

**PARASITOLOGICAL AND MOLECULAR STUDIES ON TICKS AND
BABESIA SPECIES INFECTION OF DOGS IN THE FEDERAL CAPITAL
TERRITORY, NIGERIA**

BY

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AHMADU BELLO UNIVERSITY,
ZARIA**

DECEMBER, 2017

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NIGERIA**

BY

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN
VETERINARY PARASITOLOGY**

**DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

DECEMBER, 2017

DECLARATION

I declare that the work reported in this Thesis entitled “**Parasitological and Molecular Studies on Ticks and *Babesia* species Infection of Dogs in the Federal Capital Territory, Nigeria**” has been performed by me in the Department of Veterinary Parasitology and Entomology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for any degree or diploma at this or any other Institution.

Sylvester Sunday OBETA _____

Signature

Date

CERTIFICATION

This Thesis entitled “**PARASITOLOGICAL AND MOLECULAR STUDIES ON TICKS AND *BABESIA* SPECIES INFECTION OF DOGS IN THE FEDERAL CAPITAL TERRITORY, NIGERIA**” by Sylvester Sunday OBETA meets the regulations governing the award of the degree of Doctor of Philosophy (VETERINARY PARASITOLOGY) of the Ahmadu Bello University, Zaria, and is approved for the scholarly contribution to knowledge and literary presentation.

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DEDICATION

To God Almighty, my family, and to all those, who through faith obtained good reports, subdued kingdoms, administered justice, obtained promised blessings, shut the mouths of lions, extinguished the power of raging fire, escaped the devourings of the sword, out of frailty and weakness won strength and became stalwart in battle and routing alien hosts.

ACKNOWLEDGEMENT

TO GOD THE FATHER, THE SON AND THE HOLY SPIRIT, I return all the glory for empowering me to receive this academic excellence.

This successful study would not have been possible without the contributions of many people, and I would like to take this opportunity to thank them all:

It is a great pleasure and honour to express my heartfelt gratitude to my amiable supervisory committee. To Prof. I. A. Lawal, I thank you for your positive mentorship, tireless guidance, enlightened views, great suggestions and uncommon patience, without which this study would have been a mirage. To Prof. A. J. Natala, my co-supervisor, I thank you for your positive approach to life, inspirational guidance, great and tireless contributions, which added up to the successful completion of this academic journey. To Dr. E. O. Balogun, my co-supervisor, I sincerely thank you for your uncommon generosity, patience and tireless corrections through out the course of the research. You thought me the basic skills of molecular techniques and providing the necessary materials to carry it out, without which the molecular aspect of the study would not have been accomplished. All your effort towards inculcating the spirit of hard work and academic excellence will continue to be a source of inspiration in my academic journey.

With profound sense of gratitude, I am deeply thankful to Prof. J. O Ayo of the Department of Veterinary Physiology for being a beacon of encouragement, your tireless corrections and valuable suggestions, contributed immensely to the successful completion of this study. I express my heartiest thanks to Profs O. P Ajagbonna of Department of Veterinary Pharmacology and Toxicology; O. C Jegede and M. N. Opara of Department of Veterinary Parasitology and Entomology, University of Abuja, for their tireless encouragement, valuable suggestions and moral support, throughout the course of this study. I warmly thank Prof. R. I. S. Agbede, Head of Department, my colleagues; Drs. B.M. Rabi, M. S. Kawe, and E. C. Ejiofor; and the entire staff of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Abuja, for their warm support and immense contributions in the course of the study.

I express my deep sense of gratitude and profound indebtedness to Prof. S. Mailafia, Drs N. I. Ogo., C. Uchendu., J. Igwe, A. Mgbeahurike, P. Ehizibolo, J. Musa, P. Umakuana, S. Idoko, and E. Abieyre, for their contributions, solidarity and diverse encouragements throughout the course of the study. I warmly thank my colleagues and entire staff of Veterinary Clinics in the Federal Capital Territory, in Bwari, Municipal, Kwali, Kuje, Abaji and Gwagwalada Area Councils; management and staff of Vet-World Limited, Mac-Acee Limited, Good Shepherd Veterinary and Kings Veterinary Services, for their great assistance and co-operation, throughout the sample collections.

I extend my warm appreciations to the followings: Miss Kate, Mallams Lawal and Saidu of the Protozoology Labs, for their tireless assistance in the reading of the slides. I warmly, thank Messrs. Benjamin and Simon Otobo of the Entomology Labs, for their assistance in tick

identifications and haemolymph smear techniques. I am very grateful to the entire staff of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, ABU, Zaria, for their diverse assistance, friendship, and unreserved support in the course of this study.

I use this privileged opportunity to give kudos to my scientist friends: Solomon Umukoro, David Dangton, and Israel Siobise, for your great support in this successful academic journey. To my reliable friend, Kingsley Mou, I salute you, for your support and encouragement all through the stressful days of this research. Indeed together we are winners.

Finally, I deeply express my indebtedness to the entire members of my family, especially, my darling wife; Chinyere, my mother; Christiana; my children; Chidinma, Chinwe, Chinonso, Chidiebere, and Tochukwu, for the years of waiting and hands-in fervent prayers, for my safety and successful completion of the study. Together we have overcome.

The study was graciously sponsored by the Tertiary Education Trust Fund (TETFUND), Study Fellowship of the University of Abuja, Abuja, Nigeria.

ABSTRACT

The study aimed at determining the prevalence of *Babesia* species infection in dogs in the Federal Capital Territory, Abuja, Nigeria and identification of ticks of the sampled animals. It further evaluated the parasitological and molecular characteristics of the ticks and *Babesia* species. A total of 480 asymptomatic dogs were sampled for tick and blood, using standard method. Ticks collected were identified to generic level using morphological features in a dissecting microscope. Thick and thin blood smears were prepared, Geimsa stained and examined microscopically, for intra-erythrocytic merozoites of *Babesia* spp. Haematological parameters of sampled dogs were evaluated using automated haematology analyser. Genomic DNA was extracted from 30 *Rhipicephalus* spp. of ticks and 15 *B. canis* positive whole blood, followed by partial amplification of 18S rRNA gene using genus specific primers. The PCR products were purified and sequenced. The chromatograms were trimmed in ApE software, and aligned to generate a consensus sequence for each isolate. The nucleotide sequence BLAST analysis was carried out in NCBI database. Correct sequences of 9 *R. sanguineus* and 3 *B. c. vogeli* were obtained and deposited in the GenBank using Bankit tool. Multiple sequence alignments was carried out using ClustralW in MEGA7.0 software. Phylogenetic trees were constructed for *Rhipicephalus* and *Babesia* species using neighbour-joining and maximum likelihood methods respectively. The results showed an overall prevalence of 10.8% and 3.1% *B. canis* infection in dogs for thick and thin smears techniques respectively. The prevalence of *Babesia* infection in FCT based on sampled location was highest (12.5%) in Gwagwalada, Abuja Municipal and Kwali Area Councils and lowest (6.3%) in Abaji Area Council. The prevalence of *B. canis* infection based on sex showed that males had a higher (13.7%) than the females with 8.3% infection rate. The prevalence based on breed showed that exotic breeds had

the highest (12.9%) while the cross breed had the lowest of 9.4% *B. canis* infection rate. The prevalence based on purpose showed that hunting dogs had the highest (11.3%) while the pet dogs recorded no *Babesia* spp. infections. The prevalence based on season showed that the rainy season had higher (14.6%) than the dry season with 7.1% of *B. canis* infection. The prevalence of *Babesia* infection based on tick infestations showed that tick infested dogs had higher (17.1%) than dogs without ticks, with 3.5% infection rate. The percentage of tick attachment based on predilection sites showed that the ear region had the highest (45%), while the scrotal and the mammary region had the lowest with 4.0%. There was significant ($p < 0.05$) association between rate of *Babesia* infection and age group, purpose, season and tick infestation. No significant association was observed between *Babesia* infection and sex, breed, and sampled locations. The haematological parameters showed higher values for packed cell volume, haemoglobin concentration, red blood cell count and platelets in *Babesia* negative dogs. However, red blood cell count, lymphocytes and monocytes values were lower when compared with *Babesia* positive dogs. Out of the 2,043 ticks collected from sampled dogs, 99.9% were *Rhipicephalus* spp. and 0.1% were *Amblyomma* spp. The PCR amplified at 173bp and 612bp gene segments on gel electrophorogram for *Rhipicephalus* spp. and *B. canis* respectively. The BLAST analysis showed 96-100% and 100% similarity with *R. sanguineus* and *B. c. vogeli* respectively in the GenBank. The phylogenetic trees showed that sequences of *R. sanguineus* isolates from the study area (accession numbers: KY 799078 – KY799086) clustered together while the sequences of *B. c. vogeli* isolates (accession numbers: MF000388, MF000389, MF000390), separated into two different clades. The study concludes that *R. sanguineus* was the common ticks infesting dogs and *B. canis vogeli* was the subspecies of *B. canis* infecting the sampled dogs in the study area.

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LIST OF ABBREVIATIONS

ApE	A Plasmid Editor
BLAST	Basic Local Alignment Tool
CCHF	Cremean-Congo Haemorrhagic Fever
CVBD	Canine Vector Borne Disease
CME	Canine Monocytic Ehrlichiosis
DNA	Deoxyribonucleic Acid
dNTPs	dinucleotide triphosphates
EDTA	Ethylene diaminetetraacetic acid
ELISA	Enzyme Link Immunosorbent assay
FCT	Federal Capital Territory
IFAT	Immune Fluorescence Antibody Test
ICT	Immunochromatographic Test
LAMP	Loop Mediated Isothermal Amplification
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MEGA7.0	Molecular Evolutionary Genetics Analysis version7.0
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
PCR-RFLP	PCR-Restriction Fragment Length Polymorphism

RBC	Red Blood Cell
RLB	Reverse line blot hybridization
rRNA	ribosomal Ribonucleic Acid
RT-PCR	Real Time Polymerase Chain Reaction
TBD	Tick-Borne Disease
WBC	White Blood Cell

CHAPTER ONE

1.0

INTRODUCTION

1.1

Background of the Study

Ticks are amongst the most important arthropod ecto-parasites of animals, being adapted to different climates and host species (Dantas-Torres *et al.*, 2012). Hard ticks (order Ixodida, family Ixodidae) represent the most diverse group, occurring in tropical, temperate and even arctic regions (Estrada-Peña *et al.*, 2012). Their medical and veterinary importance is mostly due to their great capacity to transmit infectious agents to vertebrate animals, which may cause a diverse range of conditions, commonly referred to as Tick-Borne Diseases (TBDs). As haematophagous obligate parasites, ticks are major vectors for a wide range of pathogens (e.g., viruses, bacteria and protozoa) of companion animals, livestock and humans (de la Fuente *et al.*, 2008; Dantas-Torres *et al.*, 2012). Tick-borne pathogens include species of *Babesia*, *Anaplasma*, *Rickettsia*, *Ehrlichia*, *Theileria* and *Coxiella* (Uilenberg, 1995). *Babesia* species are tick-borne Apicomplexan protozoan parasites of erythrocytes that infect a wide range of vertebrate hosts (Irwin, 2009), resulting to a disease condition known as babesiosis, with a worldwide distribution (Parnell *et al.*, 2008; Otranto *et al.*, 2009). The *Babesia* parasites replicate in the red blood cells and initiate a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes (Zygner *et al.*, 2007).

The disease is characterised by fever, splenomegaly, inappetence, weakness, lethargy, generalised lymphadenopathy, anaemia, haemoglobinuria and collapse associated with

intra-and extravascular haemolysis, hypoxic injury, systemic inflammation, thrombocytopenia, and pigmenturia (Irwin, 2009).

Canine *Babesia* infection (formerly canine piroplasmosis) is one of the most important tick-borne diseases of domestic and wild canidae (Abd Rani, 2011). It is characterized by a wide range of clinical manifestations; from subclinical disease to life-threatening conditions, with severity of clinical signs, depending on the species involved and host immune response against the infection (Otranto *et al.*, 2010; De Tommasi *et al.*, 2013). The diagnosis of canine *Babesia* infections and identification of each species has traditionally been based on host specificity and the morphology of the intra-erythrocytic forms in stained blood smear. The *Babesiacanis* and *Babesiagibsoni* are the major species infecting dogs, causing anaemia. *Babesia canis* is the large piroplasm (4-5µm), usually occurs as a single pear-shaped piroplasm or in pairs or in multiple merozoites divided by binary fission within the erythrocyte (Soulsby, 1982).

Recent advances in molecular biology techniques like Polymerase Chain Reaction (PCR) have made it possible to detect and identify piroplasms with greater sensitivity and specificity than the traditional methods (Birkenheuer *et al.*, 2003 and Jefferies *et al.*, 2003). Molecular biology studies have revealed that, on the basis of differences in the geographical distribution, vector specificity, and antigenic properties (Hauschild *et al.*, 1995), *B.canis* is subdivided into three subspecies: namely, *B. canis canis* transmitted by *Dermacentorreticulatus* (in Europe), *B.canis vogeli* transmitted by *Rhipicephalussanguineus* (in tropical and sub-tropical regions), and *B.canis rossi* transmitted by *Haemaphysaliselliptica* (in South Africa)(Birkenheuer *et al.*, 1999). There are also differences in their pathogenicity, as *B.canis rossi* causes a frequently fatal

infection in domestic dogs, even after treatment; *B. canis vogeli* gives a moderate and often clinically inapparent infection, and *B. canis canis* infections result in a more variable pathogenicity intermediate between *B. canis rossi* and *B. canis vogeli* (Uilenberg *et al.*, 2006). *Babesia gibsoni* is the small *Babesia* that commonly appears as individual ring forms or pyriform bodies ranging between 1.0 and 2.5µm in size (Conrad *et al.*, 1991). The *B. gibsoni* was found to be associated with infection of dogs in Asia, North America, northern and eastern Africa, and Europe (Conrad *et al.*, 1991).

Dogs are most common domestic animals seen in almost every human settlement performing valuable roles in the society. In Nigeria, dogs are kept as pets, guards, for hunting, herding, breeding, as well as a source of animal protein among some ethnic groups (Aiyedun and Olugasa, 2012; Hambolu *et al.*, 2014). Canine *Babesia* infection was first encountered in Nigeria, in 1962, and documented in the Veterinary Department annual report, as a disease associated frequently with higher severity among imported dogs than the indigenous breeds Leeflang and Ilemobade (1977). Several publications have shown different prevalence reports of the infection in different parts of the country based on microscopy (Ajayi *et al.*, 2009; Ogo *et al.*, 2011; Adamu *et al.*, 2012; Okubanjo *et al.*, 2013; Konto *et al.*, 2014). Recent studies using molecular techniques have shown the presence of all the three *Babesia canis* subspecies: *Babesia canis*, *Babesia vogeli* and *Babesia rossi* in Nigeria (Sasaki *et al.*, 2007; Kamani *et al.*, 2010; Takeet *et al.*, 2017) with evidence of co-infections with *Ehrlichia canis*, *Hepatozoon canis*, *Anaplasma platys*, *Theileria* sp., *Candidatus Neoehrlichia mikurensis* and *Rickettsia conorii israelensis* (Kamani *et al.* 2013; Adamu *et al.* 2014).

In the Federal Capital Territory, only few reports of *Babesia canis* infections exist (Obeta *et al.*, 2009; Jegede *et al.*, 2014). Molecular techniques are being utilized in the diagnosis and in differentiating different infections caused by various *Babesia* subspecies (Oyamada *et al.*, 2005).

1.2 Statement of Research Problem

Current global changes, such as climate change, deforestation, changes in land use, urbanization, increased trade and travel are affecting animal host populations worldwide (Dantas-Torres, 2015), favouring the establishment of ticks and their associated pathogens into previously free areas. Due to the ability of ticks to survive under different climatic conditions and ecological niches, they have a cosmopolitan distribution and their control is still a major challenge for veterinarians and pet owners (Cafarchia *et al.*, 2015).

Dantas-Torres *et al.* (2012), reported that among ixodid ticks, *Rhipicephalus sanguineus sensu lato* (s.l.) is a vector of *Ehrlichia canis*, *Babesia vogeli* and *Hepatozoon canis* in dogs and *Rickettsia conorii* and *Rickettsia rickettsii* in humans. The *Rhipicephalus sanguineus*, (brown dog tick), is a good example of 'parasite globalization' as is considered the most cosmopolitan species among the families, transmitting a wide range of pathogens to dogs and other animals including humans (Fourie *et al.*, 2013). It can transmit a variety of pathogens to dogs, including *Babesia canis vogeli*, *Ehrlichia canis*, *Anaplasma platys* and *Hepatozoon canis* and also capable of transmitting pathogens to humans such as *Rickettsia conorii*, the agent of Mediterranean spotted fever (Chomel, 2011). Reports have shown that *Babesia* infections in dogs occur worldwide (Solano-Gallego and Baneth 2011; Schnittger *et al.*, 2012). There are over a hundred species of *Babesia* infecting vertebrate hosts and twelve have been documented to infect dogs (Irwin, 2010). There is a speculation that all

vertebrates, including human, can be infected with *Babesia*, largely depending on their suitability as hosts for tick vectors (Schnittger *et al.*, 2012). Some TBDs of companion animals are zoonotic (Cito *et al.*, 2015) and may place human owners at risk of infection (Rijks *et al.*, 2015).

In Nigeria, studies have shown that *Rhipicephalus*, *Boophilus*, *Hyalomma* and *Amblyomma* species can infest dogs (Konto *et al.*, 2014). However, the *Rhipicephalus sanguineus* is the most common ticks infesting dogs while *Babesia* is the predominant haemoparasite of dogs in the country (Amuta *et al.*, 2010; Opara *et al.*, 2017). The tick fauna was reported to peaks around the rainy and is attributed to the high humidity of 80% and optimum temperature of 27⁰C required for the hatching of eggs (Natala *et al.*, 2009). Adamu *et al.* (2014), recorded 72% prevalence of haemoparasites infections in dogs from Jos, Plateau State. Amuta *et al.* (2010), reported 10.2% prevalence of canine babesiosis in Makurdi, Nigeria. Sasaki *et al.* (2007), reported the presence of *Babesia canis rossi* and *B canis vogeli* in 2.0% and 0.3% respectively using nested PCR and sequence analysis. Kamani *et al.* (2010), reported for the first time in an untraveled Nigerian dog the presence of *Babesia canis canis* on the African continent, as a co-infection with *Babesia canis rossi*, which is a rare occurrence. In areas where canine tick-borne diseases are endemic, dogs can be simultaneously infected with more than one tick-borne pathogen (Cardoso *et al.*, 2010). Infection with *Babesia canis* common in Nigerian dogs due to high prevalence of the tick vector, *Rhipencephalus sanguineus* (Abdullahi *et al.*, 1990). Studies have shown an increasing prevalence of tick-borne pathogens in local dogs, with a recent report of *Candidatus Neoerlichiamikurensis* as an emerging disease agent of humans, in *Rhipicephalus sanguineus* and *Haemaphysalis leachi* ticks (Kamani *et al.*, 2013). *Rickettsia*

conorii israelensis, the causative agent of Mediterranean spotted fever was also detected in *Rhipicephalus sanguineus* ticks using molecular techniques (Kamani *et al.*, 2013). An earlier study of 400 randomly sampled dogs, from different parts of Nigeria, showed 2.3% positive for *B. canis rossi*, while a single dog was positive for *B. canis vogeli* (Sasaki *et al.*, 2007). Of the 500 blood samples from dogs presented to Veterinary Clinics in Ibadan, Oyo State; 26.0% were infected with *B. canis* while 20.2% were infected with *B. gibsoni* under microscopic examination (Oduye and Dipeolu, 1976). *Babesia canis (sensulato)* infection had earlier been reported in dogs from Zaria, Kaduna State (Useh *et al.*, 2003). A low prevalence of 2.8% *B. canis* infection was recorded in a thin blood-smear survey of slaughtered dogs in Maiduguri, Borno State (Adamu *et al.*, 2012). A prevalence of 4.8% to 14.6% *B. canis rossi* infection was recorded in dogs presented to veterinary hospitals in four states, comprising; Rivers, Plateau, Kaduna and Nasarrawa (Kamani *et al.*, 2010). Other reports include *B. canis vogeli* infection in a dog in Kaduna; *B. canis (sensu stricto)* and *B. canis rossi* co-infection in a dog domiciled in Vom, Plateau State (Kamani *et al.*, 2010). These documented reports being the first confirmation of the occurrence of *B. canis canis* in a region where the tick-vector, *Derma-centorreticulatus*, had not been reported, have stimulated a renewed interest in the epidemiology of canine babesiosis in Nigeria (Kamani *et al.*, 2010).

1.3

Justification for the Research

Despite huge resources committed towards tick control projects, especially for companion animals and livestock, the incidence of TBDs has been reported to be on the increase

globally (Dantas-Torres *et al.*, 2012). Reports have shown that due to the ability of ticks to survive under different climatic conditions and ecological niches, they have a cosmopolitan distribution and their control is still a major challenge for veterinarians and pet owners (Cafarchia *et al.*, 2015). The efforts towards the production of effective anti-tick vaccines have not been successful and dogs appear to develop no immunity against ticks (Nuttall *et al.*, 2006).

While our understanding of *Babesia* is improving, diagnosis and treatment of *Babesia* infections remain challenging. The morphological similarity between species and subspecies of *Babesia* has led to much confusion over accurate diagnosis using microscopy (Jefferies *et al.*, 2007). The use of molecular tools; such as PCR and sequence analyses has increased the understanding of the distribution of TBDs and the role of ticks in the infection (Dantas-Torres *et al.*, 2012). The techniques also offers an effective and rapid means of differentiating between species (Jefferies *et al.*, 2007; Gulanber *et al.*, 2006). Cross antigenicity seen in the *B. canis* subspecies is thought to be responsible for vaccine failures in the field, thus the possibility of developing potent vaccines against canine babesiosis will be dependent on the proper identification of the subspecies (Schetters *et al.*, 1995). The pathogenicity of *Babesia* is believed to vary in different regions due to host factors and/or differences in the species present. Therefore, accurate identification of tick vectors and the *Babesia* species infections is an important factor in the monitoring, management and control of tick-borne diseases (Abdel Aziz *et al.*, 2014). Combination of PCR and nucleotide sequence analysis may increase information about subspecies or strains of the parasite. Knowledge obtained from cross-species or cross-genus comparisons may be

helpful in improving existing prophylaxis, treatment, and management of the disease and may provide a better understanding of their underlying biology and evolution (Lau, 2009).

Being companion animals, the diseases of dogs are of great concern, both directly because of financial burden for owners and the negative impact on animal health, and indirectly because of transmission of pathogens to humans and animals (Fourie *et al.*, 2013). It has also been reported that Nigeria does not consider canine babesiosis as a reportable disease and as such does not enforce screening of dogs imported into the country, which may possibly introduce foreign tick vectors and their associated parasites/diseases (Ogo *et al.*, 2011). There is a paucity of information on the molecular characterisation of ticks and *Babesia* spp of dogs in the study area. This research was conducted with the aim of determining the prevalence distributions of *Babesia* species infection of dogs in the Federal Capital Territory, identify the ticks found on them using morphological features, evaluate the haematological indices of sampled dogs, and further characterize the ticks and *Babesia* sp. in the blood of infected dogs using Polymerase Chain Reaction and Sequence analysis.

1.4

Research Questions

1. What is the prevalence of *Babesia* infection in dogs in FCT?
2. What are the tick species infesting sampled dogs in the study area?
3. What are the haematological indices of dogs sampled for ticks and *Babesia* spp. in the FCT?
4. What are the molecular characteristics ticks and *Babesia* species from sampled dogs in FCT?

5. What is the relationship of the sequences of ticks and *Babesia*spp. from dogs in the FCT, with reference sequences in the GenBank?

1.5

Aim of the Study

To conduct parasitological and molecular study on ticks and *Babesia* species in dogs in Federal Capital Territory, Abuja, Nigeria.

1.6

The Specific Objectives of the Study

1. Determine the prevalence of *Babesia*spp. in dogs in the FCT
2. Identify tick species in sampled dogs in the FCT using morphological features.
3. Determine the haematological parameters of sampled dogs in the study area.
4. To evaluate the molecular characteristics of ticks and *Babesia* spp. of the sampled dogs in FCT using Molecular techniques.
5. To determine the relationship of the sequences of ticks and *Babesia* from the study area with reference sequences in the GenBank.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of *Babesia* spp. parasite

Infections of *Babesia* organism was first reported in 1888, towards the end of 19th Century, by Viktor Babes in Romania when he detected the presence of round, intra-erythrocytic bodies in the blood of infected cattle. Babes (as cited by Solano-Gallego *et al.*, 2016) associated the disease with bovine haemoglobinuria or “red water fever” but failed to report the presence of ticks in sick cattle. It was until 1893, when Theobald Smith and Frederick Kilborne of the Bureau of Animal Industry of the United States, published their results of a series of experiments demonstrating that the southern cattle tick *Boophilus (Rhipicephalus) annulatus* from infected cattle, were responsible for transmitting a disease called the “tick fever” to susceptible cattle (Smith and Kilbourne, 1893). Their studies was considered as the first documented arthropod vector-borne disease. The observations of Smith and Kilborne (1893), were strengthened by Cooper Curtice’s own hypothesis that eliminating the cattle tick would eliminate the disease, and they were the basis for the establishment of a tick eradication program, which culminated with the eradication of the southern cattle tick and babesiosis from the United States territory in 1943 (Graham and Hourrigan, 1977). The infectious agent was first called *Pyrosomabigeminum* but later named *Babesia bigemina* (Uilenberg, 2006). Not long after these observations in ruminants the first description of *Babesia* spp in dogs was reported in Italy in 1895 by Piana and Galli-Valerio (cited by Uilenberg, 2006). In 1901, Lousby (cited by Briekenheur *et al.*, 2004) documented *Haemaphysalis leachi* to be the vector for *B.canis rossi*. Patton in 1910 was the first

researcher to explain a small piroplasm infecting canids and named the organism as *Piroplasma gibsoni* in honour of Dr. F. Maitland Gibson who first saw the parasite. Parasites of the genus *Babesia* infect a wide variety of domestic and wild mammals as well as man. To date, more than 100 species have been identified, infecting many mammalian and some avian species (Gray and Weiss, 2008), occurring worldwide (Boozer and Macintire, 2003; Solano-Gallego and Baneth, 2011).

