blood parasites considered world-wide (Homer et al. 2000). More than 100 *Babesia* species have been identified (Homer et al. 2000), which infect many vertebrate hosts, including dogs, birds and rodents. Besides *B. rossi* and *B. vogeli*, the well documented *Babesia* species include *B. microti*, *B. bovis*, *B. bigemina*, *B. caballi*, *B. canis*, *B. divergens* and *B. gibsoni*.

The piroplasms (sporozoites) of *Babesia* are intra-erythrocytic and pear shaped, usually appearing in pairs. They are loosely thrown into groups termed the ‘large *Babesias*’ and the ‘small *Babesias*’, based on the size of their piroplasms (Simões et al. 2011). After infection, the piroplasms multiply in the vertebrate host’s erythrocytes (Homer et al. 2000).

In order to transfer a successful infection, the *Babesial* parasites require both a competent vertebrate and invertebrate host. The success of the *Babesia* sp. is based upon the survival of both hosts. All *Babesial* parasites described to date are transmitted by the invertebrate Ixodid tick-vectors (Homer et al. 2000). Briefly, transmission follows a pattern as illustrated by this life cycle (Fig 1).

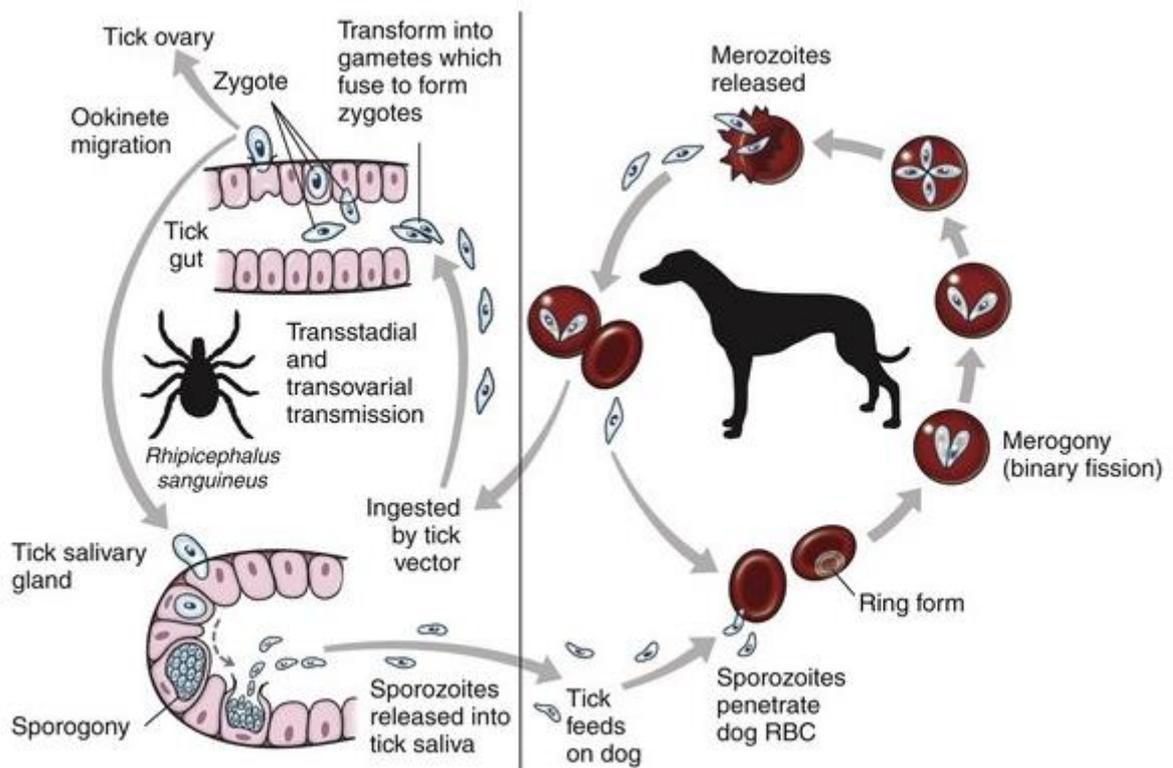


Figure 1 Life cycle of *Babesia canis* (Birkenheuer 2016)

Sporozoites are released from the tick salivary glands, and as they feed enter the bloodstream of the vertebrate host. The sporozoites attach to erythrocytes in the host. Asexual reproduction (merogony) occurs and the daughter cells infect uninfected erythrocytes. An uninfected tick then feeds on the vertebrate host, ingests infected erythrocytes, and becomes infected. The merozoites transform into the gametocyte. In the tick, the sexual reproduction phase occurs when the gametocytes fuse to form the zygote. The zygote invades epithelial cells of the tick gut. Ookinetes migrate from the epithelial cells and invade the salivary gland or the ovary of the tick. Sporogony occurs in the salivary gland. Transstadial or transovarial transmission then occurs (Birkenheuer 2016).

1.4.1.1 *Distribution of canine babesiosis*

Canine babesiosis has been reported widely almost everywhere tick vectors are found. Causative agents of canine babesiosis include *B. canis*, *B. vogeli*, *B. rossi* and *B. gibsoni*. The large *Babesias* (4-5 μm) are *B. canis* found in Europe and Asia, *B. vogeli* distributed in the United States, Europe and Africa, and *B. rossi*, the most virulent of the group predominantly found in northern and southern Africa (Matjila et al. 2008a; Furlanello et al. 2005; Böhm et al. 2006; Penzhorn 2011). These three species are morphologically indistinguishable from each other and further investigation is required to confirm their presence in the blood of the animal (Furlanello et al. 2005).

The small piroplasms are morphologically distinguishable from the large *Babesias* but not from other 'small piroplasms' of dogs. *Babesia gibsoni* piroplasms are considered the main small *Babesias* of dogs (Kjemtrup et al. 2000; Matjila et al. 2008a; Simões et al. 2011), occurring in the Middle East, Southern Asia, Japan, North America, South America, (Schoeman 2009).

Three genetically distinct small piroplasms have been identified in dogs in the USA (Californian), Spain and Japan (Asian) (Kjemtrup et al. 2000). The USA (Californian) isolate named *Babesia conradae* is part of the clade containing western piroplasms (Kjemtrup and Conrad 2006). The *B. microti*-type Spanish isolate, *Babesia vulpes* sp., previously

known as *Theileria annae* is part of the *B. microti* clade (Kjemtrup and Conrad 2006); Matjila et al. 2007; Baneth et al. 2015). The Japanese isolate, *B. gibsoni* from Asia and the Midwestern US is part of the *Babesia sensu stricto* clade (Kjemtrup and Conrad 2006).

Canine babesiosis is an important disease in dogs in South Africa. *Babesia rossi*, transmitted by *H. elliptica* ticks and *B. vogeli* transmitted by *R. sanguineus* ticks have both been identified, and are considered endemic in South Africa (Matjila et al. 2004; Matjila et al. 2008a).

Canine babesiosis in South Africa is most often caused by the virulent *B. rossi* organism. This pathogen frequently causes life-threatening disease in dogs even after treatment (Matjila et al. 2004). An observational study was conducted in 2005, where 100 dogs were naturally infected with *B. rossi*. It was determined that a high parasitaemia is significantly associated with mortality in *B. rossi* infections in dogs (Böhm et al. 2006). *Babesia vogeli* infections are endemic in South Africa, have a low parasitaemia in dogs and cause a moderate, subclinical infection (Matjila et al. 2004; Schoeman 2009).

Between 1988 and 1993, an annual average of 1170 dogs were positively diagnosed at Onderstepoort Veterinary Academic Hospital's (OVAH) in South Africa (Böhm et al. 2006). Another large babesiosis study performed at the OVAH indicated a mortality rate of between 12 and 15% in infected dogs (Böhm et al. 2006).

1.4.1.2 Pathogenesis of canine babesiosis

Canine babesiosis, also referred to as malignant jaundice or bilious fever (Homer et al. 2000; Jacobson 2006; Penzhorn 2011), has different clinical stages which are known as the peracute, acute and chronic stages. The peracute stage of the disease is immediate and violent. The acute stage of the disease has a sudden onset of clinical signs. The chronic stage is persistent with longer lasting clinical syndrome. Most canines with babesiosis develop a varying degree of haemolytic anaemia (which is not proportional to the amount of parasites present), normochromia, normocytosis, non-regenerative anaemia, possible thrombocytopenia, apathy, dehydration, fever, decreased appetite,

pallor, pigmenturia, jaundice, splenomegaly, hypoxia, weakness and possible collapse (Jacobson 2006; Irwin 2010; Furlanello et al. 2005). Immune status and secondary infections compound the illness and the age of the animal plays a role as well. For example, in puppies, *B. vogeli* causes a severe infection, whereas in adult dogs the infection is mild (Irwin 2010; Penzhorn 2011). The species of piroplasm responsible for causing the disease determines to what extent the animal gets sick. For example, *B. rossi*, causes acute and peracute disease, and is considered the most detrimental infection of the *Babesia*, whereas *B. vogeli* causes a more chronic disease in adult dogs. Severe manifestations of a *B. rossi* infection may include hypoglycaemia, acute respiratory distress, shock and single or even multiple organ failure with possible mortality which occurs in 12% of cases (Jacobson 2006; Penzhorn 2011). Despite successful treatment plans, most infected dogs will become carriers for life. Clinical signs may be absent most of the time, but may flare up during stressful periods or if the dog has been immunocompromised (Irwin 2010).

In some instances, an acute case of infection, such as that caused by *B. rossi*, may clear the symptoms without treatment, or remain sub-clinical for many months or years (Schoeman 2009). A sub-clinical infection may be asymptomatic, or become chronic with the dog suffering ongoing poor health such as chronic anaemia or thrombocytopaenia (Irwin 2010).

1.4.1.3 *Detection of canine babesiosis*

The urgent diagnostic capability of babesiosis is imperative. There are a number of tests used to detect *Babesia* including light microscopy, serological testing and molecular testing.

Microscopic detection is quick and inexpensive for on-site diagnosis of acute disease. Microscopic examination is performed on a capillary smear or an ear prick smear. The capillary smear is favoured over a venous blood smear because the capillary has a significantly higher parasitaemia (Böse et al. 1995; Böhm et al. 2006). Slides can be stained with Giemsa or a differential quick-type stain using eosin and methylene blue.

Thin smears should be properly prepared as the quality of the slide influences the artifacts seen. Artifacts such as stain deposits and debris can look very similar to tick-borne pathogens, so this differentiation is important. The observation of the piroplasm is a definite positive result; however, the parasites disappear from the peripheral blood on day ten post bite as determined by a touch-down PCR assay (Sobczyk et al. 2005). Consequently, parasites are not always easily detected by light microscopy, hence more sensitive molecular and serological assays such as Polymerase Chain Reaction (PCR) and Immunofluorescent Assay (IFA), respectively, can be used. Studies have shown that PCR and IFA offer better detection rates than light microscopy (Böse et al. 1995; Birkenheuer et al. 2003).