2.2 Babesiaspp. Infection in Dogs in Nigeria

Leeflang and Ilemobade (1977), first reported canine *Babesia* infection as documented in the Veterinary Department annual report of 1926, as a disease associated frequently with higher severity among imported dogs (cited by Shitta and James-rugu, 2013). Abdullahi (1990) and Dipeolu (1975) reported that infections of *Babesia* spp. are endemic in Nigeria due to high presence of brown dog ticks in most parts of the country. However, there appears to be conflicting reports among several authors as regards to the actual prevalence of the disease in Nigeria. Several authors have reported variations in the prevalence of the disease in different parts of the country: Oduye and Dipeolu (1976), reported from blood smears of 500 dogs in Ibadan, South-West Nigeria that *Babesia canis* accounted for 53% parasitemia. Saror *et al.* (1979) on examining 254 dogs in Zaria for blood parasites reported 22% of *Babesia canis* infection; Odewunmi and Uzoukwu (1979) while investigating the prevalence of blood parasites in 116 dogs at Enugu reported that *Babesia canis* accounted for 55.1%. Other authors had in different occasions reported *Babesia* spp. infection rate in

Nigeria to range between 2% and 43.6% (Dipeolu, 1975; Bodade *et al.*, 1989; Amuta *et al.*, 2010).

Diagnosis is usually by microscopy which is based on morphological identification of the parasite either as large (*Babesia canis*) or small (*Babesia gibsoni*), relating to their size within the erythrocytes (Adeyanju and Aliu, 1982; Bodade *et al.*, 1989). Reports have shown that the prevalence of the disease in Nigeria has been studied extensively by microscopy (Ajayi *et al.* 2009; Ogo *et al.* 2011; Adamu *et al.* 2012; Okubanjo *et al.* 2013; Jegede *et al.* 2014). However, microscopy may be less useful in low parasitaemic situation, the method cannot discriminate species of *B. canis*, *B. rossi* and *B. vogeli* because of their morphological similarity (Ogo *et al.* 2011; Salem and Farag 2014). Serological diagnosis by Enzyme Link Immunosorbent assay (ELISA) is rarely conducted on clinical cases, while molecular studies are occasional (Sasaki *et al.*, 2007; Kamani *et al.*, 2010). The few molecular studies that were undertaken provided information on subspecies of *Babesia canis* present in Nigerian dogs. Sasaki *et al.*, (2007) first reported the occurrence of *Babesia canis rossi* and *B. canis vogeli* in 2.0% and 0.3% respectively in Nigerian dogs using nested PCR and sequence analysis. Kamani *et al.* (2010) using specific PCR for *Babesia* spp. and DNA sequencing reported for the first time in an untraveled Nigerian dog the presence of *Babesia canis* on the African continent as a coinfection with *Babesia canis rossi*, also a rare occurrence. Kamani *et al.* (2013), reported high prevalence of vector-borne pathogens of humans and animals in Nigeria and West Africa where the incidence of tick-borne infections appears to be underestimated. The reports of Sasaki *et al.*, 2007; Kamani *et al.*, 2013., Adamu *et al.* 2014) employed nested and quantitative PCR to shed light on the prevalence of tick-transmitted pathogens of dogs in the South West and North Central parts

of the country with different rate of infections (Table 2.1). However, reports of the studies showed that only the *B. rossi* from North Central were characterized. In a recent study, Takeet *et al.* (2017), reported a prevalence of 7.7% *Babesia* merozoites using microscopy and 18.7% *Babesia canis rossi* infection rate by PCR and sequencing among 206 dogs screened at the veterinary hospitals in Abeokuta, South-West, Nigeria. Ogo *et al.* (2011), reviewed the status of canine babesiosis in Nigeria and described the disease as an emerging veterinary problem worldwide (Ogo *et al.*, 2011). Several literatures of the disease occurrence using molecular tools exist in Nigeria affirming the presence of all the three large *Babesia* subspecies: *Babesia canis*, *Babesia vogeli* and *Babesia rossi* in the country (Sasaki *et al.*, 2007; Kamani *et al.*, 2010), an information that is of grave consequence to the animals and of importance to veterinarians considering the various clinical presentations of different species (Ogo *et al.*, 2011).

Table 2.1: Available data on molecular survey of *Babesia* spp. infection of dogs in

S/N	No.of Dog Sampled	<i>B. canis canis</i>	<i>B. canis rossi</i>	<i>B. canis vogeli</i>	References
1	100	-	5.3	-	Adamu <i>et al.</i> (2014)
2	181	-	6.6	0.6	Kamani <i>et al.</i> (2013)
3	400	-	2.0	0.3	Sasaki <i>et al.</i> (2007)
4	209	-	18.7	-	Takeet <i>et al.</i> (2017)
<hr/>					
Nigeria					

2.3

Classification of *Babesia* spp.

Levine (1988), placed *Babesia* in the Phylum Apicomplexa, Class Sporozoa, Order Piroplasmida, and family Babesiidae. The family Babesiidae contains three genera, *Babesia*, *Theileria* and *Cytauxzoon*. Hunfeld *et al.*, (2008) reported that the grouping of *Babesia* spp. was mainly classified on the basis of their morphology, host/vector specificity, and susceptibility to drugs. Morphologically, *Babesia* spp. is divided into the small babesias (trophozoites of 1.0–2.5 μm ; including *Babesia gibsoni*, *B. microti*, and *B. rodhaini*, and the large babesias (2.5–5.0 μm ; including *Babesia bovis*, *B. caballi*, and *B. canis*). Further understanding of the taxonomic classification of these parasites was delayed for close to a hundred years by the fact that within these general size ranges, their morphological features did not permit further differentiation. The first suggestion that all *B. canis* isolates were not identical species came from the German protozoologist, Eduard Reichenow, who recognised differences in pathogenicity of “*B. canis*” isolates from France and North Africa (Reichenow *et al.*, 1937). It was until the late 1980s, with the advent of molecular phylogenetic analysis, in particular that of the 18S rRNA gene, ushered clarifications and it was recognized that the subspecies are in fact distinct species, mainly *B. rossi*, *B. canis*, and *B. vogeli* (Schnittger *et al.* 2012). More recently an unnamed fourth

“large” *Babesia* sp. (*coco*) has been found in dogs in North Carolina in the US (Marks *et al.*, 2012). Genetic analyses have also revealed a number of small *Babesia* spp. that is genotypically distinct from *B. gibsoni*. These include *Babesia conradae* and *Babesia microti*-like piroplasm (named *Theileria annae*), which are in separate lineages or clades (Schnittger *et al.*, 2012). The morphological classifications are generally consistent with the phylogenetic characterisation based on nuclear small subunit- ribosomal RNA gene (18S rDNA) sequences, which shows that the large and small babesias fall into two phylogenetic clusters, with the small babesias being more related to *Theileria* spp. than the large (with the exception of *B. divergens*, which appears small on blood smears (0.4–1.5 µm) but is genetically related to large babesias (Homer *et al.*, 2000). To date, more than 100 *Babesia* species have been identified, infecting many mammalian and some avian species (Gray and Weiss, 2008). Two morphologically distinct forms of the erythrocytic stage in the canine host were recognised in early studies that led to the naming of the larger form, measuring approximately 3-5 µm as *B. canis*, and the smaller (1-3 µm) as *B. gibsoni*.

Babesia canis” was reclassified into three sub-species (*B. canis canis*, *B. canis rossi* and *B. canis vogeli*) on the basis of cross-immunity, serological testing, vector specificity and pathogenicity; these parasites are now considered to be separate species in their own right (Zahler *et al.*, 1998; Carret *et al.*, 1999). With regard to small piroplasms, three genetically and clinically distinct species are currently recognised to cause disease in dogs; *Babesia gibsoni*, *Babesia conradae* and a *Babesia microti*-like piroplasm (named *Theileria annae*) (Zahler *et al.*, 2000; Camacho *et al.*, 2001). Traditionally, the morphology of the protozoan (piroplasm merozoites) within the red blood cell was used as the chief taxonomic determinant (Solano-Gallego *et al.*, 2016). The orientation of the parasite in the red blood

cells (RBCs) depends on its size because large pyriform parasites meet at their pointed ends at an acute angle to each other and small forms make an obtuse angle to each other.

2.4 Geographical Distribution of *Babesia* spp.

Babesia canis canis is transmitted by *Dermacentor reticulatus* in Europe, *B. canis vogeli* transmitted by *Rhipicephalus sanguineus* in tropical and subtropical regions, and highly pathogenic *B. canis rossi* transmissible by *Haemaphysalis leachi* in South Africa (Uilenberg 2006). *Babesia canis canis* is the most important agent of babesiosis in Europe. *Babesia gibsoni* has been found to occur in Asia, North America, Northern and Eastern Africa (Criado-Fornelio *et al.*, 2003). In Central Europe, autochthonous canine babesiosis due to *B. canis* was recorded in several countries. In Slovakia, first cases of autochthonous babesiosis started to emerge in 1997; the first case of babesiosis in dog was documented in 2000 (Chandoga *et al.*, 2002). The incidence of babesiosis nearest to the Czech Republic was observed in the neighborhood of Malacky, Slovakia. So far, only imported babesiosis has been observed in the Czech Republic. Dynamics of the spreading of canine babesiosis in Europe markedly changed in the last few years. This is largely connected with the expanding area of *D. reticulatus* distribution. In fact, the expansion of the vector's area and the increasing number of clinical cases of babesiosis has been observed also in all adjacent

countries. Babesiosis has spread to Germany, Austria, Hungary and Poland as well as Switzerland (Hornok and Farkas, 2009).

Table 2.2: Distribution, vectors, morphological and molecular characteristics of canine babesial species.

Size	Species	Geographical distribution	Vectors in dog
Large	<i>B. canis</i>	Europe, Asia	<i>Dermacentorreticulatus</i>
	<i>B.rossi</i>	Africa,Nigeria, Sudan	<i>Haemaphysaliselliptica</i> (formerly <i>H. leachi</i>)
	<i>B. vogeli</i>	Worldwide	<i>Rhipicephalussanguineus</i>
	<i>Babesia</i> <i>spp.</i>	North Carolina, USA	Unknown
Small	<i>B. gibsoni</i>	Worldwide	<i>Rhipicephalussanguineus?</i>
	<i>B. conradae</i>	California, USA	<i>Rhipicephalussanguineus?</i>
	<i>B.microt-like</i> (<i>Theileriaanna</i> syn. <i>B. vulpes</i>)	Southern Europe	Most probably <i>Ixodeshexagonus?</i>
	<i>Theileria equi</i>	Africa, Europe, Asia	Unknown
	<i>Theileria annulata</i>	Africa, Europe, Asia	Unknown

(Matijatko *et al.*, 2012).

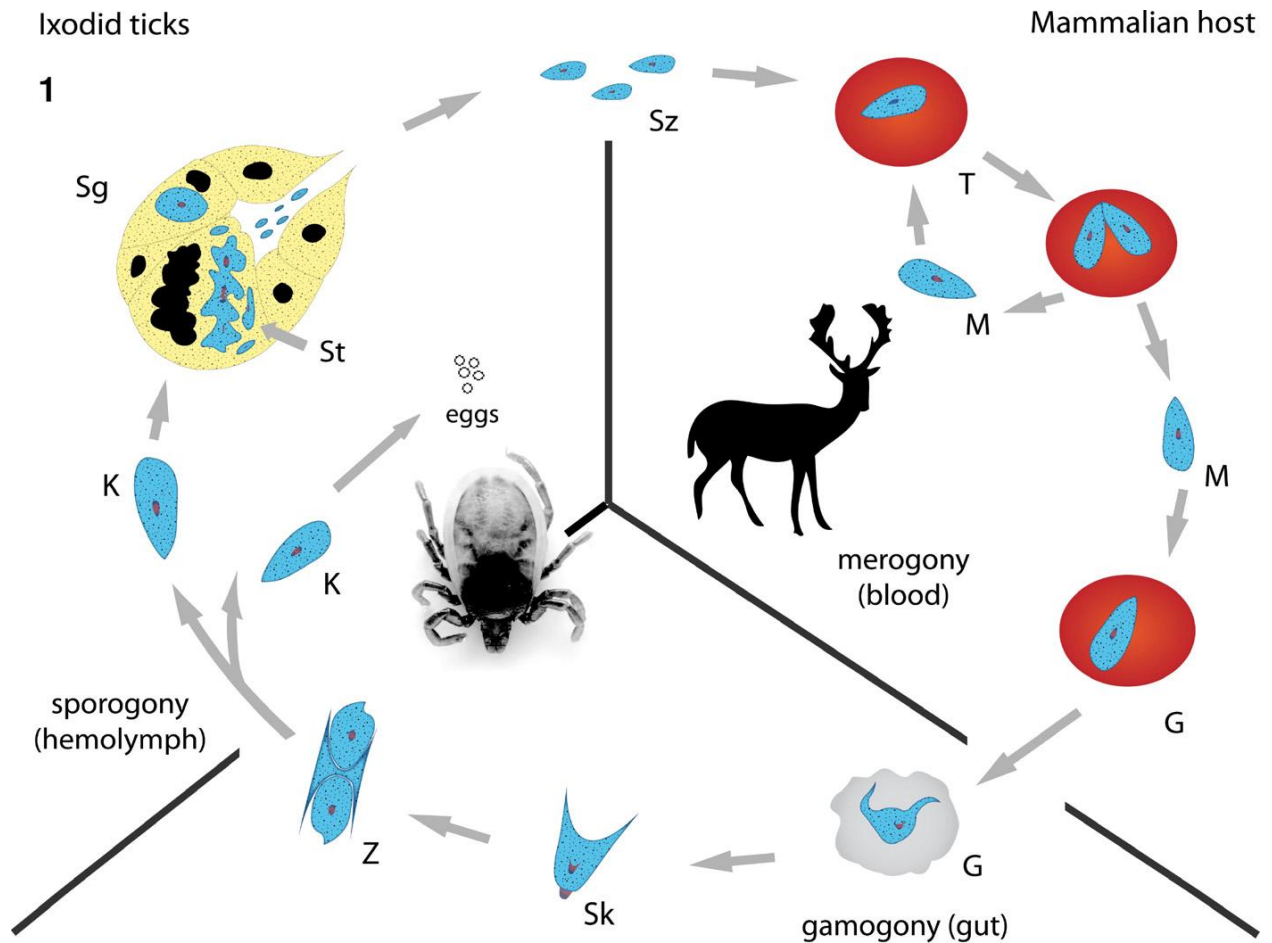


Figure 2.1: General life cycle of *Babesia* species

<https://www.google.com.ng/search?q=life+cycle+of+Babesia+species+in+dog>

Simplified general life cycle of *Babesia* spp. (modified from Mehlhorn and Piekarski, 2002). *Babesia* life cycles consist of merogony, gamogony, and sporogony. Infection is acquired when sporozoites (Sz) are transferred during tick feeding. Sporozoites then invade erythrocytes and develop into trophozoites (T). Trophozoites divide by binary fission and produce merozoites (M) which continue infection and reinitiate the replicative cycle in the host. Some trophozoites develop into gametocytes (G) which can initiate infection in the tick vector. In the tick gut gametocytes develop into "Strahlenkörper" (Sk) which fuse to form a zygote (Z) develops into a kinete (K). Kinetes gain access to the hemolymph of the tick, replicate, and invade various organs. Note that members of the *Babesia* spp. sensu stricto groups can infect the ovaries and be transmitted transovarially via the eggs (E), so that all stages (larvae, nymphs and adults females) are potentially infective, whereas members of the *Babesia microti*-like groups are only transmitted from one stage to the next (transstadially), so that larvae are rarely if ever infected. Sporogony is initiated when kinetes invade the salivary glands (Sg). Here, the parasite forms a multinucleated sporoblast (St). Newly developed sporozoites (Sz) will then be injected into the host with tick saliva upon the next blood meal.

2.5.1 Development of *Babesia* parasite in the ticks

When ticks ingest *Babesia*-infected erythrocytes from the host, most of the parasites are degenerated and destroyed. However, “pre-gametocytes” are not degenerated and they develop into gametocytes. Within several hours of gestation, elongated bodies characterised by an arrowhead-shaped ray begin to appear. The gametes fuse in the lumen of the tick digestive system. After zygote internalization, it transforms into a motile form –an ookinete. The ookinete exits in the midgut epithelium and invades the tick tissues and organs. The invasion of the ovary in female ticks results in infection of tick eggs. The developmental cycle of *Babesia* includes asexual multiplication, sporogony and the development of kinetes. Sporogony occurs in salivary glands of ticks where sporozoites are produced. These are passed into a mammalian host to close the cycle (Schnittger *et al.*, 2012).

2.5.2 Development of *Babesia* spp. in the vertebrate host

Vertebrate hosts are infected by sporozoites from saliva after a tick bite. *Babesia* spp. sporozoites directly penetrate the red blood cells and their parasitic development occurs (Schnittger *et al.*, 2012). Two merozoites are produced by binary fission. After erythrocytes are lysed, each merozoite invades a new erythrocyte and successive merogonies occur. The multiplication is asynchronous and various divisional stages of the parasite are present in the blood system at the same time. The location and size of merozoites differ between *Babesia* and host species. The size of merozoites determines the affiliation of *Babesia* spp. into large or small groups (Table 2) (Gray *et al.*, 2010). In a large group *Babesia* spp. merozoites are larger than the erythrocyte radius, whereas in a small group the merozoites are smaller than it. This is not consistent with the genetic basis. Further, interspecific

phenotypic variability, with different parasite sizes or forms in human, bovine, or rodent blood cells was described for *B. divergens* (Schnittger *et al.*, 2012).

2.6 Transmission of *Babesia* spp. Infection

Schoeman(2009), reported that various species of ticks such as *Rhipicephalus sanguineus*, *Dermacentor* spp. and *Haemaphysalis ellipticum* can transmit the large *Babesia* of dogs, whereas *B. gibsoni* transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis longicornis*. *Babesia annae* is thought to be transmitted by *Ixodes hexagonus* (Lobetti, 2006). Both trans-stadial and trans-ovarial transmission can occur and ticks are believed to remain infective for several generations. Although protozoans of the genus *Babesia* undergo part of their life-cycles in the tick vector, the merozoites circulating in the blood may be transmitted to a healthy host directly by blood transfusion. This scenario has been described for *B. gibsoni* infection (Stegeman *et al.*, 2003), which can also be transmitted vertically, from dam to offspring (Birkenheuer *et al.*, 2005) and by direct contact between dogs through wounds among fighting dogs, saliva or blood ingestion (Birkenheuer *et al.*, 2005; Jefferies *et al.* 2007; Yeagley *et al.*, 2009).

2.7 Pathogenesis of *Babesia* spp. Infection

The incubation period for babesiosis caused by *B. canis* varies from 10- 21 days (Schoeman, 2009). The severity of babesiosis in dogs ranges from subclinical infection, the development of mild anaemia to widespread organ failure and death. The critical determinant of this variable pathogenesis is the *Babesia* species, yet other factors such as

the age and immune status of the host and concurrent infections also plays a role. All species may cause pyrexia, anorexia, splenomegaly, anaemia and thrombocytopenia. Direct parasite-induced red-cell damage, increased osmotic fragility of infected cells, oxidative and secondary immune-mediated injury of the erythrocyte membrane result in a combination of intravascular and extravascular haemolysis. In broad terms it is generally agreed that the least pathogenic of the well-recognized canine piroplasm species is *Babesia vogeli*, at least in adult dogs, and the most virulent is *Babesia rossi* in Africa (Lobetti, 1998; Solano-Gallego *et al.*, 2008). The pathogenicity of *Babesia canis*, *Babesia gibsoni*, *Theileria annae* and *Babesia conradae* is moderate to severe in dogs, but it should be stressed again that a wide range of clinical signs of varying severity can be observed in individuals. *Babesia conradae* is considered to be more pathogenic than *B. gibsoni*, resulting in higher parasitaemias and more severe anaemia (Kjemtrup *et al.*, 2006). The clinical consequences of chronic babesial infection are unclear and while most dogs appear to tolerate this state of premunity with few ill effects, theoretically they remain at risk of developing immune-mediated complications and recrudescence of clinical disease (and parasitaemia) if immunocompromised at a later time. Chronic infection may be inconsequential in some dogs and may be even beneficial for hosts living in endemic regions by protecting them from further disease (Brandao *et al.*, 2003). Typical symptoms of acute babesiosis include apathy, anorexia, fever and general weakness (Jarmila *et al.*, 2012). Clinical signs also include anaemia, pale mucous membranes, depression and splenomegaly (Schoeman, 2009). *Babesia canis* infections result in a more variable pathogenicity, intermediate between *B. rossi* and *B. vogeli*. A study from Italy, presumably describing *B. canis* infection reported anaemia in the majority of dogs and thrombocytopenia in all cases (Furlanello *et al.*, 2005). The disease leads to haemolytic

anaemia along with thrombocytopaenia, lymphadenopathy and splenomegaly. Jaundice and haematuria can occur as well. Clinical signs are often very variable and the disease can have mild to per- acute course that results in death within 2 days. The infection induces an antibody reaction which is usually not strong enough to eliminate all babesia parasites in a host organism. Animals therefore become chronic carriers of the infection (Vercammen *et al.* 1997). Antibodies acquired after the infection with one *Babesia* species do not protect against the infection with other species (Uilenberg 2006). Animals that recover from the infection and live in endemic localities acquire non- sterile immunity (Jarmila *et al.*, 2012).

2.8 Clinical Signs of *Babesia* spp. Infection

The clinical signs of babesiosis ranged from subclinical to severe life-threatening signs (Birkenheuer, 2014). The wide range of clinical manifestations depends very much on the species of *Babesia* causing infection and other factors that affect the severity of the disease, including age, splenectomy, immune competence, and concomitant infection or disease (Solano-Gallego *et al.*, 2011). Bohm *et al.* (2006), reported an association of the disease severity with parasite density in *B. rossi* infection. However, limited information is available regarding disease severity and parasite density in other *Babesia* species. In a recent study, parasite density was not different between survivors and non-survivors in dogs infected with *B. canis* (Eichenberger *et al.*, 2016). Solano-Gallego *et al.*, (2016), has postulated that it is likely that different *Babesia* spp. might result in different parasitemias due to differences in disease severity, but further studies need to confirm this hypothesis. Differences in virulence have been described among *Babesia* species infecting dogs. In general, it is assumed that the least pathogenic large-sized species of *Babesia* is *B. vogeli*,

at least for adult dogs, and that the most virulent species is *B. rossi*. The pathogenicity of small-sized *Babesia* spp., such as *B. gibsoni* and *B. microti-like* sp., is moderate to severe (Irwin, 2009; Solano-Gallego *et al.*, 2011). Frequent clinical signs associated with canine babesiosis are apathy, weakness, anorexia, pale mucous membranes and a poor general condition. All *Babesia* species can cause fever, enlarged lymph nodes and spleen, anaemia, thrombocytopenia, jaundice and pigmenturia. Thrombocytopenia is frequently detected and when present, varies from mild to severe, as does anaemia. Other abnormalities that can be detected include hypoalbuminemia and hyperbilirubinemia (Solano-Gallego *et al.*, 2011). Depending on the infective species and the course of infection, anaemia can be regenerative; nonregenerative anaemia is more typically associated with *B. canis* (Solano-Gallego *et al.*, 2008). In all species, anaemia is caused by a combination of intravascular and extravascular hemolysis resulting from parasite-caused injury and rupture of red blood cells, the cells' increased osmotic fragility, and the activity of secondary immune-mediated processes. Many dogs could present other clinical signs that are not directly related to hemolysis by piroplasms but that demonstrate the involvement of other organs. These complications are especially prevalent following infection by *B. rossi*. A non-exhaustive list includes weight loss, acute or chronic nephropathy, glomerulonephritis, coagulation disorders (disseminated intravascular coagulation), jaundice from liver disease, immune-mediated hemolysis or thrombocytopenia, hemoconcentration, shock, metabolic and/or respiratory alkalosis, and/or acidosis, gastrointestinal disorders (vomiting or diarrhea), pancreatitis, ascites, ocular lesions (uveitis or blindness), myalgia, rhabdomyolysis and respiratory problems (edema or acute respiratory distress) (Jacobson, 2006). It must be noted that many "carrier" dogs with chronic infections will not present with any clinical signs as the result of premunition or concomitant immunity unless their health deteriorates,

for example from immunosuppressive treatment, splenectomy, or any other immune-compromised situation. This phenomenon was reported in Greyhounds infected by *B. vogeli* and in Pit Bull. It results from the inability of the immune system to eliminate the infection, which then establishes itself with more rigour when the immune system is in abatement (Solano-Gallego *et al.*, 2011).

2.9 Diagnosis of *Babesia* spp. Infection

Canine babesiosis, which ranges in severity from relatively mild to fatal depending on the causative *Babesia* species, is a tick-borne infection of world-wide importance. The diagnosis of *Babesia* spp. infection is based usually on the morphology of the intraerythrocytic stages in blood films, but the similarity between species and subspecies has been a limiting factor (Kjemtrup *et al.*, 2000; Jefferies *et al.*, 2007). It has been classically diagnosed by demonstrating intra-erythrocytic trophozoites in stained blood smears. Currently, molecular methods have contributed to the delineation between species and subspecies of *Babesia* organisms as described in epidemiological studies in different regions (Zahler *et al.*, 1998; Caccio' *et al.*, 2002; Matjila *et al.*, 2004).

In Nigeria canine babesiosis is endemic (Abdullahi, 1990) and in abundant (Kamani *et al.*, 2013). On the basis of presumed vector specificity, pathogenicity and morphological aspects, it was admitted that *B. canis* was the agent involved in canine babesiosis cases in the Federal Capital Territory (Jegade *et al.*, 2014; Opara *et al.*, 2017). However, studies on the incidence, the prevalence and the characterization of *B. canis* subspecies are completely lacking.

Abdel Aziz *et al.*, (2014), reported that accurate and correct diagnosis of babesial infections plays an important role in monitoring, management and control. A large diversity of diagnostic techniques exist, each of which has its own limitations. The diagnostic tests for babesiosis can be divided into three categories; traditional methods, including microscopy and culture; serological techniques; and molecular based methods. It is increasingly recognized that a combination of detection techniques is necessary for accurate diagnosis. Limitations of clinical data, parasite morphology and serological cross-reactivity, have led to an increased interest in molecular based methods and highlight the need for their application in clinical medicine (Jefferies 2006).

2.9.1 Light microscopy

Babesia parasites have a complex life cycle, therefore identification of the different stages of the parasite in mammalian or arthropod host tissues can be used for direct diagnosis purposes. Microscopy was the first technique utilized to identify piroplasms and remained the most common diagnostic technique for researchers and clinicians for the diagnosis of babesiosis.

2.9.1.1 Thin and thick blood smears technique

Thin blood smear was the first method to detect *Babesia* parasites in clinical samples, a method still used today very effectively in most diagnostic laboratories. It is easy to do and inexpensive but requires an experienced microscopist to differentiate species and is reliable only if the amount of parasites in the blood is high enough to be detected, which is usually only possible during acute cases. Special attention must be paid to the source of the blood; peripheral blood is useful only for species like *B. bigemina*, *B. divergens*, or *B. gibsoni*,

which do not adhere to the vascular endothelium. Some species like *B. bovis* or *B. canis* adhere to endothelial cells (Jacobson, 2006) and their diagnosis using this method is feasible if the blood sample is taken directly from a blood capillary from the ear or the skin of the tail, compared with peripheral blood taken from the jugular or caudal veins, since capillary blood contains a higher percentage of infected erythrocytes for these species. The observation of paired intraerythrocytic merozoites is indicative of infection but there are other stages of the parasite like the trophozoites, which present different forms and sizes depending on the species, and these make their detection difficult and time-consuming.

Another technique developed to detect low levels of parasitemia, especially in cases where *B. bovis* is involved, is based on thick smears of infected blood stained with Giemsa (Boseet *al.*, 1995). The advantage of the thick smear is that a large amount of erythrocytes is analyzed in a reduced amount of space; therefore the probability of finding infected cells is ten times higher than in the thin smear (Bishop and Adams, 1973). The method is usually recommended when *B. bovis* infections are suspected or when a subclinical disease occurs. This method relies on a very experienced microscopist who must identify the *Babesia*-infected cells among a mass of conglomerated erythrocytes.

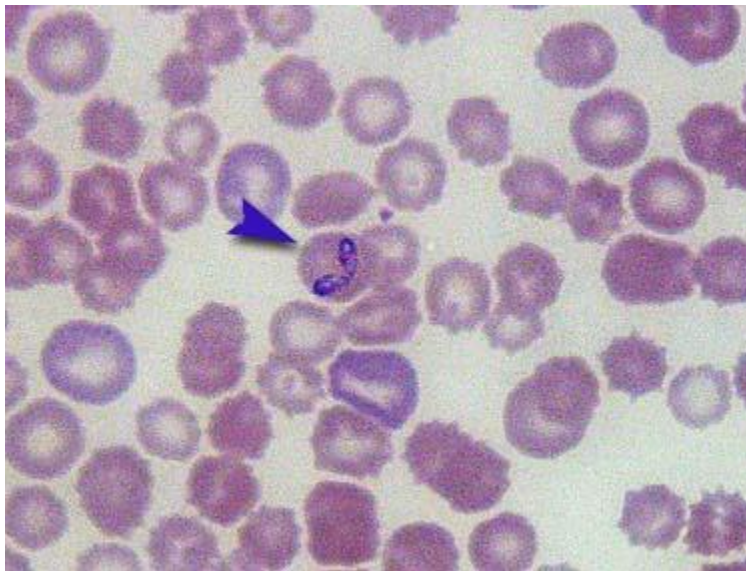
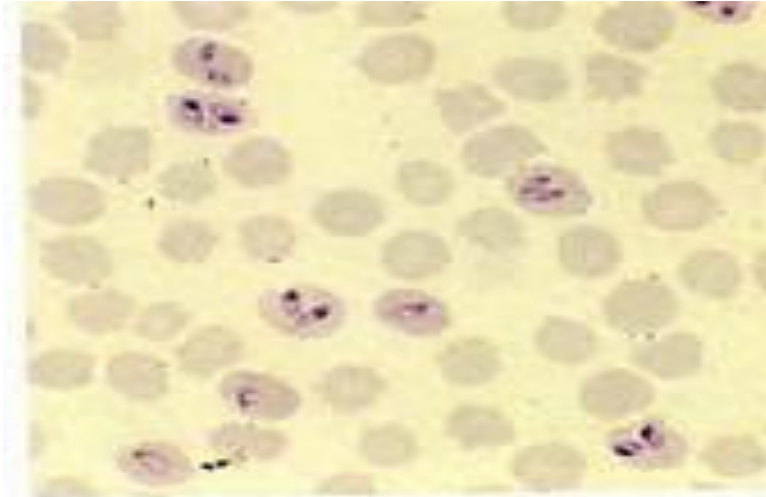


Figure 2.2: *Babesia canis* in a Giemsa-stained thin blood smear
(<http://www.yamagiku.co.jp/pathology/index.htm>)

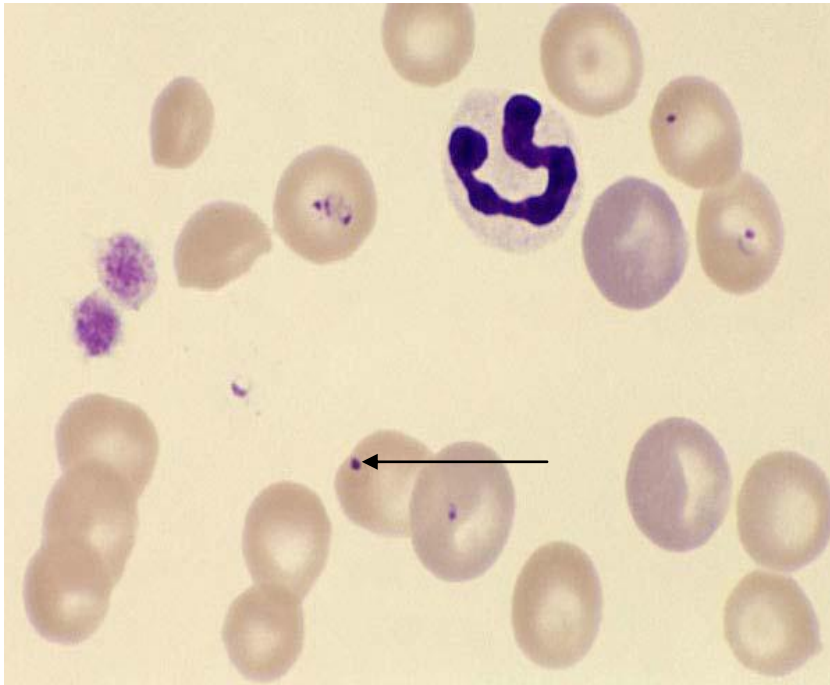


Figure 2.3:*Babesia gibsoni* in a Giemsa-stained thin blood smear (<http://www.yamagiku.co.jp/pathology/index.htm>).

2.9.1.2 Brain smears

When the death of an animal is presumed to be from babesiosis caused by *B. bovis* due to presence of nervous clinical signs, identification of the parasite can be done by brain smears. In this case, a small sample of grey matter of the cerebral cortex is placed on a slide and the tissue is smeared using another slide. The brain tissue is fixed and Geimsa stained as mentioned above. The diagnosis is based upon observation of brain capillaries filled with infected erythrocytes (Ristic, 1981). Almost one hundred percent of erythrocytes present in the brain capillaries are infected (Uilenberg, 1972). Smears of other organs as the kidney or liver can also be carried out with good results.

2.9.1.3 Haemolymph smears

As a tick-transmitted pathogen, *Babesia* parasites infect several tick tissues. Immediately after repletion with a blood meal, the tick acquires intracellular parasites, which soon escape from the erythrocytes and remain in the gut lumen for a short period of time. Sexual reproduction occurs and infective diploid cells penetrate the midgut cells of the tick and transform to motile stages called kinetes after 72 h post-repletion (Riek, 1964). Kinetes are motile stages of the parasite, which reach the haemolymph of the tick and infect several organs including the ovary of mated females. A good approach to identify adult females infected with *Babesia* is detecting kinete stages in the haemolymph by performing the haemolymph test. The haemolymph test requires an experienced microscopist since female ticks collected from cattle in endemic areas have a very low amount of kinetes.

2.9.1.4 Animal inoculation test

Sensitive and specific tests such as sub-innoculation of infected blood into rodents or susceptible splenectomized animals have been used in clinical trials and research settings, but are impractical for routine diagnostic purposes

2.9.2 Immunological methods

2.9.2.1 Indirect fluorescent antibody test

When the number of *Babesia* parasites in the blood is too low to be detected, searching for antibodies against proteins of blood stages has proven to be a reliable tool to identify infected carriers or previously exposed animals. The IFAT was first described by Ristic and Sibinovic in 1964 to detect antibodies against *B. caballi* in chronically infected horses

(Ristic and Sibinovic, 1964). Since then, it has been adapted to all *Babesia* species and has a good level of specificity and sensitivity (Duh *et al.*, 2007). The IFAT is based on the recognition of parasite antigens by serum antibodies in the blood of the tested animal. Bound antibodies are detected by a fluorochrome-labelled antibody anti-Ig (secondary antibody).

2.9.2.2 Enzyme-linked immunosorbent assay

The Enzyme-linked immunosorbent assay (ELISA), has the advantage of non-subjectivity, capacity to read a large number of samples easily and presents higher specificity than the IFAT. There are several ELISA versions for the detection of several *Babesia* species including *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, *B. canis*, *B. gibsoni*, and *B. microti* (Weiland, 1986). This ELISA method was initially performed using purified crude antigen from infected blood, but the cross-reactivity with serum proteins was very high (Morzaria *et al.*, 1992). Recent ELISA methods include the use of recombinant antigens and monoclonal antibodies, thus increasing specificity and diminishing unspecific binding and signal. Modern biotechnology allows the expression of antigenic pathogen proteins, which can be bound on ELISA wells and used to evaluate the presence of anti-babesia antibodies using an anti-IgG conjugated with an enzyme, usually peroxidase (Fukumoto *et al.*, 2001).

2.9.2.3 Immunochromatography test

The immunochromatographic test (ICT), is a rapid diagnostic device that detects antibodies against a specific antigen in a small amount of serum by means of specific antibody and a recombinant antigen both impregnated on a nitrocellulose membrane-based test strip

(Weigl *et al.*, 2008). The ICT is very convenient because it is very easy to perform and read, therefore does not require a trained technician; it does not use any special equipment, so it can be implemented in the field and it has a low cost comparable with other techniques; it is a fast test taking only ten to fifteen minutes to complete and it is very stable under different temperatures (Wongsrichanalai *et al.*, 2007).

2.9.3 Molecular methods

Molecular methods aimed to detect nucleic acids have been very useful when immunological methods do not work. Detecting nucleic acids is an indirect way of detecting the parasite so they are still considered indirect methods. However, the sensitivity and specificity of these methods are very high and over the past years many different approaches have been developed to detect *Babesia* species in their hosts and their vectors.

2.9.3.1 Polymerase chain reaction

The diagnosis of babesiosis has been revolutionized with the advent of molecular biology particularly the polymerase chain reaction (PCR). Fahrimal, (1992), described the first PCR test for *Babesia* and since that time, a number of PCR based tests have been developed (Carret *et al.*, 1999b; Jefferies, 2006). The majority of these tests have been based on 18S rRNA gene sequences, but other genes such as the internal transcribed spacer ITS1 and ITS2 regions, cytochrome b, and P50 have also been used as gene targets. Studies comparing the sensitivity of PCR tests for diagnosis of babesiosis to detection by microscopy have found that PCR is more sensitive in most cases (Takeet *et al.*, 2017). The PCR is highly sensitive and specific. Primers used in the test can be designed to be genus specific or can amplify species-specific sequences of DNA, so can detect a single species.

Fukomuto *et al.* (2001) and Jefferies *et al.* (2003), assessed the PCR sensitivity for detecting canine *Babesia* by serially diluting blood samples of a known percentage parasitaemia (ranging from 0.000118- 0.00000073%). The high degree of sensitivity of PCR is important in diagnosing sub-clinical infections when the parasitaemia is low (i.e. <1%). Krause *et al.* (1996), showed that PCR is more sensitive than blood smear examination and IFAT for the diagnosis of acute *Babesia* infections.

2.9.3.2 Reverse line blot hybridization

For the detection of multiple genera, species or strains in a sample of blood or tissue, there is a better option than PCR. Reverse line blot hybridization is a technique initially developed for the diagnosis of sickle cell anaemia (Saiki *et al.*, 1988), then it was used to detect multiple serotypes of *Streptococci* (Kaufhold *et al.*, 1994) and three genotypes of *Borrelia* (Rijpkema *et al.*, 1995). Visualisation of a dot indicates the spot where the amplified PCR product recognized and bound the specific oligonucleotide (Criado-Fornelio, 2007). This method has been used for the detection of several species of *Babesia* including *B. bovis*, *B. bigemina*, *B. divergens*, *B. major*, *B. motasi*, *B. crassa*, and *B. caballi*, although this technique is mostly used for the combined detection of different genera and species in epidemiological studies (Niu *et al.*, 2009). The most recognized advantage of RLB is that the membranes can be re-used up to 20 times, thus reducing the overall costs of the procedure. Certainly this technique is valuable when several pathogens, species or strains are present in the same sample.

2.9.3.3 Real time polymerase chain reaction

Real Time PCR (RT-PCR), is a technique that amplifies and quantifies a specific DNA fragment. The DNA amplified is quantified as it is being generated (in “real time”), therefore it determines whether a specific sequence is present in the sample, and it also determines the number of copies of that sequence. The RT-PCR does this by detecting a fluorescent signal emitted during the PCR reaction as an indicator of the production of the sequence being generated in each cycle; this is opposite to what happens during the end-point PCR, where the detection of the product is at the end of the reaction. RT-PCR has many advantages over conventional PCR; it does not require post-PCR analysis because the signal is detected as it is generated, therefore, it is faster and it does not generate expenses due to electrophoretic analysis or photo-documentation. Additionally, the fact that the positive fluorescent signal is detected by the thermocycler, the sensitivity is higher compared with the in-gel analysis of ethidium bromide-stained DNA detected in a conventional PCR, and it has been reported to be at least fourfold (Chiang *et al.*, 1996).

There are several formats for RT-PCR, and the most common are based on the use of SYBR Green or TaqMan probes. The first RT-PCR method reported for the quantification of *Babesia* was in 2003 when SYBR Green was used to quantify the transcription of the *Babesia bigemina rap-1* locus genes (Suarez *et al.*, 2003). Since then several protocols have been published for the quantification of *B. gibsoni*, *B. microti*, *B. bovis*, *B. bigemina*, *B. caballi*, *B. canis*, and *B. orientalis*, (Peleg *et al.*, 2009; Ramos *et al.*, 2011; He *et al.*, 2011). The sensitivity of RT-PCR has been reported to be also higher than that of conventional PCR, for example, for *B. bovis* and *B. bigemina*, it was reported to detect 0.75 copies of DNA per μl of blood (Buling *et al.*, 2007). Probably the only disadvantage of this

methodology is the higher costs of the equipment, which usually doubles or triples the cost of a conventional PCR machine.

2.9.3.4 Loop mediated isothermal amplification

The Loop Mediated Isothermal Amplification (LAMP) is a detection method that amplifies DNA under isothermal conditions with high efficiency, specificity and speed. LAMP is based on the use of four primers specifically designed to amplify six different sequences on the same target DNA, with the aid of an isothermal DNA polymerase. By using four primers to amplify the same target sequence, the specificity of the amplification is increased, solving in part the background amplification observed in most nucleic acid amplification methods. Because LAMP produces a large amount of DNA, it can be analysed by direct observation of; a) an intercalating fluorochrome like SYBR green, ethidium bromide, etc (Notomi *et al.*, 2000), b) the turbidity generated by magnesium pyrophosphate precipitation as a result of pyrophosphate ion by-product (Mori *et al.*, 2001), or c) a newer colorimetric method using the metal ion hydroxy naphthol blue (HNB) as an indicator. This colorimetric assay is reported to be superior to the existing colorimetric assays for LAMP with regard to reducing contamination risks and expenses (Goto *et al.*, 2009). Because of this, LAMP does not require of electrophoresis and image documentation post-analysis. Additionally, the isothermal conditions required for the DNA polymerase make the use of a thermocycler dispensable, reducing overall cost and time. The sensitivity of LAMP has been estimated to be of a minimum of six copies of target DNA (Notomi *et al.*, 2000). *Babesia* species have been detected by LAMP including *B. gibsoni*, *B. caballi*, *B. bovis*, *B. bigemina*, *Babesia canis* and *B. orientalis* (Muller *et al.*, 2010).

2.9.3.5 PCR-restriction fragment length polymorphism

Studies have demonstrated that RFLP use allows the discrimination of amplified DNA products on the basis of nucleotide differences (Zahler *et al.*, 1998). Restriction enzymes are used to cleave DNA at specific sites, so as to produce different range of smaller DNA fragments which thus can be used as a means of differentiating species or genotypes. Each of the *B.canis* subspecies has also been differentiated by RFLP using a partial region of the 18S rRNA gene (Carret *et al.*, 1999b). This method reduces the time and cost of detection and differentiation as the amplified DNA does not need to be sequenced.

2.10 Treatment and Control of *Babesia* spp. Infection

Control of babesiosis can be either by tick management, immunization, and anti-babesia drugs or by a combination of these approaches (Suarez and Noh, 2011). Chemotherapy of babesiosis is important for controlling the disease either to treat field cases or to control artificially induced infections (Rodriguez and Trees, 1996). In endemic areas, sick animals should be treated as soon as possible with an anti-parasitic drug. The success of the treatment depends on early diagnosis and the prompt administration of effective drugs (Vial and Gorenflot, 2006). In addition, supportive therapy such as blood transfusions, anti-inflammatory drugs, tick removal, iron preparations, dextrose, vitamins (B complex), purgatives, and fluid replacements, may be necessary in severe cases of babesiosis (Zintlet *et al.*, 2003). The indiscriminate use of anti-*Babesia* prophylactic agents, including the administration of the drug at sub lethal blood levels to animals, can produce the development of drug resistant parasites, a problem that will require the development of new drugs (Vial and Gorenflot, 2006). New drugs with a chemotherapeutic effect against

babesiosis, with high specificity to the parasites and low toxicity to the hosts are desired to control the disease (AbouLaila *et al.*, 2010).

Drugs used for the treatment of canine *Babesia* infections includes; Imidocarb, Atavaquone, Azithromycin, Diminazene aceturate, Phenamidine isethionate, Pentamidine, Parvaquone, niridazone and Trypan blue. These are known to be unable to completely eliminate the infections, but can only reduce the severity of the clinical signs and the mortality (Birkenheuer *et al.*, 1999). Reports have documented that these drugs show varying degrees of success rates either alone or in combination in terms of eliminating the parasites or reducing the parasite load (Matjila *et al.*, 2007). Birkenheuer *et al.* (2004), reported that a combination of azithromycin and atovaquone therapy is able to treat *Babesia gibsoni* infections in dogs successfully without infected erythrocytes being seen in capillary blood smear, and blood from dogs with this combination therapy was shown to be negative on PCR assay for about 4 months. This report is encouraging when compared to single therapy with atovaquone in which *B. gibsoni* parasite DNA were intermittently detected in the blood of experimentally infected dogs 33 days after the last treatment (Matsu *et al.*, 2004), thus supporting the assertion by Choidioni *et al.* (1995) and Wittner *et al.* (1996) that recurrence of disease and decreased sensitivity to protozoa parasites (*Plasmodium falciparum* and *Babesia microti*) occurred following therapy with atovaquone alone. Although research has shown that treatment of canine *Babesia* infections due to *B. gibsoni* with diminazene and or imidocarb is ineffective (Birkenheuer *et al.*, 1999; Stageman *et al.*, 2003), however, it has been documented that imidocarb has the capacity to stop the multiplication of the intra-erythrocytic parasites and also allow the persistence of several parasites in order to induce immunity and as such are desirable for the treatment of

infection due to *B. canis* (Bourdoiseau, 2006). Ogo *et al.* (2011), reported that most, if not all anti-*Babesia* drugs are toxic to the host and should be used with the utmost caution. Toxicity with these drugs is expressed in form of CNS disorders (diminazene); vomiting, colic and diarrhoea alongside hepatic, renal or vascular complications (imidocarb). It has been recommended that supportive therapy using intravenous fluids, corticosteroids and blood transfusion be used alongside irrespective of the drug(s) used for the treatment of canine *Babesia* infections.

2.11 Vaccines against *Babesia* spp. Infection

Most research has been focused on bovine babesiosis, because it is a serious livestock health concern. The first attempts to develop a vaccine used an attenuated organism passaged in vivo in calves. The vaccine obtained proved to be a successful reducer of virulence (Callow *et al.*, 1997). Although the vaccine was produced using good manufacturing practices and has become adequately standardised, it has several drawbacks including: potential adverse reactions, handling or storage issues, antagonistic and synergistic interaction with concurrent disease, stress, and inadequate immune response (De Waal *et al.*, 2006). Another strategy for building adequate immunity to *Babesia* parasites is the subunit vaccine. Numerous proteins have been put forward as vaccine candidates, e.g. SBP-1, SBP-2, SBP-3, high molecular weight (MW) antigen I, RAP-1, 12D3, MSA-1, MSA-2, HSP family proteins, and *Babesia divergens* apical membrane antigen-1 (BdAMA-1) (Moreau *et al.*, 2015). These vaccines are very promising, because they are safe, high-throughput, eminently manufacturable, and putatively efficient. However, due to the changeable nature of this target, the design of the vaccine is complex (Zwart and Brocklesby, 1979), and in practice the solution obtained may not induce

immunity to different strains. With respect to canine babesiosis control, two commercial vaccines are available in Europe now (Schetters *et al.*, 2009). They are based on soluble antigens derived from *B. canis* and *B. rossi* cultures. However, their efficacy can be questioned because they do not prevent infection and only affect the course of the disease. Immunity lasts only up to six months and a skin reaction at the injection site have been noted (Freyburger *et al.*, 2011). Thus, vaccination does not stop the spread of *B. canis*. The efficacy of the vaccine is even lower in the control of *B. rossi* infection. Currently, there is no available vaccine against canine babesiosis caused by small *Babesia* species. Recently, Sunaga *et al.*, (2014) tested soluble antigens from an attenuated *B. gibsoni* culture for vaccine preparation. The challenge showed a reduction of parasitaemia and partial protection against infection. However, the research was carried out on a small number of animals, and now it is essential to perform proper clinical trials of the vaccine prior to its implementation. Vaccine development still poses a huge challenge to scientists worldwide, and widespread infections are still a serious issue.

2.12 Antigenic Diversity and Variation in *Babesia* spp. Infection

Vaccination experiments had shown that dogs vaccinated using soluble parasite antigens (SPA) of a *B. canis* strain were protected against a homologous challenge infection, but not against a homologous challenge infection with a heterologous *B. canis* strain B. This confirms that there are functional antigenic differences between *B. canis* strains (Schetters, 2005). This antigenic diversity may be explained by allelic diversity. Different *Babesia* strains/clones express particular proteins from a particular family. This was shown by Carcy *et al.* (2006), on MSA-1 and MSA-2 merozoite surface antigens. The antigenic diversity might be attributed to homologous recombination during sexual stages in the final

host (tick); however, this process is still not well understood and some reports based on molecular analysis of gene organisation, point to the potential role of other mechanisms. Antigenic variation is generated by a complex phenomenon, a dynamic genetic process at the clonal level. This process has been reported in *B. bovis* and other protozoa parasites (Allred and Khedery, 2004). This molecular event occurs when antigenic molecules are not expressed at the surface of infected erythrocytes. In this situation they may play a role in the specific adhesion of infected erythrocytes to the capillary endothelium of particular organs (Matijatko *et al.*, 2012). This process is called sequestration and plays a crucial role in sustaining low-level chronic infection in immune animals. This helps to maintain immune responses (Matijatko *et al.*, 2012). However, antigenic variation has not been shown for *B. canis* (Vercammen *et al.*, 1995; Matijatko *et al.*, 2012).

2.13 Classification and Taxonomy of Ticks

Ticks are obligate, haematophagous ectoparasites in the phylum Arthropoda. Within the order Parasitiformes, suborder Ixodida, superfamily Ixodoidea, all ticks are included in three families comprising 896 species: Ixodidae, Argasidae and Nuttalliellidae. In these families, the Nuttalliellidae comprises a single species, *Nuttalliella namaqua*. The remaining two families consist of the hard ticks (Ixodidae) and the soft ticks (Argasidae) (Guglielmone *et al.*, 2010). Ixodid ticks are differentiated from Argasid ticks by the presence of a sclerotized scutum. Fourteen genera and 702 species are recognized in the family Ixodidae, and species within the genera *Ixodes*, *Rhipicephalus*, *Dermacentor*, *Amblyomma*, *Hyalomma* and *Haemaphysalis* commonly infest dogs.

2.13.1 Argasid ticks

The Argasidae consists of 193 species and the most important argasid ticks of medical and veterinary importance belong to the genera *Argas*, *Ornithodoros* and *Otobius*. The Argasidae live near their favourite host (nest, pen, stable, hut, etc.), and the parasitic stages (nymphs are always parasitic, larvae and adults usually) feed for a short period only on the host (a matter of minutes to say an hour, with the exception of *Otobius*, where the immatures are parasitic for long periods), and then go back to their hiding place. There are often several nymphal stages. Adult Argasidae mate off the host; the female (again with the exception of *Otobius*, where adults are non-parasitic) feeds several times, producing a small egg batch after each meal (Guglielmone *et al.*, 2010).

2.13.2 Ixodid ticks

Ixodidae may be one-, two- or three-host species, depending on the number of host animals they attach to during their life cycle. Larvae and nymphs feed once, to engorgement, and then moult. One-host ticks moult twice on the same host animal, from larva to nymph and from nymph to adult. Two-host ticks moult once on the host, from the larval to the nymphal stage; the engorged nymph drops off, moults off the host, and the resulting adult has to find a second host animal (which may or may not be of the same species as the first). Three-host ticks do not moult on the host; the engorged larva drops off, moults to a nymph, which then has to find a second host animal on which it engorges and drops again to moult to the adult stage, which will attach to a third host animal (if it succeeds in finding one). Adult Ixodidae usually mate on the host, the female then feeds to engorgement, drops off, lays an egg batch and then dies (while the male may remain on the host for several months). Logically, the egg batch of a one-host tick contains on the average far fewer eggs than of three-host ticks,

as the latter have to run the gauntlet of finding a new host three times in their life cycle, the former only once. Two-host ticks are in between. A further detailed account of the biology of ticks is available in two classic monographs of Sonenshine (1993). The characteristics of seven main genera of ixodid ticks (*Boophilus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus* and *Amblyomma*) are given below.