Serology refers to the diagnostic identification of antibodies in the serum formed in response to infection or disease. According to Böse et al., (1995), the IFA test method is the most widely used. In addition to the IFA, other serological tests that are available for babesiosis diagnosis are the complement fixation test (CFT); enzyme linked immunosorbent assay (ELISA) and rapid agglutination test (RAT). Improvements to the ELISA test method have been limited according to Böse et al., (1995), due to the poor quality of the antigen preparations (infected erythrocyte antigen preparations). This can be overcome, however, with the production of recombinant antigens (Böse et al. 1995). Most crude preparations using contaminated host erythrocytes lead to false positives (Böse et al. 1995). ELISA test methods using recombinant DNA technology can be used to avoid the false positives mentioned above. These recombinant antigens are not contaminated by host cells and are cheap to manufacture.

These serological tests are not sensitive enough to detect all subclinical and chronic cases as the titres may be negligible. Serology methods are also limited in differentiating between current infection and previous exposure to the parasite (Birkenheuer et al. 2003).

In molecular testing, the PCR is the gold standard test for detection of *Babesia* parasites (Irwin 2009). Repeated amplification of a specific DNA sequence in the target organism guarantees an easily detectable result. The sensitivity of the standard PCR can be

increased using a nested PCR and Real-time PCR (qPCR) is also available (Böse et al. 1995). PCR testing is used to differentiate between different parasite species and, coupled with hybridization techniques such as the Reverse Line Blot (RLB) hybridization test, is able to detect multiple infections in a blood sample (Gubbels et al. 1999). The ribosomal RNA genes 18S, 5.8S, and 28S have frequently been targeted by PCR methods. The high specificity and sensitivity of PCR applications makes this test a useful tool in validating results from other methods.

1.4.2 Canine ehrlichiosis

Ehrlichiosis caused by the *Ehrlichia* organism, was originally classified as a *Rickettsia*. *Rickettsia ruminantium*, discovered in 1925 in South Africa (Cowdry 1925), was the first of what we now know today as *Ehrlichia*. *Ehrlichia* organisms, sometimes referred to as inclusion bodies, are gram negative bacteria that live within morulae in the cytoplasm of leukocytes 5-7 days post infection (Harrus et al. 1997). *Ehrlichia canis* is the aetiological agent of canine monocytic ehrlichiosis (Harrus et al. 1997; McBride et al. 1999; Kelly 2000; Tsachev 2008). The primary vector of *E. canis* is *R. sanguineus*. The association of *E. canis* with haemorrhagic disease in dogs was first described in 1969 (McBride et al. 1996).

Canine ehrlichiosis is a common disease in tropical and subtropical regions (Allsopp and Allsopp 2001), and was first demonstrated in 1935 in Algeria by Donatien and Lestoquard (Donatien and Lestoquard 1935). Soon after, it was discovered in the Middle East and then again in 1938 in Southern Africa (Lawrence 1938). *Ehrlichia canis* was first recognized in the United States in 1962 (Rikihisa 1991). Ehrlichiosis made history in an outbreak during the Vietnam War (1955-1975) when hundreds of US military dogs were lost to this disease (McBride et al. 1999). *Ehrlichia canis* now has a worldwide distribution (Kelly 2000; Rikihisa 1991), including the United States, most African countries, Europe, Southeast Asia, and India (McBride et al. 1996).

Based on the characteristics of the 16S rRNA gene sequences, the *Ehrlichia*'s are divided into three genogroups; *Neorickettsia helminthoeca*, *N. elokominicia* and *Ehrlichia risticii* (Potomac horse fever), now *Neorickettsia risticii*, make up the first group. *Ehrlichia equi*,

Human granulocytic *Ehrlichia*, *E. phagocytophila*, now *Anaplasma phagocytophilum*, and *E. platys*, now *Anaplasma platys*, make up the second. Finally, *E. canis*, *E. chaffeensis*, *E. ewingii* and *E. ruminantium* (Cowdria) make up the third (Kelly 2000; Popov et al. 1998). According to Kelly (2000), there are at least nine *Ehrlichia* species that may infect dogs, including *E. chaffeensis*, *E. ewingii*, *E. phagocytophila* and *E. platys* (McBride et al. 2001). Of these nine, only *Cowdria Ruminantium*, *Ehrlichia chaffeensis* and *Ehrlichia canis* are known to occur in South Africa (Pretorius and Kelly 1998; Kelly 2000).

Transmission follows a pattern as illustrated by this life cycle in figure 2. Ticks become infected when taking a blood meal from an infected host. The *Ehrlichia* organism is known only to be transmitted transstadially (from larvae to nymph to adult) within the tick, and not transovarially. The organisms develop in the gut epithelial cells of the tick and invade the salivary glands of the vector. The vertebrate host then becomes infected when the tick vector is taking a blood meal. The *Ehrlichia* organisms enter the monocytes as elementary bodies through a process similar to phagocytosis and divide by binary fission resulting in morulae which can then be viewed under a microscope on a blood smear (Sykes 2013; Marcelino et al. 2012).

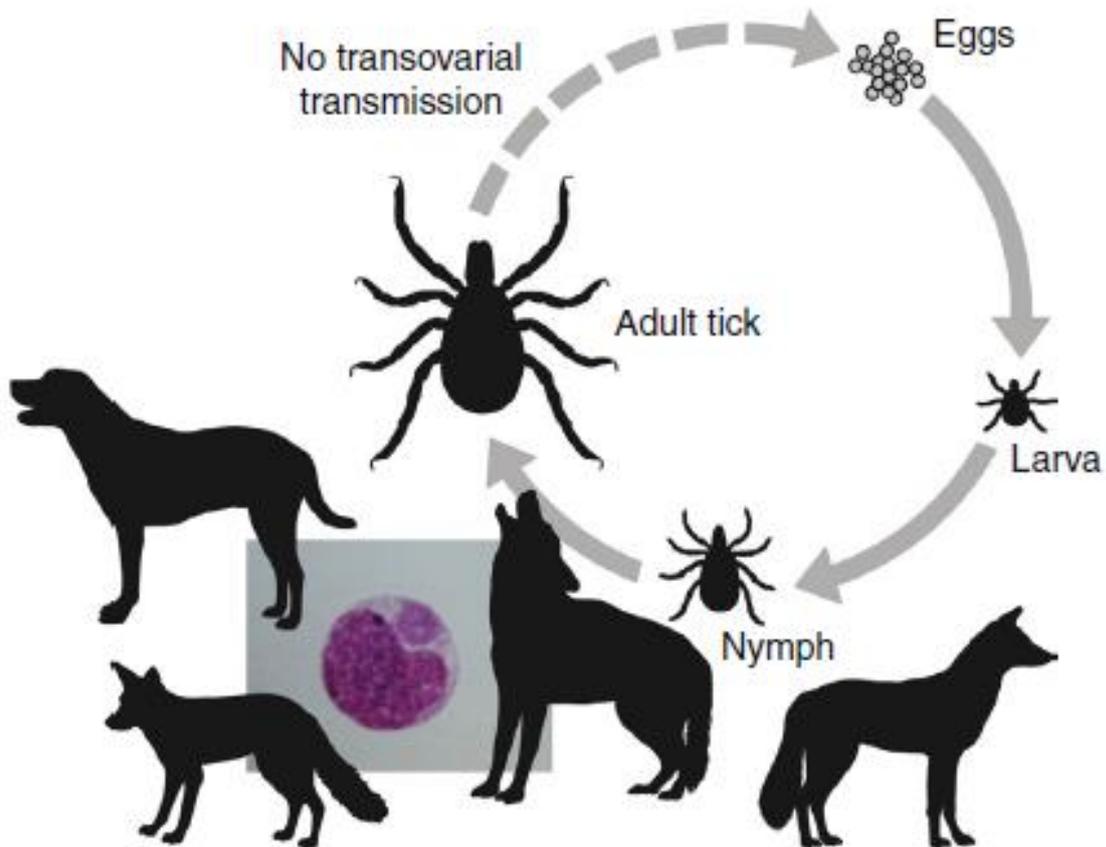


Figure 2 Life cycle of *Ehrlichia canis* (Sykes 2013)

1.4.2.1 Distribution of canine ehrlichiosis

Canine ehrlichiosis has world-wide distribution wherever infected tick-vectors are found (Harrus and Waner 2011). There seems to be considerable inconsistency in the type and severity of clinical and laboratory findings in dogs infected with *E. canis* in South Africa (Neitz and Thomas 1938; Kelly 2000), as well as other parts of the world (Hegarty et al. 1997; Kelly 2000). There is growing evidence suggesting antigenic diversity/strain variation amongst *E. canis* organisms around the world (Hegarty et al. 1997).

In a study done in Bloemfontein, South Africa, 161 dogs were serologically tested for the presence of antibodies reactive against *E. chaffeensis* and *E. canis*. Seven of the dogs had higher titres for *E. chaffeensis* than for *E. canis*. Sixty eight (42%) of the dogs had antibody titres against *E. canis* and 61 (38%) had titres against *E. chaffeensis*. It is evident

that the *E. chaffeensis* infections are rising in South Africa. This provides further evidence that the agent of human monocytic ehrlichiosis occurs in Africa (Pretorius and Kelly 1998).

1.4.2.2 *Pathogenesis of canine ehrlichiosis*

Ehrlichiosis is divided into acute, subclinical, and chronic disease phases (Breitschwerdt et al. 1998b; Harrus and Waner 2011; Iqbal et al. 1994). After an incubation period of about three weeks, the dog will enter the acute stage with the characteristic hallmark of thrombocytopenia which is said to be due to an immune-mediated mechanism. Normochromia, normocytosis, non-regenerative anaemia (similar to babesiosis), depression, high fever, lethargy, anorexia and a leukopenia, which is proposed to be due to an immune-mediated mechanism, may also occur (Breitschwerdt et al. 1998b; Waner 2008). Sometimes, the acute disease may have haemorrhagic tendencies, usually exhibited by dermal petechiae, eye lesions and retinal haemorrhage. Sub retinal haemorrhage and retinal detachment leading to blindness may also occur. Neurological clinical signs, including ataxia, paresis, seizures, altered consciousness and muscle tremor may be evident in severe cases (Breitschwerdt et al. 1998b; Harrus and Waner 2011; Iqbal et al. 1994).

When dogs suffering from ehrlichiosis enter the subclinical phase, with or without treatment, the parasite continues to multiply. The dog may seem healthy but underlying haematopathological changes may still be present. These include thrombocytopenia with enlarged platelets present, leukopenia and neutropenia, with a drop in the red cell parameters. These dogs may remain subclinical persistent carriers (Breitschwerdt et al. 1998b; Harrus et al. 1997).

The chronic stage is evident in many of the acute clinical signs and may lead to pancytopenia with a drop in all full blood count parameters. A severe case of this disease results in bone marrow hypoplasia or bone marrow suppression. In such instances, animals usually die due to secondary infection and possible bleeding. They don't respond to the usual treatment (Breitschwerdt et al. 1998b; Harrus et al. 1997; Iqbal et al. 1994).