2.13.2.1 *Boophilus* spp.

Boophilus are one-host ticks, which take about three weeks to complete their blood meal, preferably on cattle (except for *B. kohlsi*, a Near/Middle Eastern species with a predilection for small ruminants). Although *Boophilus* ticks have short mouthparts, damage to hides is considerable as the preferred feeding sites are often of good leather potential. *B. microplus* is the most important species. Originating from South-East Asia, this species has spread throughout the tropics including Australia, East and Southern Africa, and South and Central America. *B. annulatus* is present in the Mediterranean region, and occurs in southern Russia as well as in and around Middle East; this species has also extended its distribution southwards into Africa in a belt from West Africa to southern and central Sudan (Hoogstraal, 1956). As *Boophilus* spp. are one-host ticks, these ticks may become very numerous in cattle herds, especially in herds with a low degree of resistance, and cause considerable direct damage.

2.13.2.2 *Dermacentor* spp.

The genus *Dermacentor* comprises 33 species with ornate scuta, short palps, eyes and usually following a three-host cycle. *Dermacentor* spp. occur on all continents, except Australia. In Eurasia, several species (e.g. *D. marginatus* and *D. reticulatus*) infest livestock and other domestic animals. In North America, *D. variabilis* and *D. andersoni* are

important ectoparasites of livestock. In Africa, *Dermacentor* ticks do not play a significant role for livestock.

2.13.2.3 *Haemaphysalis* spp.

The genus *Haemaphysalis* contains 168 species. They are easy to differentiate from other genera by the characteristic lateral projection of palpal article 2 beyond the margins of the basis capituli. All *Haemaphysalis* spp. are eyeless, three-host ticks. Only a few species have adapted to livestock. For example, *H. bispinosa* occurs on cattle on the Indian subcontinent. *H. longicornis* is an East Asian species, occurring on cattle and other domestic animals, and has been introduced into Australia, New Zealand and New Caledonia. In Europe, *H. punctata* is common on ruminants. *Haemaphysalis* spp. are of importance to livestock in Europe and Asia.

2.13.2.4 *Hyalomma* spp.

Hyalomma species are medium to large sized ticks with long palps and eyes typically in sockets. *Hyalomma* species parasitise domestic and wild mammals, birds, and are abundant in semi-arid zones. The genus *Hyalomma* comprises 30 species, most of which follow a three-host life cycle. However, some species undergo either a two-host or a three-host cycle, depending on the host species, while *H. scupense* is a one-host tick. Adult *Hyalomma* ticks actively run out from their resting sites when a host approaches, unlike most other ixodid ticks, which wait on the vegetation.

2.13.2.5 *Ixodes* spp.

Ixodes spp. forms the largest genus of hard ticks with 243 species characterised by the anal groove curving anteriorly to the anus. The scutum lacks ornamentation and there are no

eyes and most species inhabit nests or burrows. Relatively few *Ixodes* species parasitise larger mammals, but the genus is widely distributed throughout wooded or grassy environments. The most important species in Europe and Asia are *I. ricinus* and *I. persulcatus*, and *I. scapularis* in North America. *I. persulcatus* is much more aggressive towards humans than *I. ricinus*.

2.13.2.6 *Rhipicephalus* spp.

The genus *Rhipicephalus* comprises 82 species (excluding *Boophilus* spp.). These small to medium sized ticks with short, broad palps are usually inornate with eyes and festoons. Most *Rhipicephalus* spp. are found on mammals on the African continent. These are usually three-host ticks, although some have a two-host cycle (e.g. *R. evertsi*). Taxonomic identification of rhipicephalid group ticks may cause difficulties, and a revision of the entire genus is beneficial (Walker *et al*, 1999). *R. appendiculatus*, the brown ear tick, is the most important rhipicephalid tick in East and Southern Africa, where it occurs on a wide variety of domestic and wild ruminants. This tick prefers to feed on the ears of the hosts in the adult stage.

2.13.2.7 *Amblyomma* spp.

There are 130 species of *Amblyomma* ticks, which are characterised by long mouthparts and, usually, beautifully coloured 'ornamented' scuta. Eyes are usually not housed in sockets. These three-host ticks are widespread in tropical and sub-tropical zones, where they parasitize a wide variety of mammalian hosts, and also reptiles and amphibians. Immature stages of some species infest birds, which can play an important role in dispersing the ticks. *A. variegatum* is the most important species on the African continent, being well adapted to livestock and having the widest distribution: throughout tropical sub-

Saharan Africa. *A.hebraeum* is another important pest of livestock, which inhabits the south-eastern part of the African continent. Other species of more local importance are *A. Lepidum*, *A. astrion*, *A. gemma*, *A. pomposum* and *A. cohaerens*. In addition to widespread distribution in Africa, *A. variegatum* has been introduced into the Caribbean region, presumably in the 18th or 19th century with cattle from West Africa. To date, *A. variegatum* is the only African *Amblyomma* tick to have successfully established itself outside the African continent (Camus and Barre, 1990). On the American continent, several species (including *A. americanum*, *A. cajennense* and *A. maculatum*) are of economic significance, as the adults prefer to feed on cattle.

Table 2.3The Taxonomic Status of the *Rhipicephalussanguineus*

Taxonomic position of <i>R.sanguineus</i>	
Phylum	Arthropoda
Sub-phylum	Chelicerata
Class	Arachnida
Sub-class	Acari
Order	Parasitiformes
Sub-order	Ixodida
Super-family	Ixodoidea
Family	Ixodidae
Sub-family	Rhipicephalinae
Genus	<i>Rhipicephalus</i>
Species	<i>R. sanguineus</i>

Several genetic studies have consistently reported the existence of two well-defined lineages within “*R. sanguineus*”: the southern lineage, referred to as temperate species/lineage, and the northern lineage, referred to as tropical species/lineage (Burlini *et al.*, 2010; Moraes-Filho *et al.*, 2011; Nava *et al.*, 2012; Dantas-Torres *et al.*, 2013; Hekimoğlu *et al.*, 2016; Sanches *et al.*, 2016; Zemtsova *et al.*, 2016). The taxonomic status of the brown dog tick, which until recently was referred to as *Rhipicephalus sanguineus*, is currently under debate (Nava *et al.*, 2015). At present, the genus *Rhipicephalus* contains > 70 recognized species but none are as widespread as brown dog ticks. The taxonomy of *Rhipicephalus sanguineus* is still the subject of debate (Gray *et al.*, 2013), despite several authors that have studied this species group (Camicas *et al.*, 1996). According to Camicas and colleagues (Camicas *et al.*, 1996), the so-called “*R. sanguineus* group” includes 17 species as follows: *Rhipicephalus aurantiacus*, *Rhipicephalus bergeoni*, *Rhipicephalus boueti*, *Rhipicephalus camicasi*, *Rhipicephalus guilhoni*, *Rhipicephalus leporis*, *Rhipicephalus moucheti*, *Rhipicephalus pumilio*, *Rhipicephalus pusillus*, *Rhipicephalus ramachandrai*, *Rhipicephalus rossicus*, *R. sanguineus sensu stricto* (s.s.); *Rhipicephalus schulzei*, *Rhipicephalus sulcatus*, *Rhipicephalus tetracornus*, *Rhipicephalus turanicus*, and

Rhipicephalus ziemanni. However, there is no consensus (Gray *et al.*, 2013) and the morphological similarities among ticks belonging to the *R. sanguineus* group make their identification a difficult task, even for experienced taxonomists.

Moreover, experimental transmission studies and surveys of ticks from naturally infested dogs suggest that brown dog tick lineages likely differ in their ability to transmit different pathogens, including *Ehrlichia canis*, *Hepatozoon canis*, and *Anaplasma platys* (Moraes-Filho *et al.*, 2015). Accordingly, these data may have important implications for relative risk for disease transmission, including zoonotic infections, to dogs and people in different region of the world. Dantas-Torres, (2010), described *R. sanguineus* (brown dog tick) as a near strict parasite of dogs and are well adapted to living within human dwellings as well as in gardens and kennels. It is this adaptability and close association with dogs that facilitated its worldwide distribution. The brown dog ticks are well adapted and are in close association with dogs, which facilitates its vector activities among the canine host, transmitting *Babesia* spp., being the first described arthropod vector borne disease known to man. In a recent molecular work on tick species, Lv *et al.* (2014), used the ITS 2 as a marker in identifying ticks especially in differentiating closely related genus and showed that ITS 2 has the highest interspecific divergence and a low intraspecific variation compared to other markers and therefore, concluded that ITS 2 may be the most useful DNA marker for discriminating tick species. Abdigourdarzi *et al.* (2011) noted that partial amplification of the region is good in discriminating different species of hard ticks; however, the authors asserted that it is important to have a complete sequence of the region in order to be able to resolve issues related to partial sequences that were seen in their study. *Rhipicephalus sanguineus* s. l., the only known vector of *B. vogeli*, is now thought to

be a species-complex comprising at least 17 sibling species, which may differ in vector capacity (Dantas-Torres *et al.* 2013; Dantas-Torres and Otranto 2015).

2.14

Biology of Ticks

The life cycle of ixodid ticks consists of four stages, eggs, six legged larvae, eight legged nymphs and eight legged adult ticks. Depending on the species, ixodid ticks have one- two- or three-host life cycles. All ixodid tick species, which feed on dogs, are characterized by a three-host life cycle (Figure 2.3).

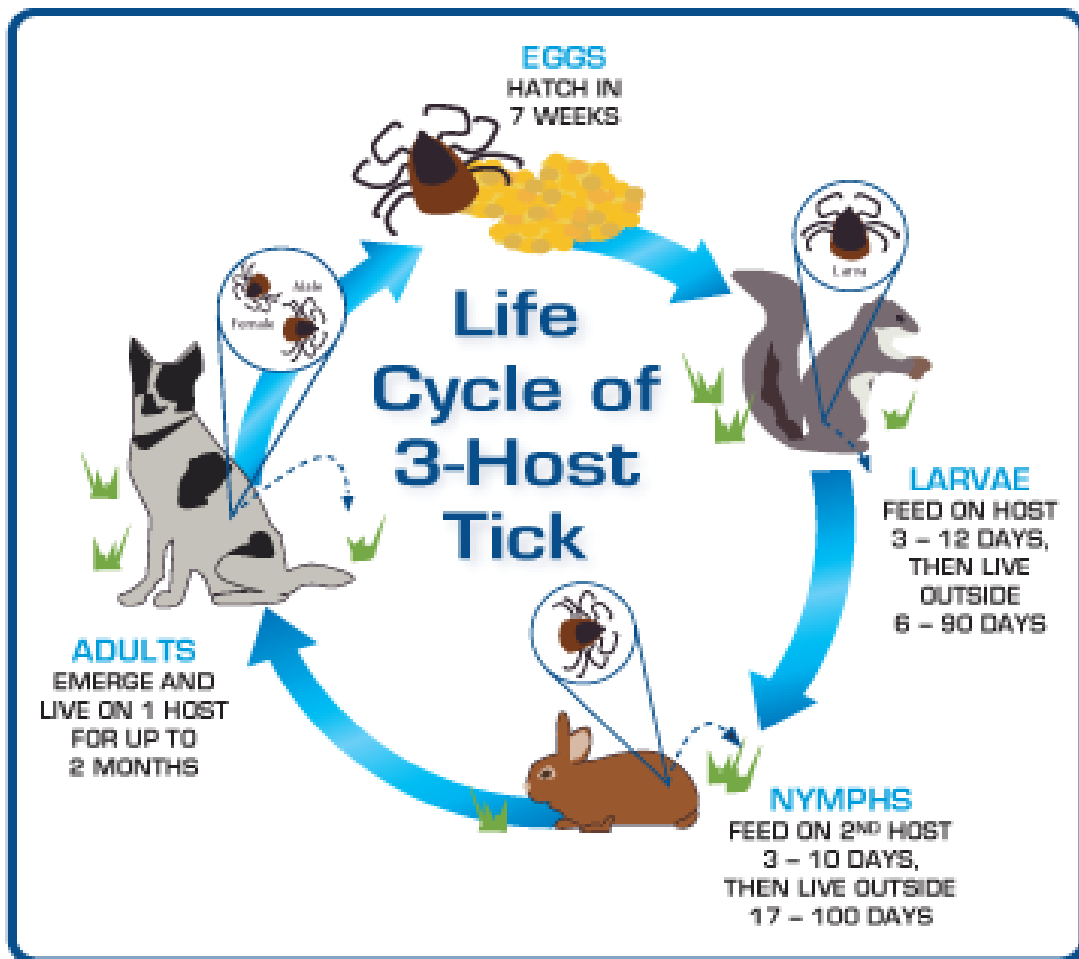


Figure 2.4: Life cycle of a three-host

tick<https://images.search.yahoo.com/yhs/search?p=picture+of+Rhipicephalus+tick+lifecycle>

Metastriate ticks which include all genera except *Ixodes* mate exclusively on the host, whereas in prostriate ticks, of which *Ixodes* is the only, gametogenesis already begins during the nymphal to adult moult, resulting in unfed adults sexually active soon after moulting. In some *Ixodes* species mating takes place off the host, before the ticks have fed, while in others mating takes place on the host. Engorgement of female metastriate and prostriate ticks on the blood of their hosts can be immense, occasionally in excess of 100 times their own unfed body weight. After engorgement, female ticks detach and drop from their hosts and commence oviposition, which results in several thousand eggs (Figure 2.4).



Figure2.5: Brown dog tick laying eggs.

<https://images.search.yahoo.com/yhs/search?p=picture+of+Rhipicephalus+tick+lifecycle>

The males of most species remain attached for varying periods of time after the females have dropped. Ticks follow two basic strategies to access suitable hosts. A passive (ambush) strategy involves a tick remaining quiescent in its habitat and depends upon contact with any host that invades this habitat. These ticks are found questing on the vegetation (figure 5).



Figure 2.6: A tick questing on the vegetation.

<https://images.search.yahoo.com/yhs/search?p=picture+of+Rhipicephalus+tick+lifecycle>

Active (hunter) host acquisition involves a tick leaving the environment where it was sheltering to actively seek a host. Once a host has been found, tactile stimuli come into play, contributing, along with short-range odourants and body heat, to the selection of a feeding site and the commencement of blood-sucking. The mouthparts are composed of a pair of hollow tubes known as the cheliceral sheaths surrounding a pair of appendages called chelicerae. The distal extremities of the chelicerae are armed with denticles which are used in a saw-like motion to cut the host's skin. The hypostome lies ventral to the chelicerae and its ventral surface is armed with variable rows of retrograde denticles. The hypostome is used to penetrate the laceration created by the chelicerae and the denticles for attachment to the host. Attachment is secured by means of a milky white fluid secreted by the salivary glands of the tick, which hardens into a latex-like cone surrounding the hypostome. This serves as the initial core of a cement cone that will eventually secure the attachment site. The secretion of this substance is continued for up to 72 hours adding cortical layers to the cement, and may even overflow onto the surface of the host's skin in some tick species, thus further consolidating the attachment site. After the establishment of

the cement cone the salivary glands enlarge and protein synthesis is accelerated. The process of penetration and probing for blood injures capillaries and small blood vessels causing an extensive haemorrhagic pool at the tick feeding site. The host's response to this type of damage to the dermis is the formation of a haemostatic plug, activation of the coagulation cascade, vasoconstriction, inflammatory responses leading to wound healing, all of which have a negative impact on tick feeding (Sonenshine,1991).

2.15 Economic Importance of Ticks

Ticks transmit a greater variety of micro-organisms, protozoa, bacteria (in particular rickettsiae and spirochaetes), viruses, and even helminths, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock, humans and companion animals. Ticks can also cause severe toxic conditions such as paralysis and toxicosis, irritation and allergy. Moreover, some ticks can cause considerable direct damage to livestock, because of sheer numbers (decrease in production by loss of blood and impact on the host metabolism) or by creating favourable conditions for secondary bacterial infections or myiasis (tick species with long and massive, deeply penetrating mouthparts (Jongejan and Uilenberg, 2004). Whereas the importance of tick-borne diseases for human and companion animals is measured by morbidity and mortality, the diseases and direct damage caused by ticks to livestock are a major constraint to animal production, predominantly in (sub) tropical areas of the world. Ticks and tick-transmitted infections have co-evolved with various wild animal hosts which often live in a state of equilibrium with them and constitute reservoir hosts for ticks and tick-borne pathogens of livestock, pets and humans. They have only become problems of domestic livestock when these wild hosts came into contact with them, either because man moved livestock into infested

regions, or moved livestock infested with the ticks into previously uninfested regions. Most ticks have a preference for feeding on certain groups of wild animals, with some even being quite host specific. Consequently, the number of species pertinent to domestic animals and/or humans is limited. As a matter of fact, relatively few species of ticks have successfully adapted to livestock or may feed on a human subject, and these have developed into efficient vectors of a range of pathogenic micro-organisms. Virtually all human TBDs are zoonoses. The following ecological criteria were used to underpin the tick-host-pathogen relationships. A tick species is considered a vector for a particular pathogen only, if it (1) will feed on an infected vertebrate host, (2) is able to acquire the pathogen during the blood meal, (3) can maintain it through one or more life stages, and (4) can pass it on to other hosts when feeding again (Kahl *et al.* 2002).

The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806), has a worldwide distribution and is found approximately between 50°N and 30°S (Walker *et al.*, 1999). This tick is a near strict parasite of dogs and is well adapted to living within human dwellings as well as in gardens and kennels (Dantas-Torres, 2010). It is this adaptability and close association with dogs that facilitated its worldwide distribution. Depending on the climate, *R. sanguineus* may be prevalent throughout the year in tropical and subtropical areas or more active during late spring to early autumn in temperate regions (Dantas-Torres, 2010). *R. sanguineus* is a vector of a number of tick-borne pathogens affecting dogs, most important of which is *Ehrlichia canis*, the causative agent of canine monocytic ehrlichiosis (CME). Canine monocytic ehrlichiosis is widely distributed and is found wherever the brown dog tick occurs. *R. sanguineus* is also a vector of protozoan tick borne pathogens, *Babesia vogeli* and *Babesia gibsoni*, causative agents of babesiosis in dogs. Furthermore, it

can transmit *Anaplasma platys*, the cause of thrombocytic anaplasmosis, *Hepatozoon canis*, the cause of hepatozoonosis and *Mycoplasma haemocanis*, causing canine haemoplasmosis (Baneth, *et al.*, 2015). Although *R. sanguineus* is in general very host specific, human parasitism does occur and appears to be more frequent in environmental conditions leading to high tick densities (Dantas-Torres, 2010). This poses a threat to human health since *R. sanguineus* is a competent vector of several *Rickettsia* species to humans. Most importantly are *Rickettsia conorii*, the causative agent of Mediterranean spotted fever and *Rickettsia rickettsii*, the agent causing Rocky Mountain spotted fever (RMSF). Mediterranean spotted fever is encountered throughout the Mediterranean region, sub-Saharan Africa, India and around the Black Sea (Fournier, 2013).

2.16 Control of Ticks

Dantas Torres (2008), had shown from extensive studies that when thinking about tick control, something should be kept in mind: only about 5% of the ticks are on the dog; the remaining 95% is in the environment. Therefore, the effective elimination of tick populations will require an integrated control strategy, targeting the canine population as well as the environment. An integrate control strategy means that all appropriate technological and management techniques are utilized, proportioning an effective decline of target populations in a cost-effective fashion. This approach includes both the use of chemical and non-chemical strategies (e.g., environmental management).

2.15.1 Chemical control

Dogs can be treated with a diverse range of veterinary preparations, such as spot-on formulations, impregnated collars, shampoos, sprays, dips, and powders (Garris, 1991).

Fipronil, amitraz, carbaryl, and pyrethroids (deltamethrin, permethrin, and cypermethrin), are among the most frequently used acaricides for controlling *R. sanguineus* ticks (Otranto *et al.*, 2005; World Health Organisation, 2006). Recent advances in ectoparasiticides (insecticides and acaricides) for veterinary use have been reviewed elsewhere (Taylor, 2001). The use of acaricides on dogs is usually effective to eliminate their tick infestations and to prevent reinfestations during a certain period of time. The frequency of treatment depends on the degree of infestation and the duration of the residual effect of the acaricide. In any case, the manufacturer's guidelines should always be followed. As 95% of the ticks are outside the host, the use of acaricides in the environment in which the dog lives is often required. There are many concerns about the use of the acaricides in the environment. The improper use of acaricides can cause environmental pollution and toxicity to humans and other non-target organisms (e.g., predators and parasites of ticks) (World Health Organization, 2006).

2.15.2 Non-chemical control

Non-chemical procedures for tick control should be implemented along with chemical control methods. Habitat change is very important in the control *R. sanguineus* ticks. Cracks and crevices should be sealed and grass and weeds should be kept cut short. It is important to note that *R. sanguineus* ticks present a marked endophilic behavior in most regions where they occur. In highly infested houses, ticks are commonly seen crawling up walls, curtains, window and door casings, and under the furniture. In this kind of situation, indoor residual application of acaricides may be necessary. Dog owners should be instructed to examine, locate, and remove ticks from their dogs periodically (Dantas-Torres and Figueredo, 2006). As ticks have many natural enemies (e.g., bacteria, fungi, and

nematodes), their biological control appears to be feasible (Samish *et al.*, 2004). It has been shown that the use of entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, may have the potential for controlling populations of certain tick species, including *R. sanguineus* (Samish *et al.*, 2004).

Currently, there is no anti-tick vaccine available for use in dogs and there is no good evidence that it is feasible. As previously discussed, dogs appear to develop no immunity against ticks and this is an important limiting factor for the development of a vaccine against *R. sanguineus* ticks. Moreover, the costbenefit relationship of the vaccination of dogs against ticks has not been fully evaluated. The progress towards the production of effective anti-tick vaccines has been low (Nuttall *et al.*, 2006). On the other hand, recent advances in molecular biology, protein chemistry and computational biology have accelerated the isolation, sequencing and analysis of transcripts and proteins from the saliva of ticks. The identification of the biological activities of proteins codified by newly isolated genes from tick saliva is likely to help the discovery of potential targets for vaccines against ticks and the disease they transmit (Valenzuela, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Ethical Considerations

Ethical approval for the study was obtained from the Ethics Committee on Animal Use and Care, Ahmadu Bello University, Zaria with approval number ABUCAUC/2016/041. All animals were sampled with the owner's consent and handled humanely.

3.2 Study Area

The study was conducted in the Federal Capital Territory, Abuja-Nigeria. The city lies between latitude 8°35' and 9° 25' north of the Equator and longitude 6° 45" and 7° 45" east of the Greenwich Meridian with a land area of about 8000 square kilometers. It has a Guinea Savannah type of vegetation; with annual rain-fall ranges from 1100 to 1600 mm. There are two major seasons: dry season (November - April) and rainy season (May – October) in each year. The maximum temperature is 37°C and the minimum 30°C (Adekayi, 2000; Balogun, 2001).The environmental conditions provide favourable and conducive conditions for the survival and propagation of ticks (Opara *et al.*, 2017). It is bordered to the north by Kaduna State, to the east by Nasarawa State; to the south by Kogi State; and to the west by Niger State. Abuja has six Area Councils namely; Abaji, Bwari, Gwagwalada, Kuje, Kwali, and Municipal. In the 2006 National census, Abuja Municipal Area Council had a population of 776,298 with an area of 890km²; Bwari Area Council had a population of 227,216 with an area of 914 km²; Gwagwalada Area Council had a population of 157,770 with an area of 1,043 km², and Kuje Area Council had a population

of 97,367 with an area of 1,644 km²; Kwali Area Council had a population of 85,837 with an area of 1,206 km² and Abaji Area Council had a population of 58,444 with an area of 992 km² (NPC, 2006).

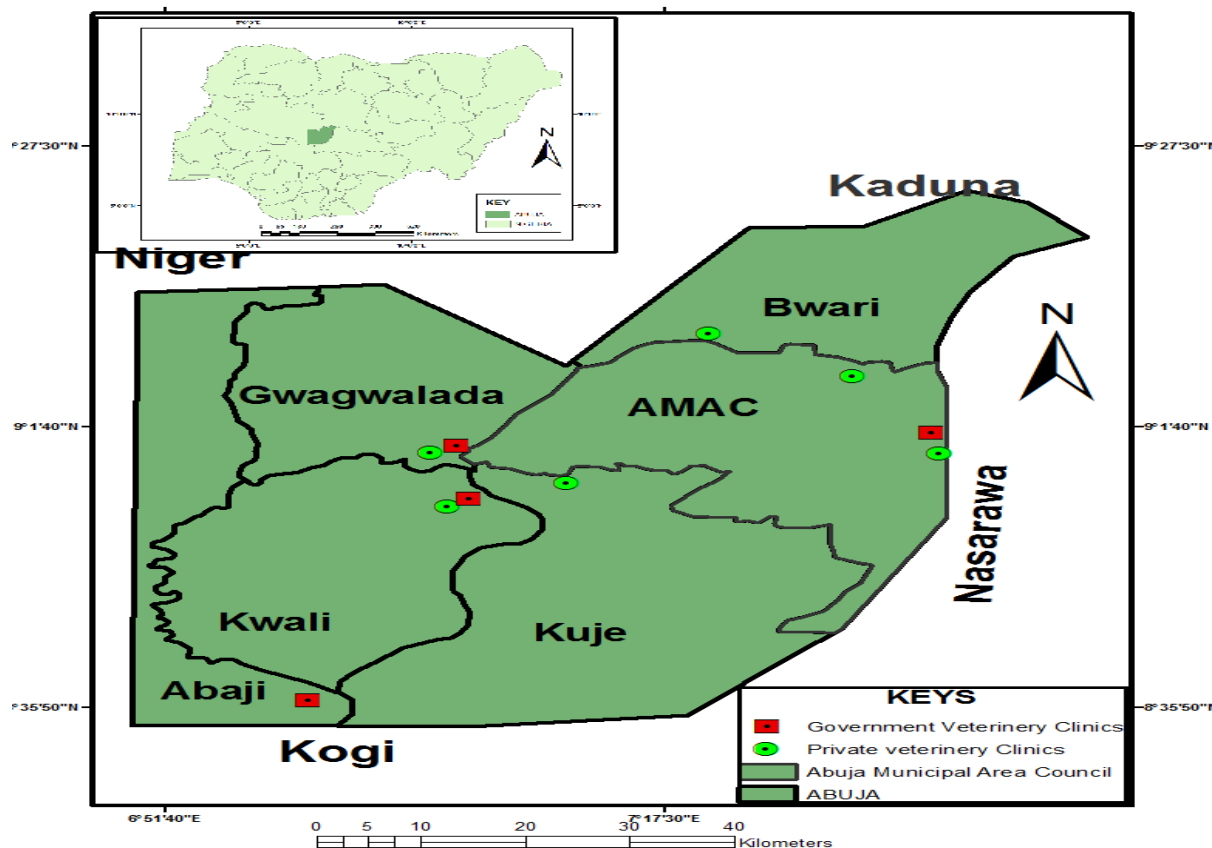


Figure 3.1: A map of the Federal Capital Territory showing the six Area Municipal Councils (Source: Administrative map of Abuja, 2015).

3.3 Study Design

Ticks and blood samples were collected by convenient sampling from 480 apparently healthy dogs in the six Area Councils of the FCT. The study was carried out for 12 months, September 2015 to August 2016, using anti rabies vaccination campaign exercise as a tool. Prior to sampling, the co-ordinates of the Government Veterinary clinics as well as private owned Veterinary Clinic in the various Area Councils were obtained.

3.3.1 Sampled animals

Animals sampled in this study, were dogs presented as patients to Government Veterinary Clinics and Private owned Veterinary Clinics within the Area Councils. Dogs from conveniently selected households in the study areas were also sampled. Information regarding their age, breed, sex, purpose for keeping, presence or absence of ticks, as well as attachment sites of tick on dogs were recorded using a sampling form.

3.3.2 Sample size determination

In estimating the minimum sample size for this study, the 8.9% prevalence rate obtained by Jegede *et al.* (2014), for *Babesia* infection in FCT was used. A minimum sample size of 125 dogs was calculated using the formula of Thrusfield, $N = Z^2 pq/d^2$ (2007), where N = minimum sample size; p = expected prevalence; d = desired absolute precision of 5% (0.05); q = complementary probability (1-P); Z = appropriate value for the standard normal deviate set at 95% confidence interval (1.96). Calculated values are shown as below

$$\begin{aligned} N = Z^2 pq/d^2 &= \frac{1.96^2 \times 0.089 \times 0.911}{0.05^2} \\ &= \frac{3.8416 \times 0.089 \times 0.911}{0.0025} \\ &= 124.5 \end{aligned}$$

A total of 480 dogs, (240 for each season) were examined in order to increase the precision.

3.3.3 Inclusion criteria

Animals included for this study were dogs of different breeds, both sexes, age limit of three months and above, those without history of travelling out of study area in the last one month whose owner's consent were approved.

3.3.4 Exclusion criteria

In this study, dogs that were below three months of age, those with history of travelling or were acquired within the month of sampling, as well as dogs whose owners' consent were not secured were not included.

3.4 Sampling Form

The sampling form was designed to capture information bordering on sample number, collection date, location, age, sex, breed, presence or absence of ticks and purpose for keeping the animal. Dogs were placed in four groups according to age. Group1, are dogs between 3 months and less than 1 year of age. Group 2, included dogs within the age ranges of 1 and less than 3 years, Group 3 included dogs within the age ranges of 3 and less than 5 years of age; Group 4 included dogs within the age ranges of 5 years and above (Bashir *et al.*, 2010). Breed of dogs was placed in three categories; local, exotic and cross breed (Okubanjo *et al.*, 2013). Dogs' purpose was placed into three categories of use; guard, hunting and pet (Obeta *et al.*, 2009).

3.5

Sampling

3.5.1. Tick sampling

Each animal was adequately restrained using a mouth gag and physically examined for ticks. The examination protocol was carried out by first, examining the head of the dog for ticks. A close attention was given to the ears, checking the pinnae and inside the external ear canal. Examination was carried out on the neck, dorsal and ventral regions, legs and inter-digital spaces. Then, the dog's hair, from head to tail, was examined manually using sufficient pressure to detect small lumps, parting the hair along the length of the body. All attached ticks were removed using a medium size steel forceps with blunt points and serrated inner surfaces. The point of attachment was dabbed with ethanol. The procedure was carefully performed to avoid destroying the mouth parts. The period of examination was estimated to last an average of 5 min per dog. The unengorged ticks collected were placed into separate, clean and properly labeled universal bottles containing 70% alcohol with 5% glycerol for preservation while the engorged were placed into separate bottles, with perforated lid and plugged with a cotton wool. At the end of each collection week, tick samples were transported to the Entomology Laboratory in the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for identification, preservation, and further investigations (Uade *et al.*, 2008).

3.5.2 Blood sample collection

The method of WHO (1991) was adopted for the collection of blood samples from dogs. Five milliliters (5mL) of blood sample was collected through the cephalic vein using 5 mL disposable syringe into a sample vial containing 1mg ethylene di-amine tetra- acetate-k

(EDTA-K) as anticoagulant. The blood samples were immediately kept inside a cool-box containing ice pack. The samples were transported to the Department of Parasitology and Entomology Laboratory, Faculty of Veterinary Medicine, University of Abuja, for the parasitological analyses.

3.6 Parasitological Examination of Blood Samples

The parasitological analyses were carried out immediately after each sampling exercise.

3.6.1 Thin blood smear

Thin blood smear technique was carried out as described by Coles, (1986). Briefly, a drop of blood approximately 5 μ L was placed at one end of a clean slide. A “spreader” was placed at a 45° angle behind the drop of blood; it spreads by capillary action along its edge. The spreader was then smoothly and rapidly slid forward the length of the slide producing a cell thick smear. The slide was air dried and fixed with absolute (100%) methanol for 3-5 minutes (Cable, 1950). The slides were stained with the Giemsa stain for 25-30 minutes (Hendrix and Robinson, 2006). Then slides were rinsed with water, air dried and examined under a light microscope using (\times 100) objective and a drop of immersion oil for the identification of *Babesia* merozoites (piroplasm) in the erythrocytes (Soulsby, 1982; Urquhart *et al.*, 1996). A sample was considered positive if intraerythrocytic, single trophozoites and/or paired piriform merozoites of a large *Babesia* sp. were detected.

3.6.2 Thick blood smear

Thick blood smear was prepared from the whole blood as described by (Soulsby, 1982). Three drops of blood was placed at the centre of a clean slide, then using an applicator stick, the blood spread out to an area of about 2 cm in diameter. The smear was allowed to

air dry. The slide was placed in a slanted position, with smear side down, in a glass beaker containing distilled water. The slide was air-dried and then stained with Giemsa stain for 30 minutes; excess stain was washed with tap water. The slides were examined under light microscope using oil immersion objective, for the presence of *Babesia* merozoites in the erythrocytes (Soulsby, 1982).

3.7 Haematological Analyses of Blood Samples

The haematological analysis of the blood was performed as described by Hasanpour *et al.*, (2008). These were carried out the same day of samples collection, in the Haematology Department of the University of Abuja Teaching Hospital, Gwagwalada, in the Federal Capital Territory. Haemoglobin (Hb) values, total erythrocyte count (TEC), total leucocyte count (TLC) and packed cell volume (PCV) were determined using (Mindray BC 5300; 5-part Differential Haematology analyzer USA) in accordance with the manufacturer's instructions. The remaining blood samples were preserved at -20°C prior to PCR analysis.

3.8 Morphologic Identification of Tick Samples

The ticks collected were transferred into petri dishe, mounted on slides and examined using a dissecting microscope at a magnification of $\times 40$ and sorted to genus, and sexes. Ticks were identified using morphological features (Hoogstraal, 1956; Walker *et al.*, 2014).

3.9 Haemolymph Staining Technique

Forty eight (48) fully engorged female ticks were cleaned with distilled water; kept in separate perforated containers, loosely plugged with cotton wool and then placed in an incubator set at temperature of 27°C and 80% relative humidity. The protocol according to

collection tube were discarded. The washing process was repeated by adding (500 μ L) Buffer AW2 and centrifuged for 3 minutes at $20,000 \times g$ (14,000 rpm), the flow-through and collection tube were discarded. The spin column was transferred into a new 1.5 centrifuge tube and the DNA was eluted by adding 200 μ L Buffer AE at the center of the spin column membrane, incubated for 1 min at room temperature (25-26 $^{\circ}$ C), and then centrifuged for 1 minute at $6000 \times g$ (8000 rpm).

The determination of DNA concentration was done using Nanodrop[®] ND 1000 Spectrophotometer according to manufacturer's protocols and recorded at 260/280 wavelength. The DNA was stored as solution samples at -20° C prior to Polymerase Chain Reaction (PCR) assay.

3.10.2 Primers design

The genus specific Oligonucleotide primers for *Rhipicephalus*spp. and *Babesia* spp. were designed to amplify the DNA at approximately 173 bp and 612 bp respectively.

Table 3.1: Oligonucleotide sequences of primers used to identify *Rhipicephalus sanguineus* and *Babesia canis vogeli* in the FCT in this study.

Primers	Sequence	References
18S-F	5'-GACAAGAAGACCCTA-3'	Zivkovic <i>et al.</i> , 2009
18S-R	5'-ATCCAACATCGAGGT-3'	
BAB-F	5'- GTGAAACTGCGAATGGGCTCA -3'	Casati <i>et al.</i> , 2006
BAB-R	5'- CCATGCTGAAGTATTCAAGAC 3'	

3.10.3 Polymerase chain reaction

Genomic DNA extracted from the 30 ticks' tissues and 15 whole blood samples were amplified using a pair of genus-specific primer designed for the amplification of partial 18S rRNA *Rhipicephalus sanguineus* (Zivkovic *et al.*, 2009), and *Babesia canis vogeli* (Casati *et al.* 2006), genes with the expected molecular size of ~173 bp and 612 bp respectively (Table 15).

The DNA amplification (PCR) was carried out in a final volume of 20 μ L containing 2 μ L of the template DNA, 0.2 μ L of each primer (forward and reverse), 2 μ L PCR buffer (including 15 mM MgCl₂), 2.0 μ L of 2mM dNTPs, 0.2 μ L of Taq DNA polymerase and 13.40 μ L Nuclease-free water (Qiagen) to complete the total volume of the reactions. The tubes were then placed into a programmed Applied Biosystems Veriti 96 well thermocycler (Germany). The PCR cycling conditions were initial denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min, and extension at 72°C for 1min, and the final extension was performed at 72°C for 10 min (Lv *et al.*, 2014). The PCR products were stored at 4°C for electrophoresis.

3.10.4 Electrophoresis and purification of pcr products

The amplified products were analyzed by electrophoresis on 1.5% Agarose gel by aliquoting 4 μ L of PCR products and 1 kb DNA ladder and run on the gel stained with SYBR Green for 1 hour at 80 volts. The PCR products were purified using MegaExtractor-PCR & Gel Clean up- (Japan) following the manufacturer's protocol. The amplicons from the performed Polymerase Chain Reaction (PCR) were electrophoresed to check the size of the amplified fragments by comparing the fragments to a standard molecular weight marker (1 kb DNA ladder). A 1.5% agarose gel was used to analyse the PCR products and it was

prepared by weighing 1.5g of agarose powder and pouring it into a conical flask containing $1 \times$ TAE buffer and heated in a microwave oven until it completely dissolved. This was removed from the oven and allowed to cool, and then 5 μ L of staining dye SYBR Green (Roche Diagnostic, Mannheim, Germany) was added and mixed thoroughly. The ends of the trays were sealed with masking tape and the combs inserted before the gel was poured, ensuring that no bubbles formed. The combs and the masking tapes were carefully removed from the gel casting tray and the tray was placed in the electrophoresis tank before $1 \times$ TAE buffer was added to cover the gel. The molecular weight marker was loaded into the first well, the nuclease-free water was loaded into the second well as negative control and then the samples were loaded into the subsequent wells. The safety cover was put in place before the power pack was switched on to run the gel for 30 minutes at 80V, after which the gel was removed and placed in the transilluminator and documentation system for capture of the gel image. The agarose block containing the DNA template was sliced into small pieces and placed into 1.5 mL microtubes. Then 400 μ L of Binding solution was added and incubated at room temperature and vortexed every 2-3 min. interval until gel pieces are completely dissolved. Then 30 μ L Magnetic Beads was added and vortexed every 10 seconds for 2 min. and each tube placed in the magnetic stand until the magnetic beads completely separated from the specimen solution. The supernatant was carefully removed after the magnetic capture. Then 600 μ L Washing solution was added to the beads and the solution vortexed for 10 seconds. Each tube was placed in the magnetic stand and the beads were collected with the magnet, and the supernatant was carefully removed after the magnetic capture. Then 1ml 75% ethanol was added to the tube and vortexed for seconds. This was repeated until the 75% ethanol was completely removed after a flash centrifugation. Then 25 μ L sterilized water was added and mixed well for 10 seconds. The

solution was incubated at room temperature for 2 min. and the tube placed in the magnetic stand after a brief vortexing, and the supernatant was collected and placed into a fresh tube.

3.10.5 Sequencing and analysis of sequences

Twenty five amplicons of *Rhipicephalus* spp. and three PCR products of *Babesiacanis* were sequenced directly using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), with the forward amplification PCR primers and AmpliTaq-FS DNA Polymerase. The chromatographs for the sequences were edited in a plasmid editor (ApE), to obtain consensus sequences (Tamura *et al.*, 2013), which were further trimmed to different sizes. For each, the forward and the reverse sequences obtained were trimmed and edited using ApE software before they were aligned to generate a single sequence product for each of our DNA product. Then, each sequence was queried in the NCBI Database and analysed in the nucleotide Basic local alignment search (BLAST) for similarity with other sequences in the GenBank was done using the NCBI database (<https://www.ncbi.nlm.nih.gov/BLAST>).

3.10.6 Submission of nucleotide sequences to the GenBank for accession number

A total of nine *Rhipicephalus sanguineus* and three *Babesia canis vogeli* sequences were edited using BioEdit software. The edited sequences were manually curated and annotated and then deposited into the GenBank using BankIt submission tool for ribosomal RNA (rRNA) sequences in <https://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank>

3.10.7 Phylogenetic analysis

Phylogenetic analysis was carried out using the Molecular Evolutionary Genetic Analysis (MEGA7.0) software program (Kumar *et al.* 2016), for *Rhipiciphalus* spp. isolates and

Babesia spp. isolates respectively. Then, a notepad page in the computer was opened and the sequences were imported and saved in FASTA format. The sequences of other *Rhipicephalus* spp isolates *Babesia canis vogeli* isolates and some genetically distanced isolates were retrieved from the GenBank and pasted in the Notepad page. Multiple alignments of all these sequences were carried out using Clustal W for pairwise comparisons. The aligned sequences were edited manually and annotated in MEGA 7.0 (Kumar *et al.* 2016). The genetic distances between pairs of sequences was calculated using Kimura's two parameter model (Tamura *et al.*, 2013), and subsequently Neighbour-joining (NJ) algorithm was used to construct a phylogenetic tree (Saitou and Nei, 1987). A bootstrap of 1000 replicates was used to evaluate the branching of the phylogenetic tree, and a bootstrap value of 80% was considered significant and as an evidence of phylogenetic grouping. Partial 18S rRNA sequences of *Rhipicephalus* spp. available in GenBank was also included. Sequences of *Amblyomma variegatum* available in GenBank (JF826436) was used to root the tree.

3.10.8 Nucleotide sequence accession number

Nine (9) sequences of partial mitochondrial 18S rRNA *Rhipicephalus sanguineus* gene and three (3) *Babesia canis vogeli* were edited using Bioedit. The sequences were assembled in notepad using Bankit method and then the corrected tick sequences were submitted in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). GenBank accession numbers from this study are KY799078 - KY799086 for *Rhipicephalus sanguineus* and MF000388 - MF000390 for *Babesia canis vogeli* (Appendix VII).

3.11

Data Analyses

Data obtained were expressed as percentages and presented in forms of tables and charts. Haematological data were expressed as means \pm SEM, and thereafter, subjected to Chi-square analysis. Odds ratio at 95% confidence interval was used to assess the risk factors (age, sex, breed and season). Values of $P < 0.05$ were considered significant. A tool of Statistical Package for Social Science (SPSS, Chicago, Ill USA, and Version 20) was used. The percentage of nucleotide variation among sequences of a given species was calculated by pairwise comparison (Kimura 2-parameter model) (Kimura, 1980) using the MEGA7.0 software (Kumar *et al.* 2016). The pairwise comparison of sequence differences (D) among *Rhipicephalus* spp. and *Babesia* spp. consensus sequences were calculated using the formula $D = 1 - (M/L)$, where M is the number of alignment positions at which the two sequences have a base in common, and L is the total number of alignment positions over which the two sequences are compared (Chilton *et al.*, 1995).

CHAPTER FOUR

4.0

RESULTS

4.1

Prevalence of *Babesia* spp. Infection of Dogs in the Study Area

The prevalence of *Babesia canis* infection in dogs sampled from the six Area Councils of the Federal Capital Territory, using parasitological method (thin and thick blood smears) as shown in Table 4.1. Out of the 480 dogs sampled, 15(3.1%) showed *B. canis* in thin blood smear techniques and 52(10.8%) showed *B. canis* in thick blood smears techniques (Table 4.1). The large *Babesia* was identified morphologically in Giemsa stained blood smears as piroplasms of typical pear-shaped, single, or paired (pyriform) or multiple merozoites in the infected erythrocytes (Figure 4.1). There was significant ($p < 0.05$) association between the prevalence of *B. canis* infection in thin and thick blood smear techniques.

Table 4.1: Prevalence of *Babesia canis* Infection in Dogs in the FCT Based on Smear Techniques

Blood film	No of films examined	No. Positive	(%) Positive
Thin	480	14	(3.1)
Thick	480	52	(10.8)

DF = 1, P = 0.003

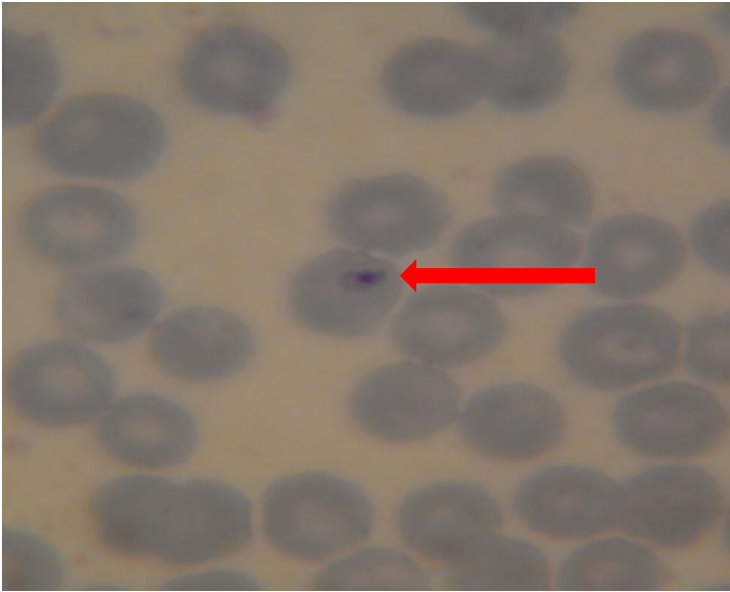


Plate I: A single *Babesia* merozoite in Giemsa-stained thin smear showing pyriform body with basophilic cytoplasm and reddish chromatin, from sampled dog in Bwari Area Council (×1000)

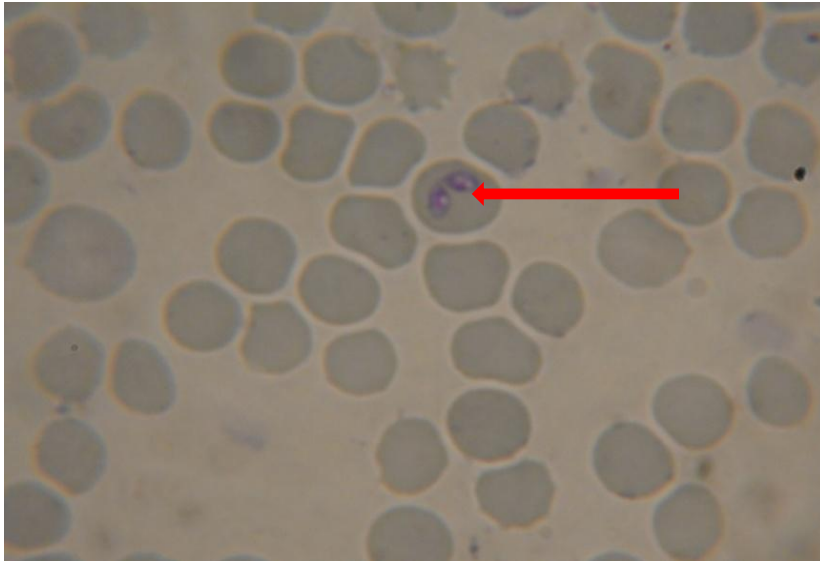


Plate II: A paired intra-erythrocytic *Babesia* merozoites in Giemsa-stained thin smear with basophilic cytoplasm and reddish chromatin, from sampled dog in Gwagwalada Area Council of the FCT ($\times 1000$).



PlateIII: Intra-erythrocytic merozoites of *B. canis* in Giemsa-stained thin smear revealing multiple pyriform bodies with basophilic cytoplasm and reddish chromatin from sampled dog in Gwagwalada Area Council ($\times 1000$).

4.2 Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Sampled Location

The prevalence of *Babesia canis* infections in the sampled locations showed 6.3%, 12.5%, 10.0%, 12.5%, 11.3%, 12.5% of the sampled dogs were positive for *B. canis* in Abaji, Abuja Municipal, Bwari, Gwagwalada, Kuje and Kwali Area Council respectively (Table 4.2). The prevalence of *Babesia canis* infections was highest (12.5%) in dogs from Kwali, Municipal and Gwagwalada and lowest 5 (6.3%) in dogs from Abaji Area Council. There was no significant ($p > 0.05$) association between sample location and the prevalence of *B. canis* infections in dogs in the FCT.

Table 4.2: Prevalence of *Babesia* spp in Dogs in the FCT Based on Sample Location

Location	No. of Dogs Sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>
Abaji	80	5	6.3
Abuja Municipal	80	10	12.5
Bwari	80	8	10.0
Gwagwalada	80	10	12.5
Kuje	80	9	11.3
Kwali	80	10	12.5
Total	480	52	10.8

DF=5, P=0.208

4.3 Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Sex

Table 4.3 showed the prevalence of *Babesia* spp. infection based on sex of the sampled dogs in the Federal Capital Territory. Of the 227(47.3%) male and 253(52.7%) female dogs sampled, 31 (13.7%) and 21 (8.3%) tested positive for *B. canis*. Odds ratio showed that male dogs were 1.2 times more prone to *Babesia canis* spp. infections than the female (Table 4.3). There was no significant ($p > 0.05$) association between sex and prevalence of *B. canis* infections in dogs in the FCT.

Table 4.3: Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Sex

Sex	No. of dogs sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio	Confedence Interval
Male	227	31	13.7	1.24	0.73 – 2.11
Female	253	21	8.3	1.00	--
Total	480	52	10.8		

DF=1, P= 0.06

4.4 Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Age

Table 4.4: The Prevalence of *Babesia canis* infection based on age of sampled dogs in the Federal Capital Territory. The 480 dogs sampled, were placed in age categories: 170 (>.25<1yr), 194 (1<3yr), 94(3<5yr) and 22(>5yr). Dogs 1 - < 3 years old had the highest prevalence of 17.0% for *Babesia canis* infection while > 5 years of age had the lowest 4.5% rate of infection. The dogs in age category of 0<1 had infection rate of 6.5% while those in age category 3<5 had 7.4% infection rate. Odds ratio analysis showed that dogs in age category of 1<3 groups are two times more prone to infections than those in other age categories (Table 4.4). There was a significant ($p < 0.05$) association between age and prevalence of *B. canis* infections in dogs in the FCT.

Table 4.4: Prevalence of *Babesia canis* Infection in the FCT Based on Age

Age (yr)	No. of dogs sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio (OR)	95% confidence interval
.25 -<1	170	11	6.5	0.58	0.28 – 1.78
1-<3	194	33	17.0	1.00	-
3-<5	94	7	7.4	0.26	0.82 – 6.42
>5	22	1	4.5	0.72	0.62 – 3.4
Total	480	52	10.8		

DF=3, P=0.003

4.5 Prevalence of *Babesia* spp. Infection in Dogs in FCT Based on Breed

Table 4.5 showed the occurrence of *Babesia canis* infection based on breed of sampled dogs in the Federal Capital Territory. Of the 480 dogs sampled 62 were exotic, 170 were crossbred and 248 were local breed. Exotic dogs had the highest of 8 (12.9%) *Babesia canis* infection while cross breed had the lowest of 9.4% infection rate. The local breed had 11.3% rate of *Babesia canis* infection. Odds ratio analysis showed that exotic dog was 1.2 times more prone to *Babesia* species infection than the local and cross breed of dogs. There was no significant ($p > 0.05$) association between breed and prevalence of *B. canis* infections in dogs in the FCT.

Table 4.5: Prevalence of *Babesia canis* Infection in Dogs in the FCT Based to Breed

Breed	No. of dogs sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio	95% confidence interval
Exotic	62	8	12.9	1.16	0.50 – 2.69
Cross	170	16	9.4	0.82	0.43 – 1.56
Local	248	28	11.3	1.00	0.22 – 1.32
Total	480	52	10.8		

DF=2, P=0.71

4.6 Prevalence of *Babesia* spp. Infection in Dogs in the FCT Based on Purpose

Table 4.6 showed the prevalence of *Babesia canis* infection based on purpose of sampled dogs in the Federal Capital Territory. Of the 395 (82.3%) guard, 71 (14.8%) hunting, and 14 (2.9%) pet dogs sampled; 44 (11.1%), 8 (11.3%) and 0 (0%) were positive for *B. canis* infection respectively. There was no significant ($p > 0.05$) association between purpose and prevalence of *B. canis* infections in dogs in the FCT.