1.4.2.3 Detection of canine ehrlichiosis

Diagnosis is challenging due to this disease having multiple clinical manifestations. Canine Monocytic Ehrlichiosis (CME) can be diagnosed using various techniques that include isolation of organisms from whole blood in tissue-culture, light microscopy, haematology, serological testing that includes the indirect fluorescent antibody test (IFAT) and molecular detection using PCR (Iqbal et al. 1994). A definitive result requires positive confirmation of a morulae, which is a vacuole full of densely packed bacteria present in the monocyte, which can be observed on a thin blood smear or a positive PCR (Harrus and Waner 2011).

Ehrlichia canis can be isolated and grown in vitro, however sensitive and reliable this approach is, it is impractical and time consuming, taking up to 10 weeks. The isolation and growth of *E. canis* is commonly used for research purposes rather than for actual diagnosis (Breitschwerdt et al. 1998b; Iqbal et al. 1994; Kelly 2000). Iqbal et al., (1994) found that tissue culture appeared to be slightly more sensitive than PCR for *E. canis* detection, particularly during the acute stage of infection (Iqbal et al. 1994).

A blood smear evaluation may detect *Ehrlichia* morulae, however, this procedure is time consuming as parasite numbers may remain low, even during the acute disease, thus being difficult to detect. This was illustrated in a study performed with 221 dogs infected with *E. canis*; morulae were observed in only 4% of the blood smears examined (Waner 2008). A buffy coat smear could also be prepared and a slide made for light microscopy. This condenses the white cells for easier focus. This is a highly sensitive method with a 66% success rate (Mylonakis et al. 2003). These types of blood smear evaluations may also detect co-infections of different pathogens, for example *Babesia*, which may influence the treatment plans. A wide range of clinical signs makes diagnosis and clinical management difficult.

An accurate thrombocyte count should be determined during a full blood count. This can be used as a screening test for CME (Canine Monocytic Ehrlichiosis) in endemic areas (Breitschwerdt et al. 1998b; Harrus and Waner 2011). This, however, is not definitive as other diseases can also present with a thrombocytopenia.

The common serological methods include IFA which tests for IgG antibodies in a serum sample and an ELISA which uses an antigen coated plate to find and identify antibodies (Iqbal et al. 1994). The ELISA's have limited use due to the antigen detection variability soon after infection. IFA is widely used and is a sensitive method, but due to possible incorrect handling, false negative results may occur easily (Iqbal et al. 1994). The IFA method is also not able to differentiate between the *Ehrlichia* species (eg: *E. canis*, *Ehrlichia ewingii*, *E. chaffeensis* and *E. ruminantium*) (Harrus and Waner 2011). The antibodies IgG and IgM only develop after more than a week post infection therefore the infection may not be detected if the test is run too early (Iqbal et al. 1994; Harrus and Waner 2011).

PCR is by far the most sensitive, accurate and effective gold standard diagnostic test for diagnosing *E. canis* (Iqbal et al. 1994; Breitschwerdt et al. 1998b; McBride et al. 1996; Harrus and Waner 2011). There seems to be a good correlation between the results obtained from organism isolation and the PCR method (Breitschwerdt et al. 1998b). It is quick, sensitive, and specific. PCR detects DNA, and not antibodies as with serology, indicating a current infection rather than possible exposure (Harrus and Waner 2011).

A Reverse line blot is also a commonly used analytical technique used in conjunction with PCR. It is used to detect and distinguish between multiple pathogens in an infection after amplification has taken place (Gubbels et al. 1999).

Quantitative real-time PCR (qPCR) is more sensitive than conventional PCR. The bacterial load may be quantified. Contaminations are less likely to occur than with conventional PCR. Real time PCR is quickly becoming the method of choice for the diagnosis of *E. canis* (Harrus and Waner 2011). More than one pathogen per sample may be detected simultaneously using real-time PCR.

1.5 Other tick-borne pathogens infecting dogs

1.5.1 *Hepatozoon* species

Hepatozoon species are found in many 'free ranging' animals including lions, leopards, domestic and wild dogs (Brockelsby and Vidler 1963; McCully et al. 1975; Matjila et al. 2008b). There are two species of *Hepatozoon* that are currently known to infect dogs; *Hepatozoon canis* occurring on all continents, and *Hepatozoon americanum* prevalent in parts of the USA (Baneth 2011).

Hepatozoon canis is a protozoa transmitted by *R. sanguineus*, causing hepatozoonosis in dogs. Ingestion of the infected tick by the animal results in infection (McCully et al. 1975). The occurrence of hepatozoonosis is closely related to the geographical distribution of its vector, *R. sanguineus*, which is found worldwide (Baneth 2011). Hepatozoonosis causes lethargy, anorexia, weight loss, severe anaemia, leukocytosis and thrombocytopaenia in infected animals (Baneth et al. 1995).

A study performed on blood samples collected from wild dogs from the Kruger national Park, showed that 26 out of 29 (93%) of the blood smears examined during this study were positive for *Hepatozoon* gametocytes, presumed to be *Hepatozoon canis* (Van Heerden et al. 1995). Another study performed in the Serengeti showed that 81.5% of wild dogs were positive for *H. canis* (Peirce et al. 1995). *Hepatozoon canis* is however non-pathogenic in healthy domestic dogs.

1.5.2 *Anaplasma* species

Canine anaplasmosis is caused by *Anaplasma platys* and *Anaplasma phagocytophilum* (Pinyoowong et al. 2008). *Anaplasma platys* is distributed globally, and it is generally assumed that all strains mainly exploit canines and rhipicephaline ticks, as vertebrate and invertebrate hosts in their life cycles (Pinyoowong et al. 2008). *Anaplasma platys* causes infectious canine cyclic thrombocytopaenia (CCT) (Pinyoowong et al. 2008). Clinical signs include bleeding disorders, cyclical fever as well as lymphadenopathy (Pinyoowong et al. 2008).

Anaplasma phagocytophilum is the causative agent of canine and human granulocytic anaplasmosis (Inokuma et al. 2005). This zoonotic parasite may cause acute or subclinical disease with symptoms that include anorexia, fever, lethargy, central nervous system dysfunction and lameness in the animal host. *Anaplasma phagocytophilum* appears to cause a less severe disease than *E. canis* (Lester et al. 2005).

1.5.3 Ehrlichia species

Ehrlichia ruminantium, formerly *Cowdria ruminantium* has been isolated in dogs, however, this parasite normally affects ruminants and the disease caused is known as heartwater or cowdriosis in cattle, sheep, goats and other ruminants. This disease occurs throughout sub-Saharan Africa as well as other parts of the world. *Ehrlichia ruminantium* is usually transmitted by the *Amblyomma* tick (Allsopp 2009). This parasite is becoming more common in dogs, especially in dogs that are allowed to roam freely with ruminants. Apparently healthy dogs may be carriers of *Ehrlichia ruminantium* (Allsopp and Allsopp 2001).

1.5.4 Theileria species

Species of the genus *Theileria* form a large group which are closely related to *Babesia*. These parasites mainly affect cattle and other ruminants. One of the economically important species is *Theileria parva* which is associated with serious diseases of cattle that include East Coast fever, Corridor disease and January disease (Muhanguzi et al. 2014). Other members of the genus that have been detected in dogs include *T. taurotragi*, *T. annulata*, (Criado et al. 2006) and *T. equi* (Criado-Fornelio et al. 2003). *Theileria* infections may cause acute or chronic disease in dogs (Slodki et al. 2001; Simões et al. 2011).

There are several species of *Theileria* that are non-pathogenic, and up until 2008, there had been no reports of pathogenic *Theileria* species identified from dogs (Matjila et al. 2008a). Matjila et al., (2008a) reported the detection of *Theileria* by PCR from a dog blood sample that was collected in 2004, from Pietermaritzburg. *Theileria* DNA was later identified in blood samples collected from dogs at OVAH in Pretoria in 2005 (Matjila et al.

2008a). However, recent evidence (Dixit et al. 2010) shows that *Theileria* may indeed be pathogenic in dogs. As mentioned previously, it was originally presumed that the only small *Babesia* organism affecting dogs was *Babesia gibsoni*. Recent research shows that a small piroplasm, genotypically and phenotypically different to *B. gibsoni* has been identified. This small piroplasm has been named *Babesia conradae* (Kjemtrup et al. 2006).

Using molecular characterization, other small piroplasms have been identified in dogs. Genotypic characterization was performed on small piroplasms found in a dog suffering from symptoms of babesiosis (Zahler et al. 2000). This molecular characterization proved that this piroplasm was only distantly related to other known genetically characterized small piroplasms, including *B. gibsoni*. This piroplasm was more closely related to *B. microti*, *B. rodhaini*, and *Theileria equi*, and named *Theileria annae* (Zahler et al. 2000). *Theileria annae* has been reclassified as *Babesia vulpes* sp. nov. (Baneth et al. 2015). Thrombocytopenia and regenerative anaemia were found to be linked to *Babesia vulpes* sp. nov. It is also suggested that kidney failure, anaemia (possibly haemolytic), splenomegaly, and a possible immune-mediated disease may be associated with these organisms (Dixit et al. 2010; Baneth et al. 2015).

1.6 Mixed infections

Mixed infections may occur when the same tick-vector serves as a reservoir for more than one pathogen (Tsachev et al. 2008), or if there is more than one type of tick-vector present on the host carrying different diseases (Kordick et al. 1999).

A high degree of co-infection has been documented in humans as well as canines (Kordick et al. 1999). Kordick et al reported that, PCR tests showed that both dogs and humans can be co-infected with various *Ehrlichia*, *Bartonella*, *Rickettsia* and *Babesia* species. Positive PCR results for any *Ehrlichia* sp. were concurrently infected with *B. canis*. 'All dogs had evidence of infection with organisms from at least two genera and three dogs had evidence of infection with at least seven different species' (Kordick et al. 1999). It

was concluded, in this study, that 'dogs with heavy tick exposure can be infected at a high rate with multiple, potentially zoonotic tick-borne pathogens'.

A different study performed at the Veterinary Teaching hospital, also in North Carolina demonstrated coinfection of different *Ehrlichia* species in dogs (Breitschwerdt et al. 1998a). PCR analysis showed that the dogs were in fact, infected with four *Ehrlichia* species namely *E. canis*, *E. chaffeensis*, *E. equi* and *E. ewingii*. In one of the dogs tested, a mixed infection with three *Ehrlichia* species (*E. canis*, *E. ewingii*, and *E. equi*) was described.

In South Africa, dogs frequently become infected with a mixed infection of *E. canis* and *B. rossi*, referred to as *Babesia canis* in the manuscript of Allsopp and Allsopp 2001. It is a common occurrence to find this co-infection of *E. canis* and *B. rossi* in South Africa as the tick-vectors, *R. sanguineus* and *H. elliptica* have overlapping distributions (Matjila et al. 2008a).