Table 4.6: Prevalence of *Babesia canis* Infection in Dogs in the FCT Based on Purpose

Purpose	No. of dogs sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio	95% confidence interval
Guard	395	44	11.1	2.78	0.46 – 16.71
Hunting	71	8	11.3	2.83	0.47 – 17.00
Pet	14	0	0	1.00	0.12 – 2.61
Total	480	52	10.8		

DF=2; P=0.37

4.7 Prevalence of *Babesia canis* Infection in Dogs in the FCT Based on Tick Infestation

Table 4.7: showed the prevalence of *Babesia canis* infection based on tick infestation of sampled dogs in the Federal Capital Territory. Of the 254 dogs infested with tick 44(17.3%) had *Babesia canis* infection while 8(3.5%) of 226 non-infested dogs were positive for *Babesia* infection. Odds ratio analysis showed that tick infested dogs were 4.9 times more prone to *Babesia canis* infection than non-infested dogs. There was a significant ($p < 0.05$) association between tick infestation and prevalence of *B. canis* infections in dogs in the FCT.

Table 4.7: Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Tick Infestation

Ticks	No. of Dogs Sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio (OR)	95% Confidence Interval
Present	254	44	17.3	4.9	2.5 – 11.8
Absent	226	8	3.5	1.0	1.22 – 7.6
Total	480	52	10.8		

DF= 1, P=0.09

4.8 Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Season.

Table 4.8: showed the prevalence of *Babesia canis* infection based on season of the year on sampled dogs in the Federal Capital Territory. Of the 240 dogs sampled (each for rainy season and dry season), 35 (14.6%) tested positive for *Babesia canis* during the rainy season and 17(3.5%) dogs during dry season. The occurrence of *Babesia canis* infection within the months of the year showed that August and September had the highest (13.5%) prevalence while January and February had the lowest 2.0% rate. There was significant ($p < 0.05$) association between season and prevalence of *B. canis* infections in the FCT. However, findings showed that *Babesia canis* infections were observed in all the months of the year studied.

Table 4.8: Prevalence of *Babesia* spp. Infection of Dogs in the FCT Based on Season

Season	No of dogs sampled	No. Positive for <i>B. canis</i>	(%) Positive for <i>B. canis</i>	Odds ratio	95% Confidence Interval
Wet season	240	35	14.6	2.08	0.56 – 1.62
Dry season	240	17	7.1	0.98	0.63 – 1.70
Total	480	52	10.8		

DF=1, P=0.022

4.9 Haematological Parameters of Dogs Examined for *Babesia* spp. Infection in the Study Area.

Table 4.9 showed the haematological parameters of dogs tested positive for *Babesia* spp. infection as compared with those tested negative for the infection. The values for haemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular haemoglobin concentration, segmented neutrophils and platelets, were higher for dogs that tested negative for *B. canis* infection than for those tested positive which had lower values. The mean corpuscular volume, white blood cell count, band neutrophils, lymphocytes, monocytes, and eosinophils values were higher in dogs tested positive for *B. canis* infection than for those tested negative that had lower values. There was significant ($p < 0.05$) association between RBCs count, HB content, PCV and *B. canis* infection among the sampled dogs. The results showed that 57.4 % of sampled dogs had RBCs count below reference values, while RBCs count was within reference values in 42.6 % of the dogs. PCV was below reference values in 63.8% of sampled dogs, while 36.2% of dogs showed PCV within reference range. The HB concentration was below reference values in 63.8% of sampled dogs, while 36.2% of dogs had hemoglobin content within normal values. The red blood cell indices showed a significant ($p < 0.05$) difference between *B. canis* infection and MCV in 28.4% of the sampled dogs, and 14.4% of dogs showed MCV below the normal range with 57.2% of the dogs having MCV within normal reference values. A decrease in MCH values was noted in 15% of sampled dogs, and increase in MCH values in 12% of dogs with 73% of the sampled dogs having MCH within reference value. Decrease in MCHC values was recorded in 32.4% of the sampled dogs, and an increase in MCHC values in 16.4% while 51.2% of the dogs had MCHC within reference values. Significant

($p < 0.05$) difference in WBCs count, lymphocytes and monocyte counts was observed in dogs tested positive for *Babesia* spp. infection as compared to those tested negative for the infection. Thrombocytopenia was observed in 89% of the dogs tested positive for *Babesia* spp. infection.

Table 4.9: Haematological parameters of sampled dogs in the study area (\pm SEM)

Haematology	Negative	Positive	Reference
WBC($\times 10^9 / \mu\text{L}$)	10.24 \pm 0.35 ^b	014.28 \pm .28 ^a	4.0 – 15.5
RBC($\times 10^6 / \mu\text{L}$)	6.08 \pm 0.05 ^a	5.11 \pm 0.13 ^a	4.8 – 9.3
Hb (g/dL)	12.2 \pm 0.26 ^a	9.71 \pm 0.27 ^a	12.1 – 20.3
PCV (%)	42.0 \pm 0.35 ^a	33.2 \pm 0.66 ^b	36 - 55
MCV(fL)	69.2 \pm 0.83 ^a	70.2 \pm 0.41 ^a	58 - 79
MCH(pg)	23.4 \pm 0.21 ^a	25.3 \pm 0.69 ^a	19 - 28
MCHC(g/dl)	33.0 \pm 0.73 ^a	32.2 \pm 0.32 ^a	30 – 38
PLT($\times 10^9 / \text{L}$)	205.23 \pm 4.50 ^a	95.64 \pm 0.95 ^b	170 - 400
NEUT (%)	61.8 \pm 0.43 ^a	42.8 \pm 0.62 ^b	60 - 77
Lymphocyte (%)	18.3 \pm 0.25 ^b	22.5 \pm 0.99 ^a	12 - 30
Monocyte (%)	2.18 \pm 0.11 ^a	3.27 \pm 0.50 ^a	3 - 10
Eosinophils (%)	3.1 \pm 0.6	2.2 \pm 0.7	1 - 3

(ab) Values with different superscripts letters on the same row indicate significant association means ($p < 0.05$); confidence interval: 95%.

4.10 Morphological Identification of *Rhipicephalus* spp. from the Study Area

Tables 4.10 – 4.12 showed that a total of 2043 ticks (2041 *Rhipicephalus* spp and 2 *Amblyomma* spp.) collected from sampled dogs in the study area, were identified to genus level using morphological features. Two genera of ticks identified were *Rhipicephalus* spp. (basis capitulum with hexagonal shape from a dorsal view); *Amblyomma* (Large mouth parts, ornamented scutum, and legs with pale rings). The categories identified in the genus *Rhipicephalus* include; 1195 male (♂): (by features of dorsal region: medium in size (2-3.2mm); covered with the scutum; regular rows of large punctuation. Ventral region: adanal plates, with wide posterior), 914 female (♀): (Dorsal region: short scutum, medium in size (3-3.5mm), and punctuation on the conscutum. Ventral region: genital aperture). The 19 nymphs (absence of anal plates and genital apparatus) and 3 larvae (3 pairs of legs) as shown (Table 4.10, Plates 1-5). The *Amblyomma variegatum* identified in the study were 2 male (♂) (ornamented scutum, legs with pale rings and well developed mouth part), as shown in (plates VI and VII).

Table 4.10: *Rhipicephalus sanguineus* from Sampled Dogs in the FCT Identified using morphological features

Location	Name	Sex	Number
Abaji	<i>R. sanguineus</i>	(♂)	155
		(♀)	128
Abuja Municipal	<i>R. sanguineus</i>	(♂)	159
		(♀)	138
Bwari	<i>R. sanguineus</i>	(♂)	192
		(♀)	168
Gwagwalada	<i>R. sanguineus</i>	(♂)	220
		(♀)	185
Kuje	<i>R. sanguineus</i>	(♂)	186
		(♀)	168
Kwali	<i>R. sanguineus</i>	(♂)	183
		(♀)	157
		Nymph	19
		Larvae	3
Total			2041

Table 4.11: *Amblyomma variegatum* from sampled dogs in the FCT identified using morphological features

Location	Name	Sex	Number
Abaji	<i>A. variegatum</i>	(♂)	0
		(♀)	0
Minicipal	<i>A. variegatum</i>	(♂)	0
		(♀)	0
Bwari	<i>A. variegatum</i>	(♂)	0
		(♀)	0
Gwagwalada	<i>A. variegatum</i>	(♂)	2
		(♀)	0
Kuje	<i>A. variegatum</i>	(♂)	0
			0
Kwali	<i>A. variegatum</i>	(♂)	0
		(♀)	0
Total		(♂)	2

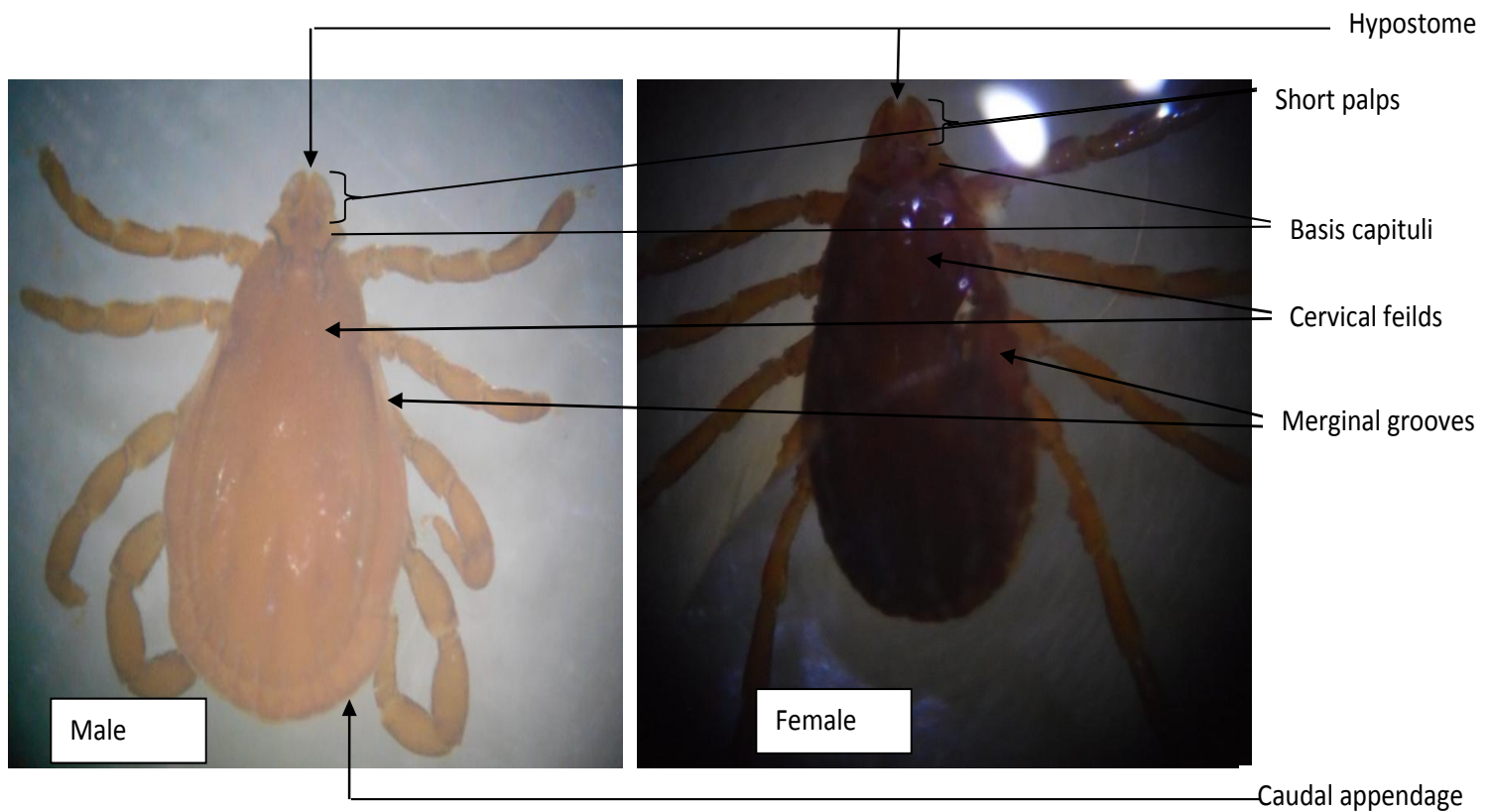


Plate IV: Dorsal view of male and female of *R. sanguineus* from Sampled Dogs in Kuje Area Council in the FCT showing the morphological features

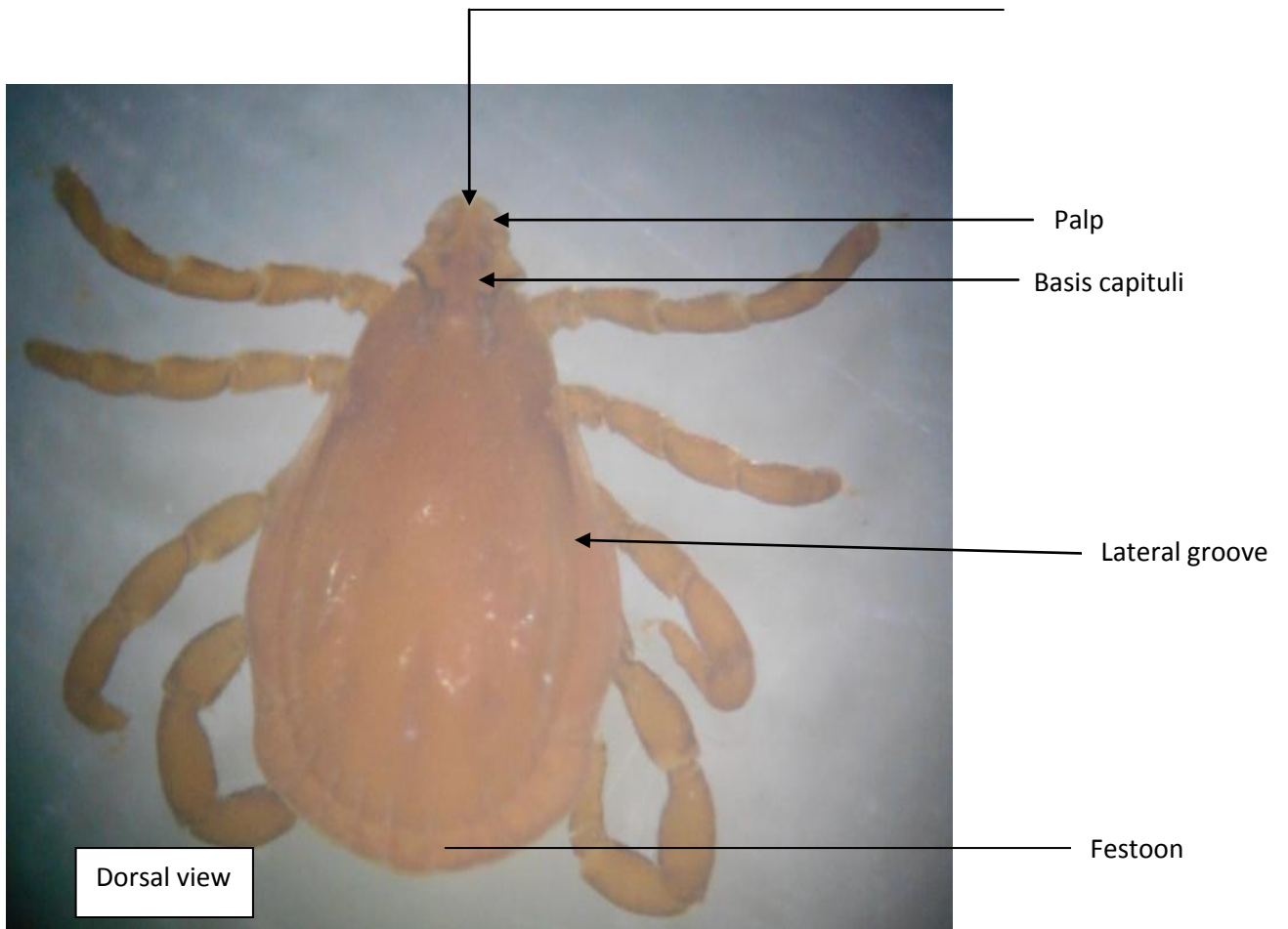


Plate V: *Rhipicephalus sanguineus* (male) from Sampled Dog in Bwari Area Council in the FCT showing morphological features



Plate VI:*Rhipicephalus sanguineus* (female) collected from Sampled Dog in Kuje Area Council in the



Plate VII: *Rhipicephalus sanguineus* (female) collected from Sampled Dog in Bwari Area Council in the FCT



Plate VIII: *Rhipicephalus sanguineus* (male) ventral region collected from Sampled Dog in Kwali Area Council in the FCT

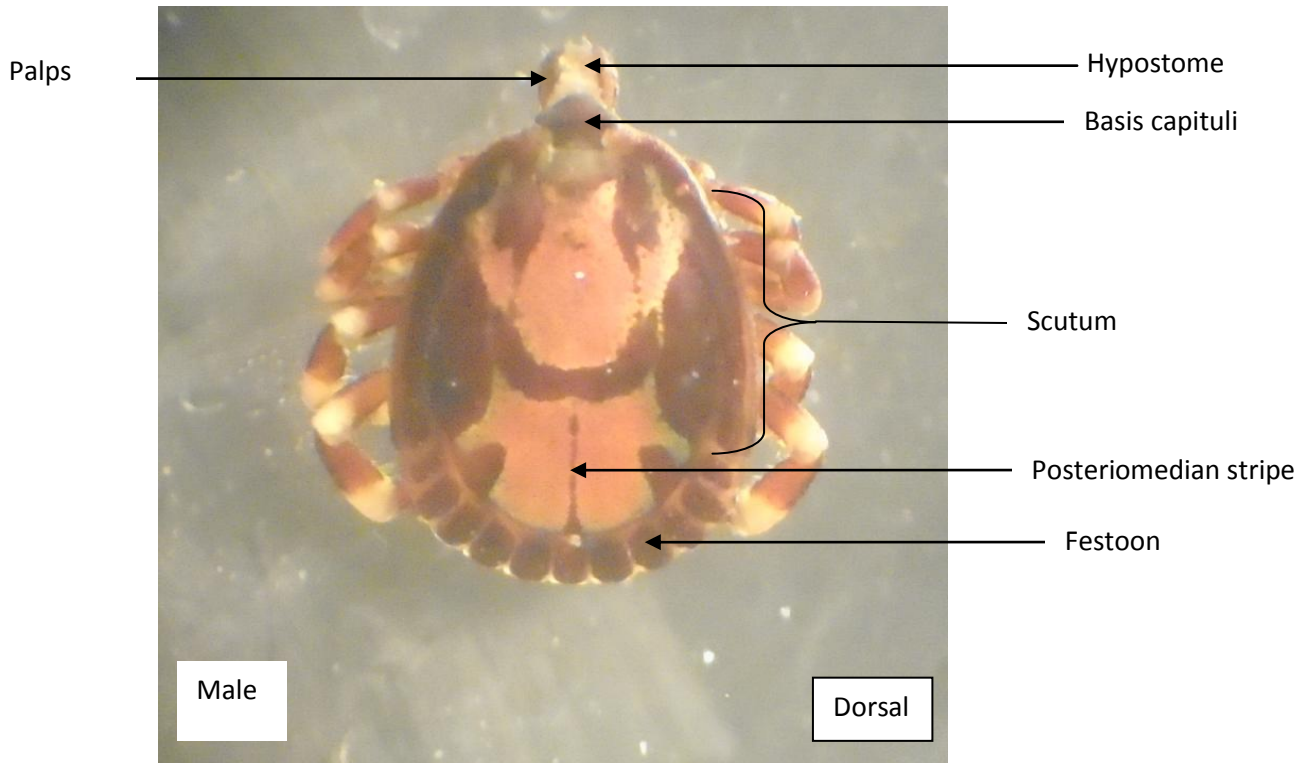


Plate IX: *Amblyomma variegatum* collected from a Hunting Dog in Gwagwalada Area Council of the Study Area

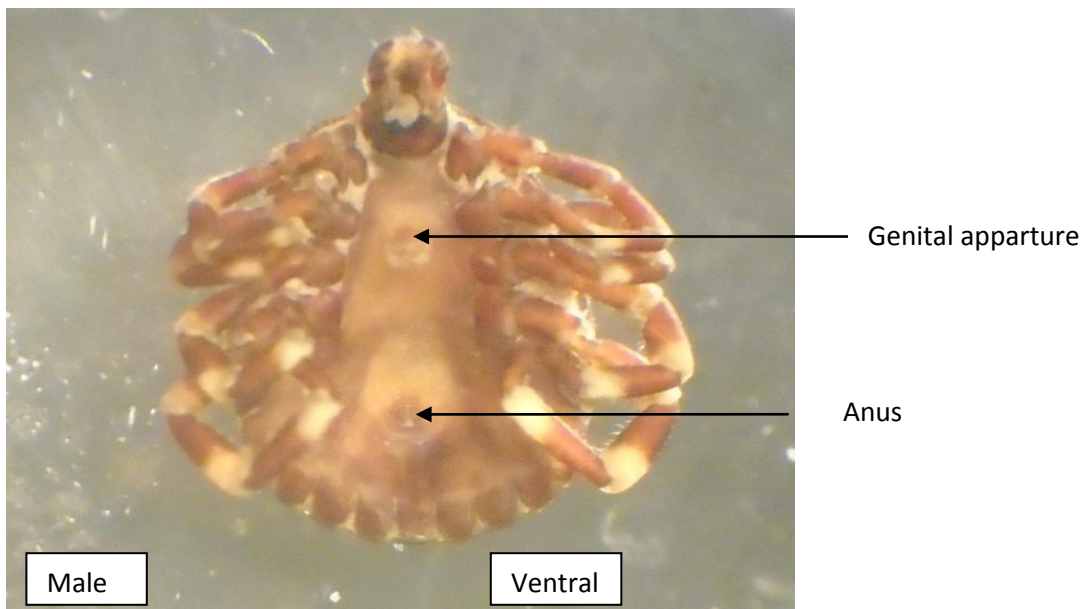


Plate X: *Amblyomma variegatum* collected from a Hunting Dog in Gwagwalada Area Council of the Study Area

Table 4.12: showed the rate of tick infestation on sampled dogs in the studied locations. Of the 480 dogs sampled, 53% (254) dogs were tick infested while 226 (47%) were free of tick infestation (Table 4.12). The tick infestation rates in Abaji, Abuja Municipal, Bwari, Gwagwalada, Kuje and Kwali Area Councils, were 47.5%, 55.0%, 52.5%, 57.5%, 55.0%, and 50.0% respectively. (Table 15). The results showed that dogs in Gwagwalada Area Council had the highest rate of tick infestation and the lowest rate was recorded in Abaji Area Council.

Table 4.12: Prevalence of tick infestation in the FCT according to sample location

Location	No of dogs sampled	N0 of dogs infested	(%)of dogs infested	Odds ratio	95% confidence interval
Kwali	80	40	50.0	0.64	
Bwari	80	42	52.5	1.60	0.09-4.66
Gwagwalada	80	46	57.5	0.32	0.03-3.60
Kuje	80	44	55.0	1.44	0.26-7.96
Municipal	80	44	55.0	0.72	0.10-5.17
Abaji	80	38	47.5	1.00	-
Total	480	254	53.0		

DF=5; p=0.27

Figure 4.1: showed the distribution of tick's attachment sites on sampled dogs. The ear was the most preferred sites with a prevalence of 45.9% while the scrotum and the mammary region had the least with (2.0%) each.

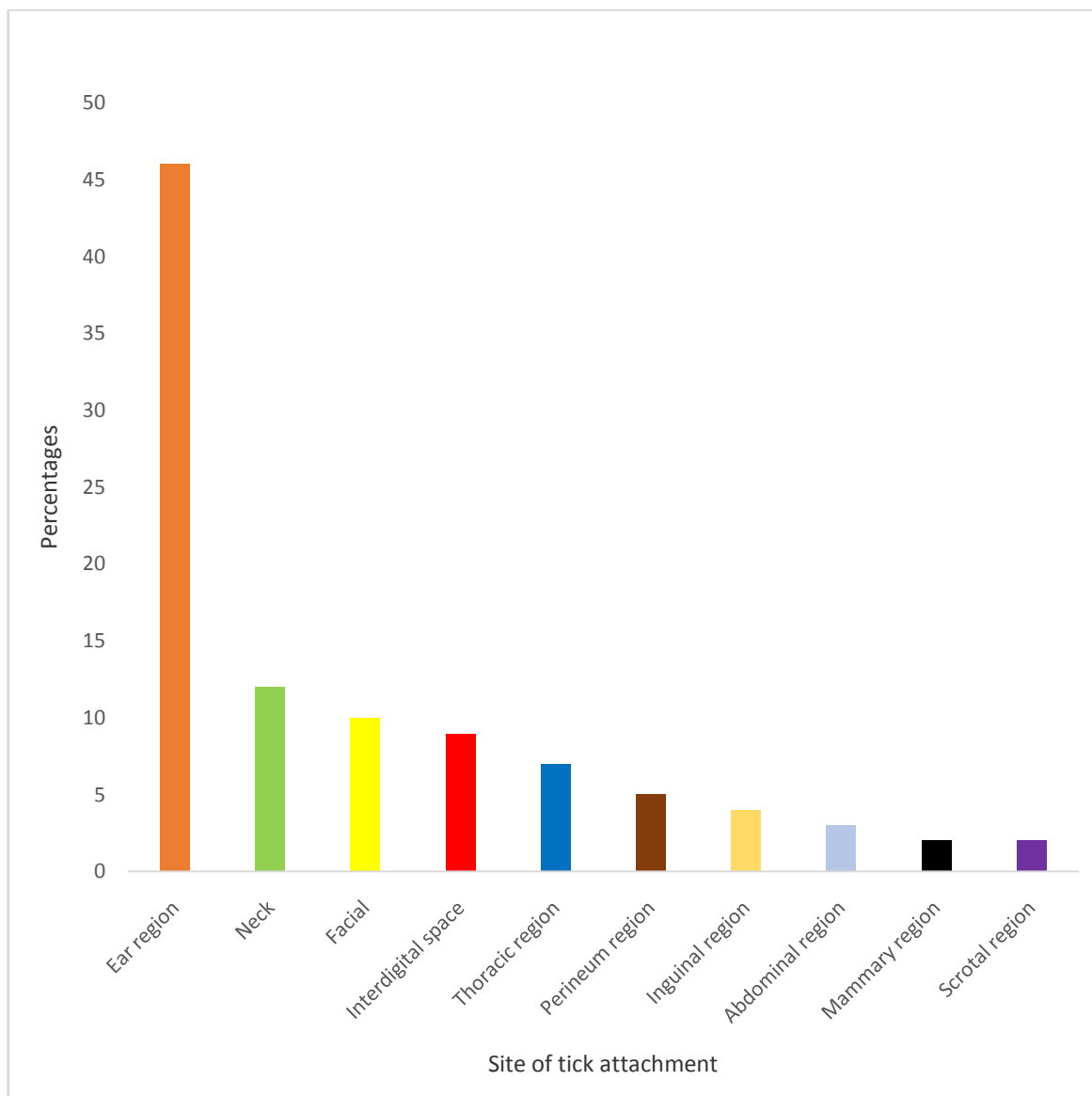


Figure 4.1: Distribution of ticks in sampled Dogs in the FCTBased on Predilection Sites



Plate XI: *Rhipicephalus sanguineus* ticks in the Ear of a Crossbred Dog Sampled in Gwagwalada Area Council in the FCT

4.11

Haemolymph Smear Result for *R. sanguineus*

From the total of 914 females *Rhipicephalus sanguineus* ticks collected from the sampled dogs in the study, (Unengorged = 866 and engorged =48). The haemolymph smears analysis was carried out on 32 fully engorged ticks. The result showed that no *Babesia*ookinete was detected in the Giemsa stained haemolymph smears under the light microscopy.