1.7 Study justification

Globally, ticks are important vectors of tick-borne pathogens, transmitting a wide variety of infectious diseases in both humans and animals. Ticks are finding new niches and becoming established in non-endemic regions. The spread of these vectors and their pathogens to different zoogeographical habitats by various means, including traveling pets, has become common. Without strict animal import control methods, the risk of establishment of non-endemic vectors and their pathogens in South Africa is increased.

Both *Haemaphysalis elliptica* and *Rhipicephalus sanguineus* are vectors of tick-borne pathogens and are endemic in South Africa. These ticks have overlapping distributions in South Africa, and can also be found in mixed infestations on the same host. The most important tick-borne pathogens of dogs causing severe clinical illness are *Babesia* and *Ehrlichia*. Ehrlichiosis and babesiosis are endemic in South Africa. The distribution of *H. elliptica* coincides with the occurrence of *B. rossi* infections, and similarly, the

distribution of *R. sanguineus* coincides with the occurrence of *E. canis* infections in dogs in South Africa.

Due to the abundant amounts of different tick species in these endemic areas, the use of diagnostic techniques ensures that the correct treatment plan for the specific pathogen is implemented. Molecular or alternatively serological testing is imperative to ensure correct treatment strategy.

With Cape Town being a popular traveller destination, and the constant movement of dogs into Cape Town, a study focusing on the investigation of tick-borne pathogens of dogs, particularly *Babesia* and *Ehrlichia*, in selected areas of the Cape Town region was proposed.

1.8 Aims and objectives

This study gives comprehension to the occurrence of tick-borne pathogens in dogs in resource-poor communities, in Cape Town. The aim was to screen for the presence of tick-borne pathogens from blood and tick samples collected from dogs in four welfare organisations and two townships in Cape Town, South Africa.

The specific objectives of this study were:

- To screen blood samples collected from dogs for the presence of tick-borne pathogens using PCR and the Reverse Line Blot (RLB) hybridization assay.
- To collect and macroscopically identify ticks infesting sampled dogs.
- To screen sampled ticks for the presence of tick-borne pathogens with PCR and the reverse line blot (RLB) hybridization assay.
- To determine the most common tick-borne pathogen in the selected welfare organisations and townships.

Chapter 2

MATERIALS AND METHODS

2.1 Sample collection areas

Samples were collected from 126 dogs from six resource-poor areas, consisting of four welfare organizations (n=83) and two townships (n=43), in the greater Cape Town region (Figure 3). Blood and tick samples were collected from the same dogs. The welfare organisations included the Animal Anti Cruelty League welfare organisations in Epping (n=18), in Bellville (n=10), Lucky Lucy Foundation in Joostenberg Vlakte (n=25), and the Emma Animal Rescue Society (TEARS), located in the Sunnydale area (n=30). The two neighbouring townships are the Asanda village (n=10) and Nomzamo (n=33), both located just outside of the Cape Town suburb of the Strand. During the study it could not be established whether the sample dogs have ever been treated for ectoparasite infestations or tick-borne pathogens.

2.2 Blood sample collection

A total of 126 whole blood samples were collected in EDTA (Ethylenediaminetetraacetic acid) anti-coagulated tubes, from apparently healthy dogs, disregarding the sex of the animal or the presence of ticks. Peripheral blood from the cephalic vein of each dog was collected into a 4 ml EDTA tube. The blood samples originating from TEARS and the Animal Anti Cruelty League, in Epping and Bellville branches, were drawn from dogs by a qualified veterinarian. At the Animal Anti Cruelty League, blood samples were taken during outreach spays and neuters. A qualified veterinary nurse was responsible for the blood collection at Lucky Lucy Foundation and the Asanda and Nomzamo townships.

2.3 Ectoparasite collection and identification

Tick collection was conducted on the same dogs as the blood sample collections. Tick collection was done from all welfare organisations with the exception of the Lucky Lucy

Foundation, where dogs were regularly dipped. Although blood sampling records were kept of dogs sampled this was not done for tick collection as not all dogs had ticks on them during the sampling periods. Ticks were collected from the whole body including the face and ears and pooled according to locality sampled (Table 2). The ticks were stored in 70% ethanol for further identification and DNA extraction, followed by PCR and Reverse line blot (RLB) hybridization assay. Tick identification was performed by Professor IG Horak from the Department of Veterinary Tropical Diseases, University of Pretoria at Onderstepoort in Pretoria.

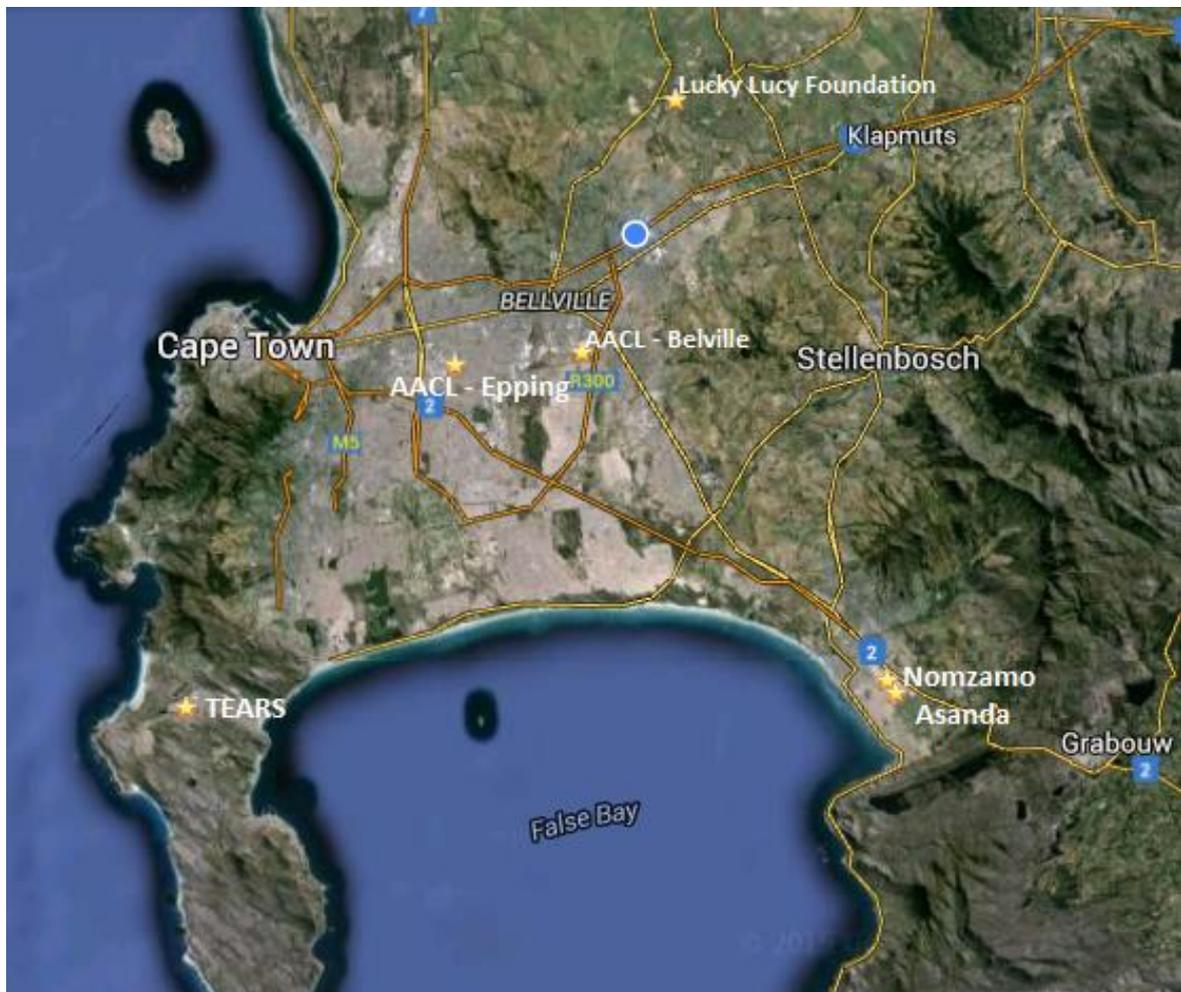


Figure 3 Map of the Greater Cape Town region indicating the six sampled localities

2.4 Blood DNA extraction

DNA was extracted from 200 µl of each of the 126 EDTA anti-coagulated whole blood samples. Total genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Southern Cross Biotechnologies, South Africa) according to the manufacturer's protocol.

Twenty microliters (20 µl) of Proteinase K (20 mg/ml) was added to 200 µl of anti-coagulated whole blood in a 1.5 ml micro-centrifuge tube. Two hundred microliters (200 µl) of Buffer AL was added and the sample was vortexed to ensure complete mixing for lysis of blood cells. The specimens were then incubated for 10 minutes at 56°C, and briefly centrifuged to remove droplets from inside the lid. Two hundred microliters (200 µl) of absolute ethanol was added. The tubes were vortexed again to ensure complete mixing, and then centrifuged for three seconds to ensure that no solution remained in the lid.

The entire mixture of sample from the micro centrifuge tube was transferred to a new QIAamp Spin Column in a clean two ml collection tube. The column was closed and centrifuged at 8000 rpm. Five hundred microliters (500 µl) of Buffer AW1 was added to the column and centrifuged at 8000 rpm for one minute.

The QIAamp Spin Column was then placed into a fresh 2 ml collection tube. Five hundred microliters (500 µl) Buffer AW2 was added to the Spin Column and centrifuged at a full speed of 14000 rpm for three minutes. The QIAamp Spin Column was placed into a new 2 ml collection tube, and the filtrate was discarded. The sample was centrifuged again at full speed of 14000 rpm for one minute to completely remove the buffers.

The QIAamp Spin Column containing the DNA was placed into a fresh 1.5 ml micro-centrifuge tube, 100 µl of the AE elution Buffer was added, and the columns incubated at room temperature (15-25°C) for one minute. To complete the elution process, the sample was centrifuged for an additional one minute at 8000 rpm. Two hundred microliters (200 µl) of whole blood yielded between 3 µg and 12 µg of DNA.

2.5 Tick-DNA extraction

The 509 ticks were identified to species level prior to DNA extraction. The ticks from each locality were equally divided into tick pools (Table 2). Since only two *H. elliptica* from Asanda and one *R. sanguineus* from TEARS were collected, these ticks were mixed in pools of the dominant species as they were too few for DNA extraction. The ticks were washed in distilled water then dried. Fifteen MagNA Lyser Green Bead tubes were marked with the specific RLB numbers. Ticks were cut into smaller pieces and transferred to each respective MagNA Lyser Green Bead tube. Three hundred micro litres (300 µl) of ATL buffer was added to each tube and the tubes were then placed on ice. To homogenize the tick material, each tube was run on the MagNA Lyser for 30 seconds at 7000 rpm. The tubes were cooled on ice before adding 20 µl of Proteinase K (20 mg/ml) for lysis at 56°C, overnight. Subsequently, 200 µl of supernatant was removed from each tube and transferred to a 2.5 ml Eppendorf tube. Two hundred microliters (200 µl) of buffer AL was then added to each tube and the solution mixed by vortex thoroughly. The samples were incubated at 70°C for 10 minutes. Two hundred microliters (200 µl) of ethanol was added to each sample, and then vortexed.

Each mixture was transferred into its respective QIAamp mini column marked with the corresponding RLB number. Subsequently the columns were centrifuged at 8000 rpm for one minute and the flow through discarded. Five hundred microliters (500 µl) of buffer AW1 was added to each column and the columns were centrifuged for one minute at 8000 rpm and the flow through discarded.

Five hundred microliters (500 µl) of buffer AW2 was added to each column and the columns then centrifuged for three minutes at 14000 rpm. The flow through was discarded and the columns were placed in a new collection tube for an additional step of centrifugation for one minute at 14000 rpm to remove residual AW buffer. The columns were placed in a 2.5 ml tube marked with the corresponding RLB number. One hundred microliters (100 µl) of buffer AE was added directly onto the QIAamp membrane. The sample was then incubated at room temperature for two minutes followed by centrifugation for one minute at 8000 rpm to elute the extracted DNA.

Table 2 Tick collection and identification from the five localities

Locality (n=number of dogs sampled)	Tick species		Tick pool
	<i>R. sanguineus</i>	<i>H. elliptica</i>	
Nomzamo Township (n=33)	400	0	T1 T2 T3 T4 T5
AACL-Epping (n=18)	14	0	T6
Asanda village (n=10)	42	2	T7 T8 T9
AACL-Bellville (n=10)	0	29	T10 T11 T12
TEARS (n=30)	1	21	T13 T14 T15
TOTAL	457	52	

2.6 Polymerase Chain Reaction (PCR) mastermix preparation

The PCR mastermix was prepared in a 'DNA uncontaminated' room, working in a laminar flow cabinet. Two mastermix solutions were prepared. The *Theileria/Babesia* mastermix consisted of the forward primer -RLBF2 (5'-GAC ACA GGG TAG TGA CAA G -3') and reverse primer - RLBR2 (biotin-5'-CTA AGA ATT TCA CCT CTG ACA GT -3') (Gubbels et al. 1999; Matjila et al. 2004). The *Ehrlichia/Anaplasma* mastermix included the forward primer Ehr-F (5'-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3') and reverse primer Ehr-R (biotin-5'-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3'). Additional components of the PCR mastermix are presented in Table 3. Twenty two and a half microliters (22.5 µl) of the mastermix solution was aliquotted into labelled 200 µl PCR tubes in preparation for the next step of adding the template DNA.

Table 3 Preparation of the PCR mastermix and volumes

PCR components	Volumes for PCR mastermix	
	Final concentration	Volume for one reaction (µl)*
*Platinum Quantitative PCR SuperMix-UDG	1 X	12.5
Forward primer (RLB F2) (20 pmol)	8 pmol	0.25
Reverse primer (RLB R2) (20 pmol)	8 pmol	0.25
Molecular grade water	-	9.5
TOTAL VOLUME		22.5

*Platinum Quantitative PCR SuperMix-UDG: 60 U/ml Platinum Taq DNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, six mM MgCl₂, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 400 µM dUTP, 40 U/ml UDG, and stabilizers

The primers in this PCR amplification reaction are designed for specific amplification of the rRNA gene of the target pathogen, and they are not complementary to the hosts or the ticks rRNA genes resulting in high specificity of the target pathogen in the PCR reaction. Both PCR primer sets for *Theileria/Babesia* as well as *Ehrlichia/Anaplasma* can be run simultaneously on the same PCR program. The *Theileria/Babesia* set of primers was used to amplify DNA fragments of size ranging between 460 and 520 base pairs of the 18S SSU rRNA spanning the V4 hypervariable region. The *Ehrlichia/Anaplasma* PCR amplified a fragment of 460 to 520 base pairs from the V1 hypervariable region of the 16S SSU rRNA gene (Bekker et al. 2002; Matjila et al. 2004).

2.7 Adding of template DNA

The template DNA was added in an UV cabinet in the DNA extraction laboratory. Each DNA sample (2.5 µl) was added to the 200 µl PCR tubes containing the *Theileria/Babesia* mastermix and the 200 µl PCR tubes containing the *Ehrlichia/Anaplasma* mastermix, resulting in two PCR reactions for each DNA sample. For positive control reactions *Babesia bovis* and *Anaplasma centrale* positive DNA samples were used as templates while water was used for a negative control reaction.

2.8 Polymerase Chain Reaction (PCR)

The PCR conditions were as described by Matjila et al., (2004). Touchdown PCR was used for amplification employing the following conditions:

The initial step of three minutes at 37°C was performed to activate the uracil DNA glycosylase (UDG), followed by one cycle of 10 minutes at 94°C to inactivate the UDG and activate *Taq*, and 10 cycles of 94°C for 20 seconds to denature the double stranded DNA template, 67°C for 30 seconds to anneal the primers, and 72°C for 30 seconds for extension of PCR products by *Taq* polymerase.

The annealing step temperature was lowered by 2°C after every second cycle, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds for extension of PCR products by *Taq* polymerase.

The Gene Amp®PCR System 9700 and 2700 (Applied Biosystems, South Africa) and the 2720 Thermal Cycler (Applied Biosystems, South Africa) were used for PCR amplification. After the PCR amplification, the aliquots of PCR products were placed onto ice until the next step of analysis.

2.9 Reverse Line Blot (RLB) hybridization assay

The Reverse line blot (RLB) hybridization assay was performed on the PCR products including test samples, positive controls and negative controls, as described by Gubbels et al., (1999). A blotting membrane was prepared in house containing oligonucleotide probes (Table 4). This membrane was activated by incubation at room temperature for five minutes under gentle shaking in 50 ml 2X SSPE/0.1% SDS (sodium dodecyl sulphate) in a plastic container. The membrane was washed for two minutes with distilled water, and placed in the MN45 miniblottedter (Immunelectrics, Cambridge, Massachusetts). The PCR products were denatured using a thermal cycler prior to dilution with 130 µl SSPE/0.1% SDS. The diluted PCR products were applied to the slots on the membrane in the miniblottedter, perpendicular to the direction of the species specific probes. Hybridization

occurred when there was a reaction between the PCR product and the species-specific oligonucleotide probe. Hybridization was performed in an incubator at a 42°C for 60 minutes. The membrane was washed twice with preheated SSPE/0.5% SDS at 50°C for 10 minutes to remove remaining PCR products. The membrane was further incubated with SSPE/0.5% SDS and 12.5 µl streptavidin-peroxidase labelled conjugate (Roche Diagnostics, South Africa) for 30 minutes at 42°C on a gentle rocker (Labnet Rocker 25). The membrane was washed twice in preheated SSPE/0.5% SDS at 42°C for 10 minutes on the gentle rocker to remove the conjugate. Ten millilitres (10 ml) of ECL detection solution (Perkin Elmer, U.S.A) (5 ml ECL1 + 5 ml ECL2) was added onto the membrane for one minute at room temperature. The membrane was placed between two plastic overhead sheets and secured into an x-ray cassette. In a dark room, an x-ray film was placed on top of the plastic sheet above the membrane, and closed into the cassette. An x-ray was developed in a developer and fixer solution then rinsed and dried. Detection is based on chemiluminescence as opposed to radioactivity in regular x-rays. The x-ray was read by placing the x-ray film on a grid and lanes of sample reaction were viewed against oligonucleotide probes.

After viewing the results on the x-ray film, the membrane was stripped of PCR products by washing twice with 200 ml preheated 1% SDS for 30 minutes at 80°C; under gentle shaking followed by a second wash with 200 ml 20 mM EDTA for 15 minutes at room temperature. The membrane was then sealed in a plastic container with 50 ml, 20 mM EDTA and stored at 4°C for re-use (Gubbels et al. 1999).

Table 4 Probe sequences for detection of specific parasite species

Genus/Species Target	Probe Sequence 5'-3'	Reference
<i>A. bovis</i>	CTT GCT ATG AGA AYA ATT AGT GGC	Bekker et al. 2002
<i>A. centrale</i>	TCG AAC GGA CCA TAC GC	Matjila et al. 2004
<i>A. marginale</i>	GAC CGT ATA CGC AGC TTG	Matjila et al. 2004
<i>A. phagocytophilum</i>	GRA TAR TTA GTG GCA GAC GGG T	Bekker et al. 2002
<i>Anaplasma sp. (Omatjenne)</i>	CGG ATT TTT ATC ATA GCT TGC	Matjila et al. 2004
<i>B. bicornis</i>	TTGGTAAATCGCCTTGTCG	Nijhof et al. 2003
<i>B. bigemina</i>	CGT TTT TTC CCT TTT GTT GG	Matjila et al. 2004
<i>B. bovis</i>	CAG GTT TCG CCT GTA TAA TTG AG	Matjila et al. 2004
<i>B. caballi</i>	GTG TTT ATC GCA GAC TTT TGT	Matjila et al. 2004
<i>B. canis</i>	TGC GTT GAC CGT TTG AC	Matjila et al. 2004
<i>B. divergens</i>	TGA CTA ATG TCG AGA TTG CAC TTC	Nijhof et al. 2003
<i>B. felis</i>	TTA TGC GTT TTC CGA CTG GC	Matjila et al. 2004
<i>B. gibsoni</i>	TAC TTG CCT TGT CTG GTT T	Matjila et al. 2004
<i>B. leo</i>	ATC TTG TTG CCT GCA GCT T	Penzhorn et al. 2001
<i>B. major</i>	CGCTGTGGCTTATCCTTTTAC	Georges et al. 2001
<i>B. microti</i>	GC TTG GCA TCW TCT GGA	Matjila et al. 2004
<i>B. occultans</i>	CCT CTT TTG GCC CAT CTC G	Oosthuizen et al. 2008
<i>B. rossi</i>	CGG TTT GTT GCC TTT GTG	Matjila et al. 2004
<i>Babesia sp. (sable)</i>	GCG TTG ACT TTG TGT CTT TAG C	Oosthuizen et al. 2008
<i>B. vogeli</i>	AGC GTG TTC GAG TTT GCC	Matjila et al. 2004
<i>Babesia genus-specific 1</i>	ATT AGA GTG TTT CAA GCA GAC	Matjila et al. 2004
<i>Babesia genus-specific 2</i>	ACT AGA GTG TTT CAA ACA GGC	Matjila et al. 2004
<i>E. canis</i>	TCT GGC TAT AGG AAA TTG TTA	Matjila et al. 2004
<i>E. chaffeensis</i>	ACC TTT TGG TTA TAA ATA ATT GTT	Matjila et al. 2004
<i>E. ruminantium</i>	AGT ATC TGT TAG TGG CAG	Matjila et al. 2004
<i>Ehrlichia/Anaplasma (E/A) genus-specific</i>	GGG GGA AAG ATT TAT CGC TA	Matjila et al. 2004
<i>T. annae</i>	CCG AAC GTA ATT TTA TTG ATT TG	Matjila et al. 2004
<i>T. annulata</i>	CCT CTG GGG TCT GTG CA	Matjila et al. 2004
<i>T. bicornis</i>	GCG TTG TGG CTT TTT TCT G	Matjila et al. 2004
<i>T. buffeli</i>	GGC TTA TTT CGG WTT GAT TTT	Matjila et al. 2004
<i>T. equi</i>	TTC GTT GAC TGC GYT TGG	Matjila et al. 2004
<i>T. lestoquardi</i>	ATT GCT TGT GTC CCT CCG	Schnittger et al. 2004
<i>T. mutans</i>	CTT GCG TCT CCG AAT GTT	Gubbels et al. 1999
<i>T. ovis</i>	GCA TTG CTT TTG CTC CTT TA	Matjila et al. 2004
<i>T. parva</i>	GGA CGG AGT TCG CTT TG	Matjila et al. 2004
<i>Theileria sp. (sable)</i>	GCT GCA TTG CCT TTT CTC C	Matjila et al. 2004
<i>T. separata</i>	GGT CGT GGT TTT CCT CGT	Schnittger et al. 2004
<i>Theileria sp. (buffalo)</i>	CAG ACG GAG TTT ACT ACT* TTG T	Matjila et al. 2004
<i>Theileria sp. (kudu)</i>	CTG CAT TGT TTC TTT CCT TTG	Matjila et al. 2004
<i>T. taurotragi</i>	TCT TGG CAC GTG GCT TTT	Matjila et al. 2004
<i>T. velifera</i>	CCT ATT CTC CTT TAC GAG T	Matjila et al. 2004
<i>Theileria genus-specific</i>	ATT AGA GTG CTC AAA GCA GGC	Matjila et al. 2004
<i>Theileria/Babesia (T/B) genus-specific</i>	TAA TGG TTA ATA GGA RCR GTT G	Matjila et al. 2004