4.12 Molecular identification of *Rhipicephalus sanguineus* ticks

A total of 23 ticks were used for PCR analysis, and out these number, 16 (70%) samples showed *Rhipicephalus* spp. 18S rRNA gene amplification at the expected range of 173bp as shown in the gel electrophoresis (Plate VII). Nine amplicons of these samples were selected for sequencing.

4.12.1 *Rhipicephalus sanguineus* sequence results

The sequence results for tick sample was received in form of an electrophorogram, and these were opened using a plasmid editor ApE[®] software. The electrophorograms graphically illustrates the individual nucleotides as waves, with the wave spikes corresponding to the different nucleotides in colours. Representative colours for the nucleotides: green, blue, black and red include Adenine (A), Cytosine (C), Guanine (G), and Thymine (T) respectively (shown in Figure 4:4). The electrophorograms were further edited to remove the noise before converting to a Fasta format (Figure 4.5), in order to obtain a consensus sequence for each isolate and subsequently conduct a blast search. The electrophorogram and the Fasta format of the nucleotides for *R. sanguineus* partial 18S rRNA gene are shown in Figures 4.4 and 4.5) respectively. The results of the query

conducted in nucleotide Blast search for the tick sequences from the FCT in the NCBI database, showed 96 – 100% similarity to ribosomal 18S rRNA gene of *R. sanguineus* with gene accession numbers (KP830113, KF958430, and KF958451) (as shown in Figure 4.6 and 4.7).

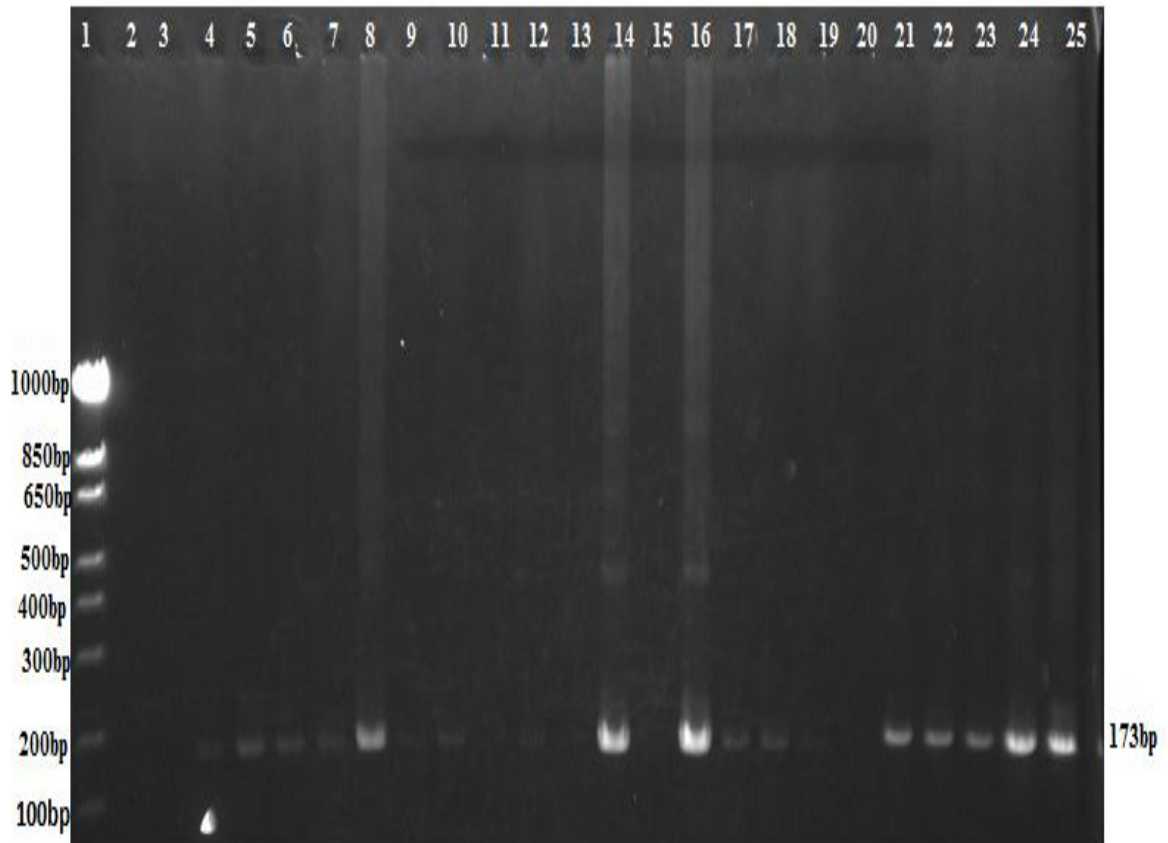


Plate XII: Agarose gel electrophoresis for *R.sanguineus* 18S rRNA gene

Lane 1- Molecular marker, Lane 2: negative control, Lanes 3 – 6 Isolates from Kuje, Lane 7-10: Isolates from Abaji, Lanes 11-14 Isolates from Kwali, Lanes 15-18 Isolates from Bwari, Lane 19-21: Isolate from Municipal, Lanes 22-25: Isolates from Gwagwalada.

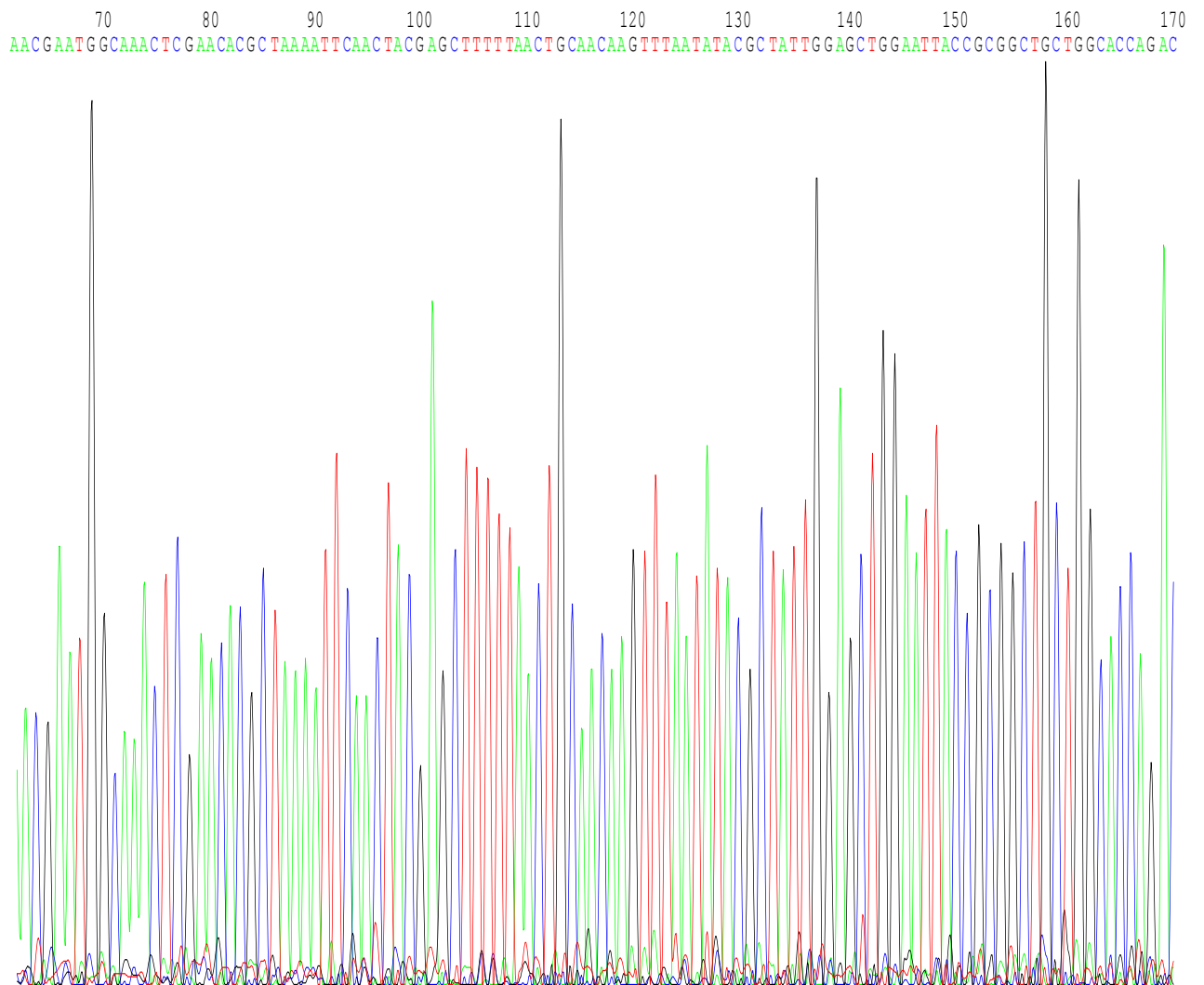


Figure 4:2 The Chromatograms of 18S rRNA gene nucleotide sequences for *R. sanguineus* collected from the Study Area, in ApE software
Representative colours for the nucleotides: Adenine (green), Cytosine (blue), Guanine (black) and Thymine (red).

>**KY799078**
CGCGTGACCATGCCCCGTTCTTCGCCCCCGGGTACTCCGTCCAAATCATTCCAA
AAGGCTGGCTCGTTAAATTATGTTAAGTTCCTTGGATCGTTTCTTCATACTTGGAA
TAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTGGGT
AACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCCGTATTGTGTGG
TGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTTTCTT
TCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGGTTGT
TACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACG
GGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAATT
GAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTG
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGC
GGTTAAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACCCG
ATGCGACGGCTCGGACTGAACATCATGCCGTTCTTTCTTGGTGCACCTTCATTG
TGTGCCTCGAGATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGC
AGCGAGTCGCCTGAATAAACTTGCATGGAATATAGAACAAGACTCGTTTCTGT
CTGTGGTTTTTGGAT

Figure: 4.3: Representative of Fasta format of nucleotide sequences for *R. sanguineus* from this study.

Score	Expect	Identities	Gaps	Strand
1504 bits(814)	0.0	814/814(100%)	0/814(0%)	Plus/Minus
Query 5	CTGGCGGGTCTGAATAGTTAAGGGATCGCCAGGTTATTACTTGTTAAAGACCGGGCGCAAC	64		
Sbjct 814	CTGGCGGGTCTGAATAGTTAAGGGATCGCCAGGTTATTACTTGTTAAAGACCGGGCGCAAC	755		
Query 65	CGAAAGGCCGCGCCGGACATCGGTCCGAAGACCTCACTAAATCATTCAATCGGTAGTAGC	124		
Sbjct 754	CGAAAGGCCGCGCCGGACATCGGTCCGAAGACCTCACTAAATCATTCAATCGGTAGTAGC	695		
Query 125	GACGGGCGGTGTGTACAAAGGGCAGGGACGTAATCAACGCGAGCTTATGACTCGCGCTTA	184		
Sbjct 694	GACGGGCGGTGTGTACAAAGGGCAGGGACGTAATCAACGCGAGCTTATGACTCGCGCTTA	635		
Query 185	CTGGGAATTCCTCGTTCAAGGGGAACAATTGCAAGCCCCTATCCCAATCACGAAAGAAGT	244		
Sbjct 634	CTGGGAATTCCTCGTTCAAGGGGAACAATTGCAAGCCCCTATCCCAATCACGAAAGAAGT	575		
Query 245	TCCACGGGTTACCCAGTCTTTTCAGACAGGGATAAAGACACGCTGCTTCCTTCAGTGTAG	304		
Sbjct 574	TCCACGGGTTACCCAGTCTTTTCAGACAGGGATAAAGACACGCTGCTTCCTTCAGTGTAG	515		
Query 305	CGCGGTGCGGCCCGGACATCTAAGGGCATCACAGACCTGTTATTGCTCTGTTTCGTGC	364		
Sbjct 514	CGCGGTGCGGCCCGGACATCTAAGGGCATCACAGACCTGTTATTGCTCTGTTTCGTGC	455		
Query 365	GGCTAGGAGCCGCTTGTCCCTCTAAGAAGGTTGTAAGGTGCTGGGAACCCCGCACCTATT	424		
Sbjct 454	GGCTAGGAGCCGCTTGTCCCTCTAAGAAGGTTGTAAGGTGCTGGGAACCCCGCACCTATT	395		
Query 425	TAATAGGCTAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAA	484		
Sbjct 394	TAATAGGCTAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAA	335		
Query 485	GAACGGCCATGCACCACCATCCACCGAATCAAGAAAGAGCTCTCAATCTGTCAATCCTCC	544		
Sbjct 334	GAACGGCCATGCACCACCATCCACCGAATCAAGAAAGAGCTCTCAATCTGTCAATCCTCC	275		

Figure 4.4: GenBank BLAST analysis for tick sample

Query represents tick from this study while subject represents *R. sanguines* (KF 958451.1), Nucleotide sequences fully aligned

Score	Expect	Identities	Gaps	Strand
1424 bits(771)	0.0	792/801(99%)	6/801(0%)	Plus/Minus
Query 11	GGTCTGAATAGTTA-AGGGATC--GCCAGGTTATTACTTGTAAAGACCGGCGCAACCGA	67		
Sbjct 806	GGTCTTATTAGTTATAGGGATCAGTGC-GGTTATTACTTGTAAAGACCGGCGCAACCGA	748		
Query 68	AAGGCCGCGCCGGACATCGGTCCGAAGACCTCACTAAATCATTCAATCGGTAGTAGCGAC	127		
Sbjct 747	AAGGCCGCGCCGGACATCGGTCCGAAGACCTCACTAAATCATTCAATCGGTAGTAGCGAC	688		
Query 128	GGGCGGTGTGTACAAAGGGCAGGGACGTAATCAACGCGAGCTTATGACTCGCGCTTACTG	187		
Sbjct 687	GGGCGGTGTGTACAAAGGGCAGGGACGTAATCAACGCGAGCTTATGACTCGCGCTTACTG	628		
Query 188	GGAATTCCTCGTTC AAGGGGAACAATTGCAAGCCCCTATCCCAATCACGAAAGAAGTTC	247		
Sbjct 627	GGAATTCCTCGTTC AAGGGGAACAATTGCAAGCCCCTATCCCAATCACGAAAGAAGTTC	568		
Query 248	ACGGGTTACCCAGTCTTTTCAGACAGGGATAAAGACACGCTGCTTCCTTCAGTGTAGCGC	307		
Sbjct 567	ACGGGTTACCCAGTCTTTTCAGACAGGGATAAAGACACGCTGCTTCCTTCAGTGTAGCGC	508		
Query 308	GCGTGCGGCCCGGACATCTAAGGGCATCACAGACCTGTTATTGCTCTGTTTCGTGCGGC	367		
Sbjct 507	GCGTGCGGCCCGGACATCTAAGGGCATCACAGACCTGTTATTGCTCTGTTTCGTGCGGC	448		
Query 368	TAGGAGCCGCTTGTCCTCTAAGAAGGTTGTAAGGTGCTGGGAACCCCGCACCTATTTAA	427		
Sbjct 447	TAGGAGCCGCTTGTCCTCTAAGAAGGTTGTAAGGTGCTGGGAACCCCGCACCTATTTAA	388		
Query 428	TAGGCTAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAAGAA	487		
Sbjct 387	TAGGCTAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAAGAA	328		
Query 488	CGGCCATGCACCACCATCCACCGAATCAAGAAAGAGCTCTCAATCTGTCAATCCTCCCAG	547		

Figure 4.5: GenBank BLAST analysis for *R. sanguineus* tick sample
Query represents tick from this study while subject represents *R. sanguineus* (KF958451.1), Nucleotide insertions and mismatches can be observed in the alignment (showed with arrows).

4.12.2 Phylogenetic analysis result for *R. sanguineus* from the study area

Sequence of *R. sanguineus* from this study and reference sequences were imported from the GenBank into notepad page and subsequently analysed in MEGA7.0 software. The sequences were aligned using multiple alignment program ClustalW (as shown in figure 4.8). The phylogenetic tree was constructed using Maximum Composite Likelihood method to compute the evolutionary distance of the tick isolates from the study area with the sequences of *Rhipicephalus sanguineus* in the GenBank. The phylogenetic tree constructed for *Rhipicephalus sanguineus* from the study area is shown in (Figure 4.9). The branch length represents evolutionary changes that have taken place over time and the amount of genetic change is represented by a scale of 0.1. The number of substitution which is related to the clustering together of the taxa as a bootstrap test, is represented by a percentage value; that is, the number of substitution per 100 nucleotide sites and is shown above the branches. The tree was rooted to the genus *Amblyomma variegatum*. The results showed that tick isolates from the studied area, *Rhipicephalus sanguineus*: KY799078 – KY799086 (FCT), from the studied area clustered together, showing common ancestral relationship. However, the second clusters were *R. sanguineus* KP830113 and KF958451 from Cuba and Israel respectively, showing common ancestral relationship between them.

DNA Sequences		Translated Protein Sequences	
Species/Abbrv	Group Name	**	* *** ** **
1. KY799078.1 Rhipicephalus sanguineus isolate Abuja FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
2. KY799079.1 Rhipicephalus sanguineus isolate Abuja FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
3. KY799080.1 Rhipicephalus sanguineus isolate Abuja 3 18S rib		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
4. KY799081.1 Rhipicephalus sanguineus isolate Abuja FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
5. KY799082.1 Rhipicephalus sanguineus isolate Bwari FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
6. KY799083.1 Rhipicephalus sanguineus isolate Gwagwalada FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
7. KY799084.1 Rhipicephalus sanguineus isolate Gwagwalada FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
8. KY799085.1 Rhipicephalus sanguineus isolate Gwagwalada FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
9. KY799086.1 Rhipicephalus sanguineus isolate Abuja FCT		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
10. KP830113.1 Rhipicephalus sanguineus isolate Cuba		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
11. KF958435.1 Rhipicephalus sanguineus isolate Israel		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
12. L76342.1 Rhipicephalus sanguineus 18S ribosomal RNA (18S r		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
13. KF958430.1 Rhipicephalus sanguineus isolate Israel		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
14. KF958451.1 Rhipicephalus curanicus isolate Israel		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	
15. JF826436.1 Amblyomma variegatum isolate		AAACGGGTCCTTACCCGACGAAACAGAGCAATACGGCTCTGTGATGCCCTTGGATGTCCGGGGCCACGCGGTTACCTGAAAGAA	

Figure 4.6: Multiple alignment of 18S rRNA gene for *R. sanguineus* sequences from this study with nucleotide sequences from imported from the GenBank in ClustalW. Representative colours for the nucleotides: Adenine (green), Cytosine (blue), Guanine (black) and Thymine (red).

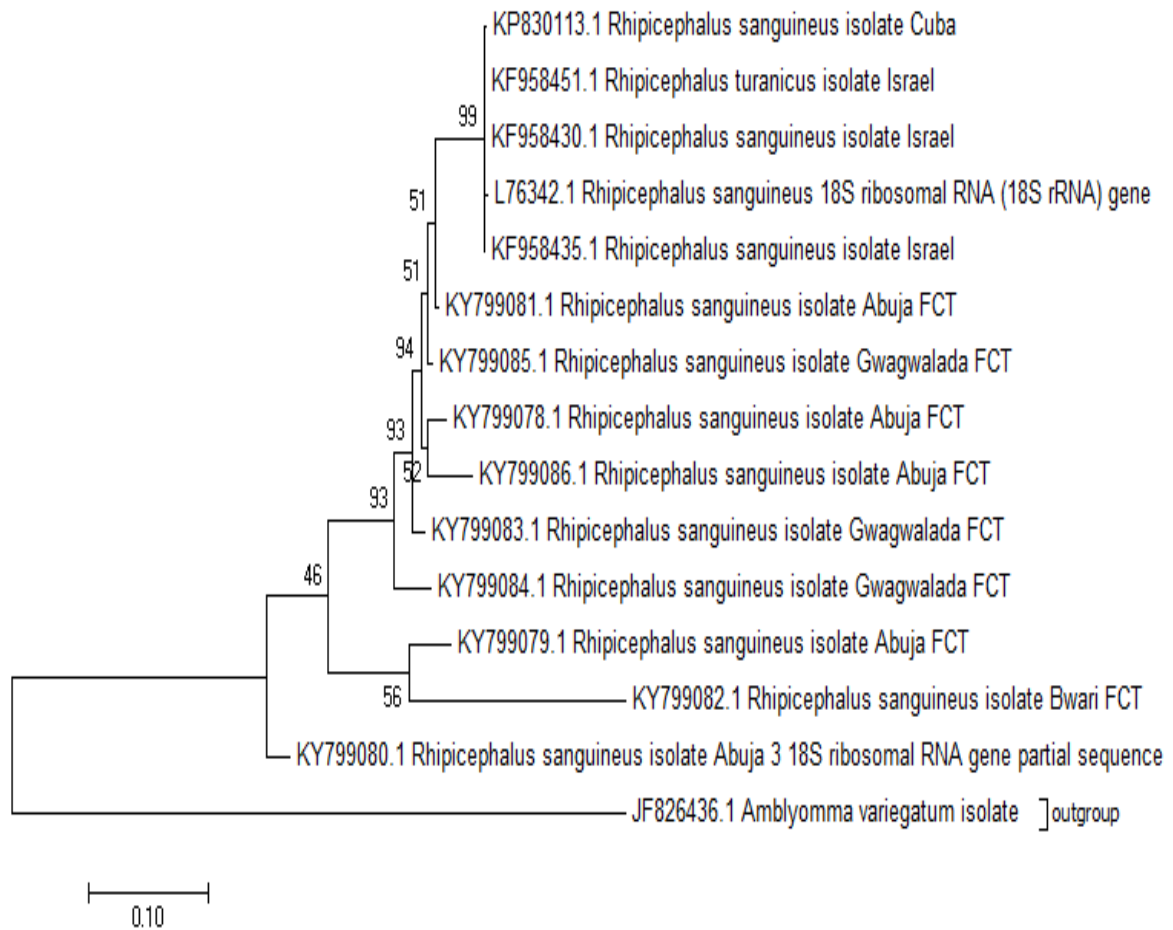


Figure 4.7: Maximum likelihood phylogenetic tree of partial nucleotide sequences (604-nt) 18S rRNA of *R. sanguines*. Numbers on the nodes indicate bootstrap support from 100 replications. Only bootstrap values >50 are shown. The scale bar represents the number of substitutions per nucleotide. The tree was rooted to the genus *A. variegatum*.

4.13 Molecular Identification of *Babesia canis vogeli* from Sampled Dogs in the FCT

A total of 15 blood samples of dogs tested positive for *Babesia canis* in the thin blood smear were used PCR analysis, and of these number 8 (53%) samples showed *Babesia* spp. The ribosomal 18S rRNA gene of *Babesia* spp. amplified in the expected range of 612bp as shown in the gel electrophoresis (Plate VIII). Three amplicons of these samples were selected for sequencing.

4.12.1 Sequence results of *Babesia* spp.

The sequence results for *Babesia* spp. samples from the study area was received in form of an electrophorogram, and these were opened using a plasmid editor ApE[®] software. The electrophorograms graphically illustrates the individual nucleotides as waves, with the wave spikes corresponding to the different nucleotides in colours. Representative colours for the nucleotides: green, blue, black and red include Adenine (A), Cytosine (C), Guanine (G), and Thymine (T) respectively (Appendix V). The electrophorograms were further edited to remove the noise before converting to a Fasta format (Appendix III), in order to obtain a correct sequence for each *Babesia* spp. isolate from the study area and subsequently carried out nucleotide blast search analysis in the NCBI database. The electrophorogram and the Fasta format of the nucleotides for *Babesia* spp. partial 18S rRNA gene are shown in Appendix V and IV) respectively. Nucleotide BLAST results of the sequences of *Babesia* spp. from the study area in the NCBI database (www.ncbi.nlm.nih.gov) shows 100% similarity to ribosomal *Babesia canis vogeli* 18S rRNA (AB083374, KT333456, AY371196, AY072915 and DQ297390) gene accession numbers (as shown in Appendix IV).