Symbols indicate degenerate positions: R=A/G, W=A/T, K=G/T

Chapter 3

RESULTS

3.1 Detection of tick-borne pathogens in blood samples

Of the 126 DNA samples screened using the Reverse line blot (RLB) hybridization assays, 46 (36.5%) tested positive for the presence of tick-borne pathogen DNA. The most occurring pathogen detected in 17 (13.5%) DNA samples was *E. canis*, followed by *B. rossi* in 16 (12.7%) dogs (Figure 4). *Babesia rossi* was the only pathogen detected in samples collected from all localities where dogs were sampled (Figure 4). Other tick-borne pathogens identified include *Ehrlichia ruminantium* which was detected in six (4.8%) samples (Figure 4).

Of the 46 samples that tested positive for the presence of tick-borne pathogens, 14 samples hybridized with the *Ehrlichia/Anaplasma* (E/A) or the *Theileria/Babesia* (T/B) genus-specific probe. Ten (7.94%) samples from AACL-Epping, AACL-Belville and TEARS, hybridized to the *Ehrlichia/Anaplasma* (E/A) genus-specific probe; three of these were found in mixed infections with *Babesia rossi*. From the 46 samples, four (3.17%) samples from the Lucky Lucy Foundation tested positive for the *Theileria/Babesia* (T/B) genus-specific probe, and one of these samples was found in a mixed infection that included *Ehrlichia canis*. Of the 14 samples testing positive on the *Ehrlichia/Anaplasma* (E/A) or the *Theileria/Babesia* (T/B) genus-specific probe, 10 samples did not show reaction with any species-specific probes (Figure 4).

The highest rate of infection was found from samples collected from the Animal Anti-Cruelty League (AACL) in Epping (n=18) (Figure 4). Of the 18 DNA samples tested from this locality, 11 (61.1%) yielded a positive result for tick-borne pathogen DNA (Figure 4). This was followed by the Animal Anti Cruelty League welfare organisation in Bellville (AACL-Bellville) (50%), and the Asanda village (40%).

The most occurring pathogens detected using RLB were *E. canis* (17/46; 37%) and *B. rossi* (16/46; 35%) (Figure 4). The majority of *B. rossi* infections (8/46; 17%) were detected

from samples from TEARS while most of the *E. canis* infections were detected from Nomzamo Township (8/46; 17%). Unexpected reactions with *E. ruminantium*, *Theileria taurotragi* and *Anaplasma sp. Omatjenne* were detected from DNA samples from Lucky Lucy Foundation, AACL-Epping, Asanda village, Nomzamo Township and TEARS. The majority of *E. ruminantium* infections (3/46; 6.5%) were detected from Asanda village and *T. taurotragi* DNA was only detected in samples from AACL-Epping. One DNA sample from TEARS had a positive reaction with the *Anaplasma sp. Omatjenne* genus-specific probe (Figure 4).

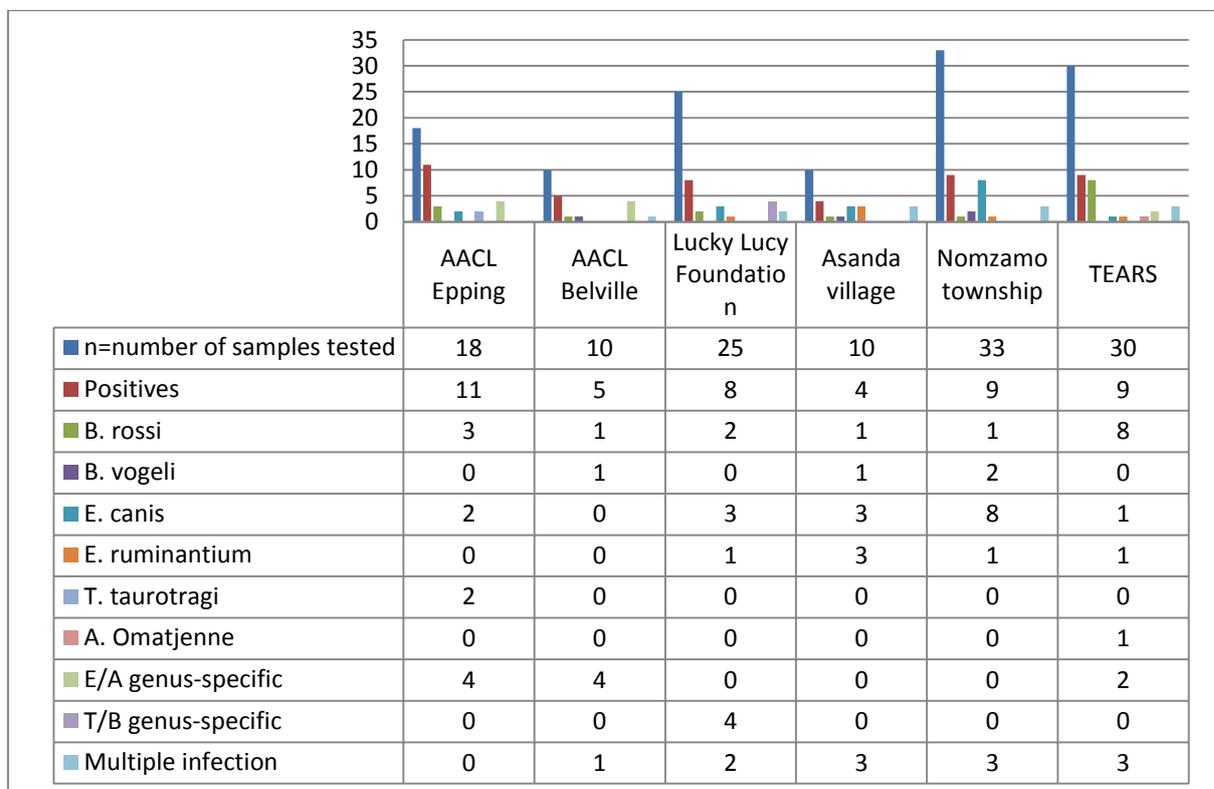


Figure 4 RLB hybridization assay results from blood samples obtained from dogs in different localities

3.2 Detection of mixed infections from blood samples

Dual and triple infections were detected from some of the samples from the different localities. Dual infections (n=10) were identified from samples from AACL Bellville (n=1), Lucky Lucy Foundation (n=2), Asanda village (n=2), Nomzamo Township (n=3) and TEARS

(n=2) (Table 5). The most common dual infection consisted of *E. ruminantium* and *E. canis* (Table 5). These tick-borne pathogens were common in the two samples with triple infections. Triple infections (n=2) were also identified from samples from Asanda village (n=1) and TEARS (n=1) (Table 5). Multiple infections were detected from samples from all localities except AACL Epping (Table 5).

Table 5 RLB hybridization assay results from mixed infections in blood samples

Organisation	n	Dual infection	Triple infection
AACL-Epping	18	0	0
AACL-Bellville	10	<i>E/A</i> genus-specific + <i>B. rossi</i> (n=1)	0
Lucky Lucy Foundation	25	<i>E. canis</i> + T/B genus-specific (n=1) <i>E. ruminantium</i> + <i>E. canis</i> (n=1)	0
Asanda village	10	<i>E. ruminantium</i> + <i>E. canis</i> (n=2)	<i>E. ruminantium</i> + <i>E. canis</i> + <i>B. vogeli</i> (n=1)
Nomzamo Township	33	<i>E. ruminantium</i> + <i>E. canis</i> (n=1) <i>E. canis</i> + <i>B. vogeli</i> (n=2)	0
TEARS	30	<i>E/A</i> genus-specific; <i>B. rossi</i> (n=2)	<i>E. canis</i> + <i>B. rossi</i> + <i>E. ruminantium</i> (n=1)
TOTAL	126	10 (7.94%)	2 (1.59%)

3.3 Tick collection and identification from the five localities

A total of 509 ticks (adults and nymphs) were collected from all localities with the exception of Lucky Lucy Foundation where regular dipping of the dogs is practiced. Only two species of ticks were identified in this study, *Rhipicephalus sanguineus* (n=457, 89.8%) and *Haemaphysalis elliptica* (n=52, 10.2%) (Table 2).

A total number of 400 ticks, all identified as *R. sanguineus* were collected from the 33 dogs from the Nomzamo Township. These ticks were randomly divided into five pools (T1-T5) for further testing. Fourteen (14) *R. sanguineus* ticks (T6) were collected from the 18 dogs sampled from the Animal Anti-Cruelty League in Epping. Both *R. sanguineus* (n=42) and *H. elliptica* ticks (n=2) were retrieved from 10 dogs from the Asanda Village.

Since only two *H. elliptica* ticks were collected from Asanda, these ticks were mixed in pools of the dominant species as they were too few for DNA extraction. These ticks were randomly divided into three tick pools (T7-9) for DNA extraction and further testing. *Haemaphysalis elliptica* (n=29) were the only tick species retrieved from the 10 dogs sampled from the AACL-Bellville. These were randomly divided into three pools (T10-T12) for this study. Both tick species were collected from 30 dogs from TEARS. One *R. sanguineus* tick as well as 21 *H. elliptica* ticks could be identified. Since only one *R. sanguineus* was collected from TEARS this tick was mixed in pools of the dominant species as they were too few for DNA extraction. These 22 ticks were randomly divided into three pools for this study (T13-T15) (Table 2).

3.4 Detection of tick-borne pathogens from ticks

A total of 15 tick pools were prepared from five localities. DNA extracted from each pool was screened for parasite DNA using the RLB hybridization assay (Table 6). *Babesia vogeli*, *B. canis* and *B. felis* were detected from the ticks from the Asanda village. Three tick pools from the AACL-Bellville containing 29 *H. elliptica* ticks were positive for the *Ehrlichia/Anaplasma* genus-specific probe only. *Babesia rossi* DNA was detected from two tick pools from TEARS. *Ehrlichia canis* was also identified from the tick pools from the Nomzamo Township. Undescribed species of *Ehrlichia* or *Anaplasma* or *Babesia* or *Theileria* were detected from all localities, as indicated by a positive reaction of some of the tick pools with *Ehrlichia/Anaplasma*, *Theileria/Babesia* and genus-specific probes (Table 6).

Table 6 Results obtained from RLB analysis of tick-DNA from dogs from different localities in Cape Town

Locality	Ticks species identified	Tick pool	Tick-DNA detected
Nomzamo Township	<i>R. sanguineus</i> (n=400)	T1	E/A genus-specific, <i>E. canis</i>
		T2	E/A genus-specific
		T3	E/A genus-specific, <i>E. canis</i> , B1 genus-specific, B2 genus-specific (vf)
		T4	No nucleic acid detected
		T5	No nucleic acid detected
AACL-Epping	<i>R. sanguineus</i> (n=14)	T6	E/A genus-specific, B1 genus-specific
Asanda village	<i>H. elliptica</i> (n=2) + <i>R. sanguineus</i> (n=42)	T7	No nucleic acid detected
		T8	E/A genus-specific, B1 genus-specific, <i>B. felis</i>
		T9	E/A genus-specific, <i>E. canis</i> , T/B genus-specific, B1 genus-specific, <i>B. vogeli</i>
AACL-Bellville	<i>H. elliptica</i> (n=29)	T10	E/A genus-specific
		T11	E/A genus-specific
		T12	E/A genus-specific
TEARS	<i>H. elliptica</i> (n=21) + <i>R. sanguineus</i> (n=1)	T13	No nucleic acid detected
		T14	E/A genus-specific, T/B genus-specific, B1 genus-specific, <i>B. rossi</i>
		T15	E/A genus-specific, T/B genus-specific, B1 genus-specific, <i>B. rossi</i>

Chapter 4

DISCUSSION

Ehrlichia canis and *B. rossi* are both clinically and economically important diseases of dogs transmitted by ticks in South Africa. The challenge with tick-borne pathogens such as these is exacerbated by the fact that in South Africa, there are many rural areas and communities that are unable to control tick-vectors.

These parasites and their vectors are endemic in South Africa and their distribution and occurrence therefore expected, more so in areas where control strategies are either limited or completely lacking such as resource poor areas or townships.

The findings of this study create an awareness of the distribution and occurrence of tick-vectors and their pathogens in resource poor areas in Cape Town. Whilst similar studies have been performed in South Africa in resource-poor communities (Bryson et al. 2000; Horak et al. 2001; Sibanda 2011), none have focused solely on *Babesia* and *Ehrlichia* occurrence.

4.1 Occurrence of tick-borne pathogens and tick species identified

The most occurring pathogen found in this study was *E. canis*. Furthermore, it was observed that the high occurrence (13.49%) of *E. canis* coincided with the occurrence of *Rhipicephalus sanguineus* which was the most occurring tick identified (Figure 4; Table 2). *Rhipicephalus sanguineus* ticks are responsible for the transmission of *E. canis* and *B. vogeli* (Tsachev et al. 2008; Waner 2008), accounting for the high occurrence of canine ehrlichiosis in dogs in this study. Seventeen dogs (13.49%) were found to be infected with *E. canis* during this investigation (Figure 4; Table 2). *Babesia vogeli* is the least virulent of the *babesia* parasites infecting dogs. It has thus been considered to have the longest association with domestic dogs (Penzhorn 2011).

The second most occurring pathogen detected was *B. rossi* while the second most occurring tick species was *Haemaphysalis elliptica* (Figure 4; Table 2). Since *B. rossi* is transmitted by *Haemaphysalis elliptica* (Irwin 2009), it would be anticipated that where *H. elliptica* is most dominant, there would be a high number of corresponding *B. rossi* pathogens. During this investigation, both *B. rossi* and *H. elliptica* were detected from dogs from all five study localities with the exception of the Lucky Lucy Foundation due to their regular dipping program. *Babesia rossi* parasitaemia has been associated with the severity of clinical signs in experimental infections (Böhm et al. 2006). *Babesia rossi* is considered to be more virulent than the other *babesia* parasites infecting dogs in South Africa. Penzhorn (2011) suggested that, from an evolutionary perspective, domesticated dogs have not had adequate time to adapt to this parasite. The implication of the occurrence of this pathogen in resource poor areas in South Africa is overwhelming. Without veterinary intervention morbidity and/or mortality caused by babesiosis is inevitable.

These results are comparable to observations of a study performed in the North West Province of South Africa by Bryson et al., (2000), which reported *R. sanguineus* as the most common vector and *Haemaphysalis* sp. as the second most common, in dogs from resource-poor communities in this province.

On the contrary, in a similar study performed in rural communities in north-eastern KwaZulu-Natal (Horak et al. 2001), it was found that the most common tick species detected in domestic dogs were *Haemaphysalis* spp. followed by immature *Amblyomma hebraeum* and adult *Rhipicephalus simus*. According to Horak et al., (2001), *R. sanguineus* is usually prevalent in dogs belonging to less affluent owners, who often live in urban or peri-urban townships; which explains the high detection of this tick species from dogs in the current study from the Western Cape area.

In this study, the tick species *R. sanguineus* and *H. elliptica* had overlapping distributions; therefore, it comes as no surprise that mixed infections of *E. canis* and *B. rossi* were detected. This result of a dual infection between these two pathogens was also obtained in a similar study (Matjila et al. 2008a).

Interestingly, none of the dogs tested in this study were infected with both *B. vogeli* and *B. rossi* contrary to what was found in results from a previous study conducted on domestic dogs in South Africa (Matjila et al. 2004). Since the tick-vectors transmitting these parasites in South Africa have an overlapping distribution, we would expect mixed infections to occur.

Another interesting finding during this study was that *E. ruminantium* was always found as a co-infection with *E. canis* (Table 5). This result is similar to observations reported in a study performed on tick-borne pathogens of domestic dogs from communal areas in Maun, Botswana, where *E. ruminantium* was detected in two samples and in both incidences as a co-infection with *E. canis* using molecular characterizations of tick-borne pathogens. PCR followed by RLB was used in this research and would have the same limitations as this study. Parasites causing disease in other animals may infect hosts accidentally and not cause disease. This may apply to this incidental finding. A conclusion drawn in this study was that further research should focus on a comparison with the prevalence of heartwater in livestock and wildlife in the study area (Sibanda 2011).

Ehrlichia ruminantium causes heartwater in ruminants and *Amblyomma hebraeum* is responsible for spreading this disease in these animals. No *A. Hebraeum* ticks were collected during this study; however, these ticks have been collected from dogs in previous studies (Bryson et al. 2000). Dogs carrying *E. ruminantium* could act as a potential reservoir for heartwater (Allsopp and Allsopp 2001).

Alternatively, detection of *E. ruminantium* in dogs may be due to the fact that most of the dogs had previously come into contact with free roaming livestock infested with *E. ruminantium*-infected tick-vectors. This result is not unexpected as dogs from townships are generally owned by keepers of livestock. These dogs are sometimes used as protection of the livestock herds (Personal observation).

Another incidental finding was the 'Ehrlichia-like agent' *Anaplasma* sp. *Omatjenne*, observed in one dog from TEARS. The presence of *A. sp. Omatjenne* has been reported previously in dogs in a study performed in Nigeria (Adamu et al. 2014). It has also been

previously isolated from hosts such as sheep and Nyala in Southern Africa (Du Plessis, 1990; Sibanda, 2011). *Anaplasma* sp. *Omatjenne* has been detected in South Africa in ruminants including goats. This has now been extended to the incidental infections in dogs in the Western Cape. A possibility for this incidental positive reaction during testing may be that cross reactivity occurred between the probes during RLB. There is also a possibility of contamination during PCR and RLB or misidentification. Parasites causing disease in other animals may infect accidental hosts and not cause disease. This may apply to this incidental finding.

However, during this study, the vectors known to transmit this *Anaplasma* sp. *Omatjenne*, (*Hyalomma truncatum* and *Amblyomma variegatum*) (Du Plessis, 1990), were not identified. This parasite's pathogenicity is not currently well known, and more research will need to be conducted to determine its importance in dogs.

4.1 Occurrence of tick-borne pathogens and tick-species identified from the study localities

Rhipicephalus sanguineus is a three-host tick of dogs with all stages feeding on the same host. These ticks are active all year round in all-weather types, be it tropical or temperate climates. These may be contributing factors to the quantity of *R. sanguineus* ticks collected from all localities. *Haemaphysalis elliptica* is a three-host tick with only the adult stage parasitizing dogs. This may account for the lower amount of these ticks identified from the localities in this study since the immature stages are found on rodents. The fewer amount of ticks collected could be related to the particular life stages that the ticks are in. *Haemaphysalis elliptica* has a variable seasonality. According to previous studies, these ticks are most abundant in the Western Cape from May to September. Samples for this study were collected from April to July 2014. This may account for the slightly lower amount of *H. elliptica*, compared to *R. sanguineus* collected during this study.

An alarming number of ticks (n=400), were collected from dogs from the Nomzamo Township; however, only one tick species, *R. sanguineus*, was identified (Table 2). Moreover, in addition to *E. canis*, commonly transmitted by *R. sanguineus*, *B. rossii*, *B. vogeli* and *E. ruminantium* were also detected. However, the detection of *B. rossii* and *E. ruminantium* in the absence of the identification of corresponding tick-vectors comes as no surprise since this locality has a large number of dogs, interacting with other animal species and herds of livestock which are host reservoirs for these pathogens. Generally, dogs in townships are not confined to a single location but mingle amongst each other and roam freely amongst the long grass and livestock. Ticks may easily drop off one host and reattach and take a blood meal on a new host, resulting in infection with additional tick-borne pathogens. Also, sampling a larger number of dogs may increase detection of low abundant ticks, which are possible vectors of other pathogens detected from dogs from this locality.

Although the highest number of ticks (n=400 ticks) was collected from dogs (n=33) from Nomzamo Township, surprisingly, this locality presented with the lowest infection rate (27.3%) (Figure 4; Table 2). This is probably due to the fact that not all the ticks removed from the dogs were infected with pathogens. In fact, of the five tick pools tested from this locality, only three gave a positive reaction for the presence of tick-borne pathogen DNA. Parasitaemia ranges in the ticks determined the detection limits during this study. Small amounts of DNA present might have fallen below the lower detection limit of our assays. This result might also suggest that the pathogen population cannot keep up with the expansion of the ever increasing tick-vector population. The pathogen population has room to grow in the increasing tick-vector population. Transmission of new pathogen species might be possible with this theory. This raises concern since the aftermath could be critical when the pathogen population catches up with the vast tick population. As mentioned previously, tick species are finding new niches and becoming established in non-endemic regions. Ticks are adapting to the warmer humid climates.

Neighbouring the Nomzamo Township is Asanda village, from which *E. canis* and *E. ruminantium* were detected, along with *B. rossii*, and *B. vogeli* (Figure 4). With the Nomzamo Township and the Asanda village being in such close proximity to each other, it

is accepted that both locations would share many of the pathogens as well as the tick-vectors. A slightly higher number of *E. ruminantium* was identified in the dogs from Asanda than at Nomzamo (Figure 4). This result might be explained if there happens to be a larger number of ruminants in the village. This detail was not recorded during the study so the fact is unknown. A large amount of *R. sanguineus* ticks were detected at this locality and this would explain the detection of *E. canis* (Table 2).

The dogs at the AACL-Epping had the highest infection rate amongst the six localities investigated (Figure 4). Interestingly, this locality presented with the lowest number of ticks (Table 2). Usually, dogs that are kept at AACL-Epping are stray dogs found by AACL inspectors from neighbouring townships. There is a high chance that at the time of sampling, these dogs had already been treated for ectoparasites. Furthermore, this area presented *B. rossi* in the absence of *H. elliptica*, their tick-vector, at least from the samples examined in this study. This may be due to the life stage of the ticks. Only the adult stages parasitize dogs. As mentioned previously, the dogs from the townships are allowed to mingle thus increasing the probability of transfer of disease-carrying vectors, from one host to the next. It can be speculated that this is the reason why *B. rossi* was detected in three of the dogs, but only *R. sanguineus* ticks removed.

Other pathogens detected from AACL-Epping and TEARS were *Theileria taurotragi* and *Anaplasma* sp. *Omatjenne* respectively (Figure 4). The detection of *T. taurotragi* as well as *A. sp. Omatjenne* DNA from the dogs in Cape Town was unexpected. Both of the tick-borne pathogens have been linked to livestock and other forms of wildlife. *Theileria taurotragi*, formerly known as *Cytauxzoon taurotragi*, is a tick-borne pathogen of eland (*Taurotragus oryx*), known to infect livestock in sub Saharan Africa (Simuunza et al. 2001; Nijhof et al. 2005; Chaisi et al. 2013). *Anaplasma* sp. *Omatjenne*, formerly known as *Ehrlichia* sp. *Omatjenne*, is a rickettsial pathogen identified to infect ruminants and has been considered non-pathogenic to livestock.

Anaplasma sp. *Omatjenne* was initially isolated from *Hyalomma truncatum*, but has also been identified in *Amblyomma* ticks (Du Plessis, 1990). This pathogen has been detected in South Africa in ruminants including sheep (Inokuma et al. 2005). This has now been

extended to the incidental finding of *A. sp. Omatjenne* in dogs in the Western Cape (Figure 4). The pathogenicity of this parasite in dogs is not well known, however, more research is needed to establish this. It is speculated that the reason for these incidental findings could be related to the fact that ruminants and other livestock exist in close proximity with dogs in the communities studied, or these could be cross-reactions from undescribed infections.

The Lucky Lucy Foundation (LLF) had three cases of *E. canis*, two cases of *B. rossi* and one of *E. ruminantium* (Figure 4). The dogs at Lucky Lucy are part of a regular dipping programme; hence no ticks were collected from dogs from this locality. However, there is a possibility that these dogs were already infected when they arrived at LLF. It can also be suggested that this establishment may not be tick-free and dogs still get exposed to infected ticks.

4.2 Tick-borne pathogens detected from tick pools from the study localities

4.2.1 Nomzamo Village

Despite Nomzamo having the largest tick quantity, the ticks yielded a very small amount of pathogen DNA (Table 6). This might be due to the fact that each tick pool contained more than 100 ticks each, diluting pathogen DNA. Alternatively, there may have been low parasitaemia in the infected ticks below the detection limit of the assay.

Only *E. canis* was identified from the tick-DNA. With the eight positive results for *E. canis* from the blood DNA it would have been expected that there would be a more definitive *E. canis* result from the tick pools. *Babesia vogeli* was detected in the blood DNA and would have been an expected result from the tick-DNA due to the high number of *R. sanguineus* ticks removed from the dogs at Nomzamo, but none was detected (Figure 4; Table 6). *Babesia vogeli* was only detected in dogs for the first time in South Africa in 2008 (Matjila et al. 2008a). It could be that *B. vogeli* is still being established as a common pathogen in dogs from this locality based on the fact that *B. vogeli* is not as widely spread compared to *B. rossi* in SA (Matjila et al. 2008a).

4.2.2 Animal Anti-cruelty League (Epping)

The lowest quantity of ticks was collected from the dogs from the Animal Anti-Cruelty League in Epping. This locality produced the highest blood pathogen infection rate from the blood DNA (Figure 4; Table 6). The fourteen ticks collected from the AACL Epping made up only one tick pool for this study (Table 6). No species-specific results were detected from pathogen DNA detected from the tick pool. DNA from the tick pool hybridized to the *Ehrlichia/Anaplasma* genus-specific and the B1 genus-specific probes indicating the presence of *Ehrlichia* or *Anaplasma* and *Babesia* species DNA in this sample (Table 6). The blood DNA showed four of the samples to be positive on the *Ehrlichia/Anaplasma* genus-specific probe (Figure 4), so this result falls in line with the tick-DNA results.

4.2.3 Asanda Village

At the Asanda village it has been assumed that the *B. felis* DNA detection was an incidental finding in the tick-DNA (Table 6). This result may be explained due to the presence of cats wondering around in these rural areas. The detection of *B. felis* from dogs is a rare incidental occurrence. No supporting literature of *B. felis* in dogs was found.

No *E. ruminantium* was detected from the ticks DNA even though three blood DNA results yielded this pathogen (Table 6). It is possible that the tick-vectors that transmitted *E. ruminantium* parasites to these dogs had already engorged and dropped off from their host. There is also a possibility that the dogs testing positive for *E. ruminantium* may be reservoirs or incidental hosts for this pathogen. The ticks identified in this study are not the usual vectors for *E. ruminantium*. This pathogen is usually transmitted by the *Amblyomma* tick (Allsopp 2009), but can be transmitted from an infected animal on the mouth parts of a different tick species. As mentioned previously, this disease is becoming more common in dogs, especially in dogs that are allowed to roam freely with ruminants. Apparently healthy dogs may be carriers of *Ehrlichia ruminantium* (Allsopp and Allsopp 2001).

4.2.4 Animal Anti-cruelty League (Bellville)

Twenty nine *H. elliptica* ticks were collected from the dogs from the Animal Anti Cruelty League in Bellville (Table 2). *Babesia rossi* would have been an expected pathogen to be detected from the tick-DNA considering that *H. elliptica* is the main tick-vector of this parasite. The three tick pools were only positive for the *Ehrlichia/Anaplasma* genus-specific probe suggesting infection with uncharacterized species of *Ehrlichia* or *Anaplasma* (Table 6). *Babesia rossi* was detected in only one DNA sample from blood. *Babesia vogeli* which was identified from blood DNA was not detected from tick-DNA samples. The *Ehrlichia/Anaplasma* genus-specific results are in line with the four *Ehrlichia/Anaplasma* genus-specific results observed in the blood DNA (Table 6).

4.2.5 TEARS

Three tick pools were made up of the twenty two ticks collected from TEARS, mainly consisting of *H. elliptica* ticks (Table 2). Analysis of DNA from these tick pools revealed the presence of uncharacterized species of *Ehrlichia/Anaplasma* genus, *Theileria/Babesia* genus and *Babesia 1* genus-specific. However, *B. rossi* was also detected, corresponding to the detection of this pathogen DNA from blood samples. The detection of the uncharacterized *Ehrlichia/Anaplasma* species confirmed the results obtained from blood DNA (Table 6).

4.3 Identification of uncharacterized tick-borne pathogen species

Uncharacterized tick-borne pathogen species were detected in the blood and tick-DNA samples. The following probes yielded a positive result in the tick and blood DNA: *Ehrlichia/Anaplasma* genus-specific; *Theileria/Babesia* genus-specific, *Babesia 1* genus-specific and *Babesia 2* genus-specific probes. The *Ehrlichia/Anaplasma* genus-specific probe had the most positive results without any specific species detected, followed by the *Theileria/Babesia* genus-specific probe, the *Babesia 1* genus-specific and the *Babesia 2* genus-specific probes. These results indicate that the hybridisation signal was too low to detect exact pathogens, or that our RLB test did not include related probes (Figure 4; Table 6).

In a similar study performed in Mnisi, Bushbuckridge, Mpumalanga Province, South Africa, similar identification of uncharacterized tick-borne pathogen species results were also detected. The *Ehrlichia/Anaplasma* genus-specific probe also yielded the highest number of positive results of uncharacterized tick-borne pathogen species; however, further analysis was done in order to establish the pathogen species (Kolo et al. 2016). In this study, no further analysis was done on these samples due to the limited scope of work. Amplification and sequencing of the variable region of the rRNA gene should be performed in order to determine the exact species of the pathogen.

Chapter 5

CONCLUSION

All dogs sampled during this study were from poor resource areas with limited veterinary care. Although they seemed to be in good health at the time of blood sample collection, the results of this study showed that the sampled dogs were infested particularly with *Rhipicephalus sanguineus* (89.8%) and *Haemaphysalis elliptica* (10.2%). Furthermore, these dogs were also found to be infected with tick-borne pathogens, mainly *Ehrlichia canis* and *Babesia rossi*.

It is well known that *E. canis* and *B. rossi* are endemic in South Africa and it can be concluded that these pathogens remain a threat to dogs in Cape Town, particularly to dogs from poor resource areas. Non-symptomatic infected dogs may also be acting as reservoirs of these pathogens.

All dogs exposed to ticks run the risk of becoming infected with tick-borne pathogens. Since the owners of dogs in resource poor areas have limited access to veterinary practitioners.

There is no doubt that the geographical range of these established pathogens, (*E. canis* and *B. rossi*) will expand due to movements of dogs in and around Cape Town as well as the expansion of tick habitats.

Cape Town has an increased number of travellers and tourists year upon year, and for this reason, it can be speculated that if the travelling pet-disease control efforts are not adhered to, the number of babesiosis and ehrlichiosis cases are likely to increase. There is an additional risk of dogs being exported out of South Africa, as *E. canis* and *B. rossi* are already endemic in this country.

As a follow-up to this study, samples that tested positive on the genus-specific probe would need to be sequenced. Furthermore, it appears as if cases of *E. ruminantium* infections are increasing. The importance of *E. ruminantium* in dogs needs to be investigated. Similar to other studies, this preliminary study has indicated that tick and tick-borne pathogens of dogs continue to be a health burden in resource-poor communities and townships around Cape Town.

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