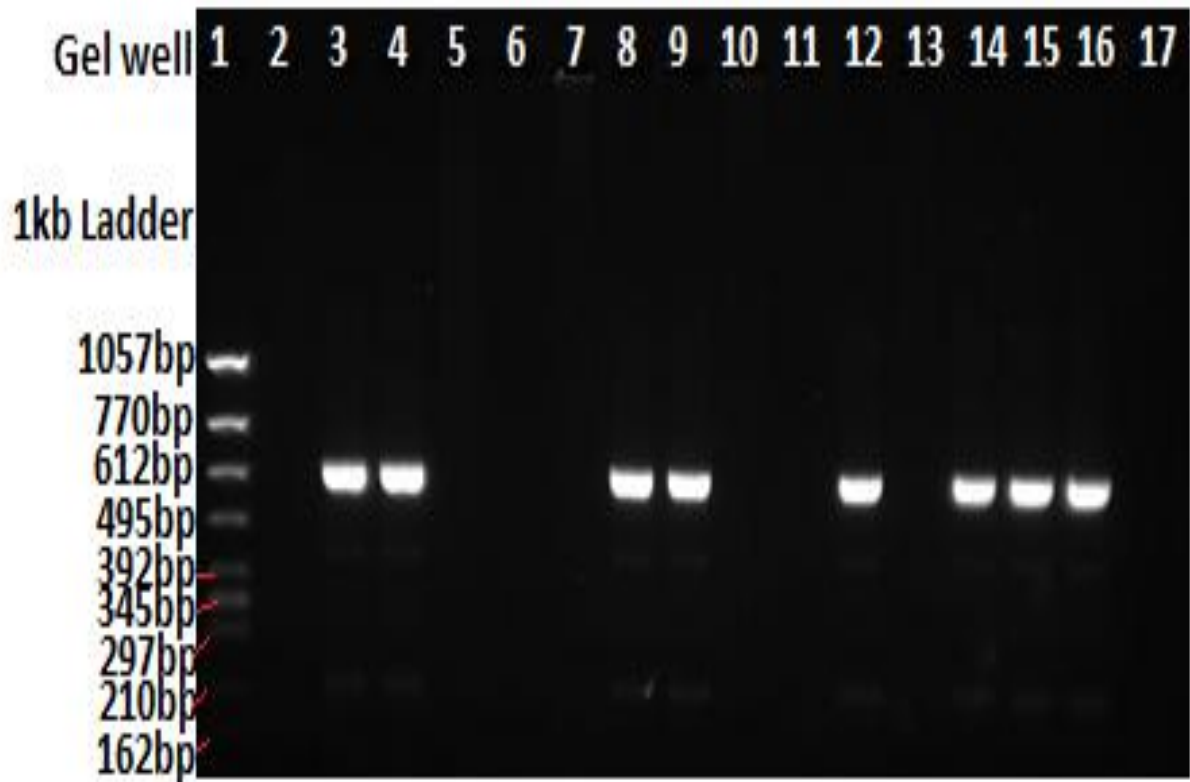


Plate XIII: Agarose gel electrophoresis for *Babesia canis vogeli* 18 S rRNA gene in PCR
Lane 1: Molecular marker, Lane 2: negative control, Lanes 3 and 4: Isolates from Kuje, Lane 5: Isolates from Abaji, Lanes 6 and 7: Isolates from Kwali, Lanes 8, 9 and 10: Isolates from Bwari, Lane 11: Isolate from Municipal Lanes 12, 13, 14, 15 and 16: Isolates from Gwagwalada

DNA Sequences		Translated Protein Sequences	
Species/Abbrv	Group Name	**	*
1. AB083374.1 Babesia canis vogeli Japan		C	G
2. DQ297390.1 Babesia canis vogeli Venezuela		C	G
3. AY371196.1 Babesia canis vogeli Brazil		C	G
4. AY072925.1 Babesia canis vogeli Italy		C	G
5. AF547387.1 Babesia canis vogeli S. Africa		C	G
6. AB303076.1 Babesia canis vogeli Nigeria		C	G
7. MF000388 Babesia canis vogeli FCT Nigeria		C	G
8. MF000389 Babesia canis vogeli FCT Nigeria		C	G
9. MF000390 Babesia canis vogeli FCT Nigeria		C	G
10. KT333456.1 Babesia canis vogeli Brazil		C	G
11. DQ786574.1 Trypanosoma evansi		C	G
12. AY072925.1 Babesia canis vogeli Italy(2)		C	G
13. AF547387.1 Babesia canis vogeli South Africa		C	G
14. JN982353.1 Babesia rossi strain NGR		C	G

Figure 4.8: Multiple sequence alignment of the *Babesia canis vogeli* with the sequences ofticks in GenBank in ClustalW

4.13.3 Phylogenetic analysis results for *B. c. vogeli* from the study area

Sequences of *Babesia canis vogeli* obtained from this study and reference sequences imported from the GenBank were assembled in notepad page and subsequently analysed in MEGA 7.0 software. The sequences were aligned using multiple alignment program ClustalW (as shown in figure 4.11). The phylogenetic tree was constructed using Neighbor-Joining method to compute the evolutionary distance of the *Babesia canis vogeli* from the study area with the sequences in the GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The phylogenetic tree constructed for *Babesia canis vogeli* from the study area is shown in (Figure 4.12). Numbers on the nodes indicate bootstrap support from 1000 replications. Only bootstrap values >50 are shown. The scale bar represents the number of substitutions per nucleotide. The tree was rooted to the genus *Trypanosoma evansi*. The results that one isolate, MF000389 *B. c. vogeli* FCT clustered with DQ297390 *B.c. vogeli* (Venezuela), AY072925 *B.c.vogeli* (Italy), AY371196 *B.c. vogeli* (Brazil) and AF547387 *B .c. vogeli* (South Africa). However, two isolates, MF000388 *B .c. vogeli* (FCT) and MF000390 *B. c. vogeli* (FCT) formed a separate clade.

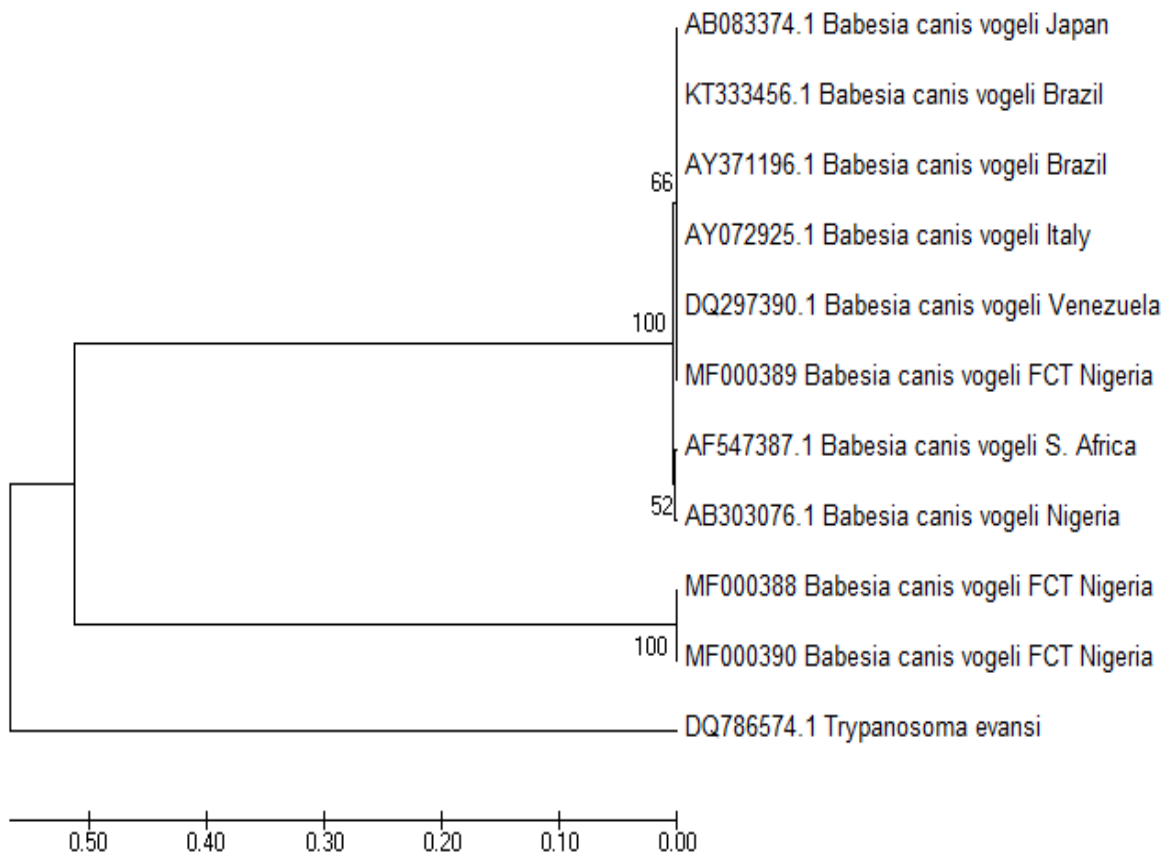


Figure 4.9: Neighbor-Joining phylogenetic tree of 18S rRNA partial sequences of *B.c. vogeli* and other piroplasms. Numbers on the nodes indicate bootstrap support from 1000 replications. Only bootstrap values >50 are shown. The scale bar represents the number of substitutions per nucleotide. The tree was rooted to the genus *Trypanosoma evansi*

CHAPTER FIVE

5.0

DISCUSSION

The study was aimed at determining the prevalence of *Babesia* species infection and further evaluate the parasitological and molecular characteristics of ticks and *Babesia* species of dogs in the Federal Capital Territory, Abuja, Nigeria. The resultsshowed that the overall prevalence of *Babesia* spp. infection in the six Area Councils in the study area was 3.1% and 10.8% in thin and thick smear techniques respectively. To the best of our knowledge, this represents the first comprehensive study that determined the prevalence of *Babesia* spp. infections in the six Area Councils for the period of one year, lasting from September, 2015 through August, 2016. Here in this study the prevalence of 3.1% *Babesia canis* infection in thin blood smear techniques was significantly lower than 10.8% in thick blood smear. Thin blood smear is the oldest technique to detect *Babesia* spp. infections in clinical samples and the most common diagnostic method still in used today in most developing countries (Mosqueda *et al.*, 2012). The sensitivity of this technique is very low as the parasite *Babesia* spp. can only be detected in one infected erythrocyte per ten thousand cells, requiring the analysis of 100-200 microscopic fields, the equivalent to 0.5 µl of blood (Morzaria *et al.*, 1992). Though the method is easy to perform and inexpensive but requires an experienced microscopist to differentiate species and is reliable only if the amount of parasites in the blood is high enough to be detected, which is possible during the acute phase of infection. The route of the blood collection is important as species like *B. bovis* or *B. canis* adhere to endothelial cells (Jacobson, 2006), and their diagnosis using microscopy is enhanced if the blood sample is taken directly from a blood capillary of the ear or tail, as compared to peripheral blood, since capillary blood contains a higher percentage of

infected erythrocytes for these parasites. The observation of paired intraerythrocytic merozoites is indicative of infection but there are other stages of the parasite like the trophozoites, which present different forms and sizes depending on the species, and these make their detection difficult and time-consuming (Potgieter and Els, 1977). Thick blood smear technique was developed to detect low levels of parasitemia, especially in cases where large *Babesia* is involved (Bose *et al.*, 1995). The advantage of the thick smear is that a large amount of erythrocytes is analyzed in a reduced amount of space; therefore the sensitivity of thick smears technique over that of thin smears of finding infected cells is ten times higher (Bose *et al.*, 1995). The method is usually recommended in subclinical disease conditions. This method relies on a very experienced microscopist who must identify the *Babesia*-infected cells among a mass of conglomerated erythrocytes. The higher prevalence of *B. canis* infection recorded in our study is an indication of continuous challenge of *Babesia canis* infection at subclinical level in the area.

On the sample locations, the results showed that the prevalence of *Babesia canis* infections was highest 10 (12.5%) in dogs from Kwali, Municipal and Gwagwalada Area Councils, and lowest 5(6.3%) in dogs from Abaji Area Council. Our findings showed that there was variation in the prevalence of *B. canis* infection in the six Area Councils understudied, though not significant. The reason for the discrepancy observed in sampled locations may be due the differences in the abundance of tick vectors and other other risk factors such as dog utility, method of husbandary, and medical history. The abundance of ticks is strongly determined by differences in climate, host availability and vegetation cover, which affects their microclimate.

The prevalence recorded in the study was relatively low when compared with the reports of Okubanjo *et al.*, (20013) and Opara *et al.* (2017), who recorded 17.3% and 57.1% prevalence of *B. canis* infections in Zaria, and Gwagwalada Area Council of the FCT respectively. The disparity in the results may be attributed to a number of reasons: transient parasitaemia particularly in early infection, as well as low and not detectable in chronic cases (Bourdoiseau, 2006). The prevalence value obtained in this study was higher when compared with the reports of Amuta *et al.* (2010); Jegede *et al.*(2014), and Takeet *et al.* (2017), who reported 10.2% 8.9% and 7.3%, of *Babesia canis* infections in Markurdi, Abuja and Abeokuta respectively. The reasons for the disparities could be due to differences in the experimental designs, study locations, duration of the study, season of the year, inclusions and the exclusions criteria employed in the studies. In some of the reports, larger sample size was employed with a longer sampling duration. Some of the researchers encountered more exotic breed of dogs which have been adjudged to be more susceptible to *Babesia* spp. infection than the local breeds. The variation in geo-climatic conditions and exposure to vectors may also be factor (Rahman, 2014). Opara *et al.* (2017), examined only local breed of dogs and suggested that the higher infections rate encountered may be due to lack of medical attention and poor husbandry. Daniel *et al.* (2016), reported 18.6% prevalence of *Babesia canis* infection in Jos, Plateau State, in which large sample size were employed in naturally infected cases presented to Veterinary Clinics in the area. In the present result, no significant difference ($p < 0.05$) was observed in the rate of *Babesia canis* infection in the six Area Councils sampled. This may be due to little or no variations in climatic conditions of temperature and relative humidity in the sampled locations.

Transovarian transmissions occur in *Babesia canis* through engorged female *Rhipicephalus sanguineus* (brown dog tick) and may transmit the infection through the eggs. The

larvae/nymph progenies may infect dogs which enhances higher prevalence of the infection. Indiscriminate use of anti-protozoan drugs by professional and non professionals may have given rise to a generation of ticks cleansed of haemoparasites infection and therefore, their high infestation on dogs does not result to corresponding high infection rates (Okubanjo *et al.*, 2013).

Risk factors associated with tick infestations in dogs are highly inconsistent in various studies. Breed, sex and age status were not found to be significant predictors of tick infestation (Jennett *et al.*, 2013). The results showed that Gwagwalada Area council had the highest tick infestation rate of 57.5% rate of tick infestation while Abaji Area Council had the lowest (47.5). The variable results obtained may be because tick attachment rate has been shown to be most strongly correlated with exposure rather than any dog physiological or phenotypic characteristics (Jennett *et al.*, 2013). Surprisingly, dogs that were restricted to urban habitats were no less likely to have ticks than dogs in the rural habitats. This corresponds with the growing number of reports of high tick infestation in urban environments (Smith *et al.*, 2013). This variation results concurs with the works of Radostits *et al.* (1997) cited by Daniel *et al.* (2016), which reported that occurrence of *Babesia* infection could be governed by the geographical distribution of the arthropod vectors responsible for their transmission. The geographical differences and period of the sampling, resulting to differences in tick availability may probably have contributed to the variations in infection prevalence. The variation in the prevalence rate representation may be due to an increased susceptibility to infection or may be due to increased exposure to potential risk factors.

With regards to age, *Babesia*spp. infection rate in dogs, has been documented to rise with age, reaching its peak between the age of 3 and 5 years and then decline (Hornok *et al.*, 2006). The higher rate of *Babesia* infection in the age ranges of 1– 3 years may probably be a reflection of higher exposure to tick vectors in the group. It could also be due to lowered maternal immunity/resistance associated with older dogs as well as frequent exposures to tick bites (Egege *et al.*, 2008). In some protozoan infections, most of the neonates of chronically infected mothers show a higher degree of immunity to the homologous parasites than those born of normal mothers. The high exposure of dogs of 1– 3 years may be as a result of lack of attention of some owners to this group (Daniel *et al.*, 2016) especially local dogs. The report of this study is in agreement with previous works of (Obeta *et al.* 2009; Opara *et al.*, 2017), suggesting that the presence of maternal immunity in younger dogs helps them to resist infections with blood parasites (Taylor *et al.*, 2007; Ivanov and Tsachev, 2008). It is our opinion that dogs in this group are more agile and might likely roam about indiscriminately, if given the opportunity, which could predispose them to tick infestations. In addition, their habit of playing on the grasses may contribute to their predisposition to questing ticks. The congregation of dogs usually during mating season could also contribute in exposing them to tick infestation. However, there are contrary reports from other researchers (Taylor *et al.*, 2007; Ivanov and Tsachev, 2008; Okubanjo *et al.*, 2013), who reported that younger dogs were more susceptible to *Babesia* parasites infection than the adult dogs, due to their underdeveloped immune system. Studies have shown that canine babesiosis is a disease of young dogs even as young as 3 weeks and that older dogs coming from a *Babesia*-free zone can develop the disease when contact with an infected tick during a brief stay in an endemic zone (Ogo *et al.*, 2011).

The sex of an animal may have influence in the occurrence of vector borne parasitic diseases. In the present study, male dogs were 1.24 times more prone to *B. canis* infection than the female dogs. Martinod *et al.* (1986), found no difference in sex related susceptibility. The aggressiveness and hormonal status of male dogs may be a contributory factor as it could limit the attention and care given them even by their owners. Studies have shown an increased risk of canine babesiosis for both intact and neutered males and neutered female dogs as compared to intact females suggesting that the increased susceptibility may be due to differences in environmental exposure, such as increased roaming behavior, or by sex-related genetic or hormonal influences on disease Mellanby *et al.*, 2011). Further epidemiological and genetic studies are required to evaluate these factors. The present findings are in agreement with the reports of Daniel *et al.* (2016), who reported higher rate of infection of *B. canis* infections among male than the female dogs. The reports have shown that male dogs were more exposed to tick infestations due to higher tendency to roam about in search of mates and establish territories, than the female counterparts which were considered to receive better management from their owners for monetary gain from their puppies. The present study disagrees with some earlier studies of (Obeta *et al.*, 2009; Omudu *et al.*, 2010; Okubanjo *et al.*, 2013; Jegede *et al.*, 2014; Opara *et al.*, 2017) who reported that the prevalence of haemoprotozoa was higher in the female than male dogs, as female dogs are usually more sedentary especially during nursing of their offspring, which predisposes them to increased infestation with tick vector. Also, the peculiar reproductive activities in the female animals may lead to stress and subsequent reduction in their immunity and resistance to diseases.

The present study reported higher prevalence of *B. canis* infection in the exotic breeds than the cross and local breeds of dogs. There is an increasing trend in the acquisition and use of exotic breeds for security and breeding purposes. The higher prevalence of infection among the exotic groups may be due to an increased susceptibility to infection or increased exposure to potential risk factors. It is our opinion that both factors may have added to our findings, as dogs coming from a *Babesia*-free zone into an endemic zone like ours may have high susceptibility to infection (Ogo *et al.*, 2011). Studies have demonstrated that *Babesia* spp. infection is most frequent with higher severity among imported dogs than indigenous breeds (Leeflang and Ilemobade, 1977). However, no significant ($p > 0.05$) association was observed between the rate of infection and breeds of dog from our study. Mellanby *et al.* (2011), reported that all breeds of dogs are not uniformly at risk for developing babesiosis, and documented that Toy breeds had lower risk than other breed of dogs. The reason for the protected status of several Toy breeds is unclear but may be related to decreased exposure to tick vectors as a result of differences in husbandry or may be related to genetic differences in susceptibility to the development of disease. Several reports have shown that breed predisposition to *Babesia* spp infections do occur, citing the vulnerability of the German shepherd breeds to developing babesiosis due to *B. canis* (Hornoket *et al.*, 2006). Predisposition of breeds of dogs to *B. canis* (Martinod *et al.*, 1986), *B. vogeli* (Taboada *et al.*, 1992), *B. gibsoni* (Birkenheuer *et al.*, 2005) and *B. rossi* (Mellanby *et al.*, 2011) infections has been described in different parts of the world. The results of the present study agree with the work of Hemmer (2000) who reported that infection with *B. canis* is common among imported dogs. However, our findings is not in agreement with that of Jegede *et al.*, (2014) who reported a higher prevalence of *Babesia canis* infection in local dogs than exotic, though statistically not significant, and suggested

that nonchalant attitude of local dog owners towards the medical needs of their animals could be the reason. Our findings showed that local dogs had a higher risk of acquiring the infection than the cross breeds. The higher rate of infection in the local breed of dogs was inline with the work of Daniel *et al.* (2016), who reported higher infection rate in local breeds as they are not expensive to acquire, owners tends to abuse them by letting them to roam about and scavenge which increases their exposure to tick vectors. It was also suggested that due to the high economic value of the exotic breeds, their owners are prompted to giving them better medical attention. There is growing interest in the use of canine babesiosis as a naturally occurring model of falciparum malaria in humans (Reyers *et al.*, 1998). Both diseases share numerous clinical and pathophysiologic characteristics such as hemolysis, hypoglycemia, severe systemic inflammation and associated proinflammatory cytokine production (Kelleret *al.*, 2004). There is an increased understanding of the genetic factors involved in host susceptibility to malaria (Verraet *al.*, 2009). Further evaluation of the role of host genetics and environment in influencing the risk of developing babesiosis may offer insights into the pathogenesis of falciparum malaria in humans, because the effect of parasite genotype might be as important as host predisposition.

The occurrence of canine *Babesia* infections was significantly higher in rainy seasons than in the dry season. The probable reason may be correlated to the seasonal activity of the brown dog ticks, *Rhipicephalus sanguineus* which is most abundant in wet and humid period of the year, thus resulting to probable higher transmission rate and thus higher incidence of *B. canis* infections in the season (Soulsby, 1982). This result was in agreement with findings of Konto *et al.* (2014), who reported high tick infestations during the months of wet season than the dry season months. The reason could be due to availability of

vegetation cover which provides favourable environment for ticks during rainy season, as ticks are noted to drop off the host to moult and quest on blades and attach on hosts that come in contact with them during grazing or movement (Shitta *et al.*, 2011). It has also been reported that optimum temperature and high humidity may encourage high fecundity in ticks (Konto *et al.*, 2014).

The present study describes the haematological changes in dogs tested positive for *Babesia canis* infection and those tested negative and compared them with the reference values. Anaemia which is defined by a low red blood cell count, haematocrit and haemoglobin concentration was found in 66 % of *Babesia* infected dogs examined. Anaemia, nucleated erythrocytes, and thrombocytopenia have been demonstrated as consistent laboratory findings in canine babesiosis by researchers (Salem and Farag, 2014) and this study also revealed that anaemia and thrombocytopenia were consistent in dogs positive for *Babesiacanis*. Though the clinical presentation of canine babesiosis can be highly variable, the classical presentation can be safely described as febrile illness with apparent anaemia (Schetters *et al.*, 2009). Anemia, thrombocytopenia, and monocytosis were the most hematologic alterations observed. The destruction of circulating red cells by auto antibodies is directed against infected and non infected red cell membranes resulting in intravascular and extravascular haemolysis (Irwin, 2005). However, Taboada and Lobetti (2006), proposed that direct parasitic damage contributes to anemia. Nevertheless, induction of serum hemolytic factors increased erythrophagocytic activity of macrophages and damage induced by secondary immune system. The formation of anti-erythrocyte membrane antibodies also proved important in the pathogenesis of anemia. Thrombocytopenia with no obvious hemorrhage observed either by the owner or during the examination, platelet

destruction, increase platelet sequestration, and decrease platelet production, could be linked to thrombocytopenia (Feldman *et al.*, 1988). Omobowale *et al.* (2017) recorded much higher percentages of anaemia in a population of *Babesia* positive dogs, though the dogs investigated were those with clinical signs of the disease presented to the Veterinary Teaching Hospital for treatments. Canine babesiosis was regarded as a disease of only the red blood cells but reports have shown that the disease can result into multiple organ dysfunctions and therefore has been classified into uncomplicated and the complicated forms (Matijatko *et al.*, 2007). The leukocytosis observed in this study is consistent with other findings that suggest a systemic inflammatory reaction which has been reported to be a major pathophysiologic mechanism in *Babesiacanis* infection (Welzl *et al.*, 2001). Previous reports have shown that neutrophillia and lymphocytopaenia ratio were typical phenomena observed in canine babesiosis (Boozer and Mcintire, 2003), and could serve as a ready diagnostic index for the clinician in differentiating complicated and uncomplicated babesiosis (Omobowale *et al.*, 2017). A recent study has reported that multiple organ dysfunctions are associated with complicated babesiosis in Nigerian dogs (Omobowale *et al.*, 2017, similar to a number of haematozan diseases including malaria (Gomes *et al.*, 2011). Santos *et al.* (2009) and Brown *et al.* (2006) had earlier observed a significant correlation ($p = 0.01$) between a decrease in platelets and *B. vogeli* occurrence. The mechanism of platelet damage by *B. vogeli* is poorly understood.

Ticks are ubiquitous haematophagous ectoparasites of animals and humans and therefore vectors of plethora of pathogens that cause severe infectious diseases in animals and humans (Lawrence *et al.*, 2004). Current global change (e.g. climate change, deforestation, changes in land use, urbanization, increased trade and travel) are affecting animal host

populations worldwide (Dantas-Torres, 2015), favouring the establishment of ticks and their associated pathogens into previously free areas.

In Nigeria, *Rhipicephalus sanguineus*, *Rhipicephalus (Boophilus) decoloratus* and *Amblyomma variegatum* ticks are the most abundant and destructive on domestic animals and pets (Ogo *et al.*, 2012). These tick species are associated with the transmission of diseases such as babesiosis, anaplasmosis, ehrlichiosis and rickettsiosis, which are of great importance in the pets and livestock industry (Natala *et al.*, 2009). There is need therefore for accurate identification of ticks in order to develop better control measures.

In the present study, ticks collected from sampled dogs in the study area were identified morphologically to belong to two genera: 1. *Amblyomma* spp. constitute 0.1% and 2.) *Rhipicephalus* spp. constitute 99.9%. The *Amblyomma* spp. were easily identified using Walker's (2013), identification features. The male *Amblyomma* spp. was identified by its colourful ornamentation, long mouth parts, large in size, legs with pale rings and festoons. The *Rhipicephalus* spp. was identified using the basis capitulum that appears hexagonal in shape from a dorsal view, small in size, usually of a dull yellow colour but some populations may be a mid brown colour. They are inornate and have short palps. An identifying character for the brown dog tick is the hexagonal basis capitulum. The male has the entire dorsum covered with fine to large punctuation of the scutum; regular rows of large punctuation on the dorsal portion and possess triangular and elongated adanal plates; with wide posterior on the ventral region. The female is distinguished on the dorsal region by fine and large sparse punctuation, with one third covered with scutum. The ventral region has U-shaped genital aperture. *Rhipicephalus sanguineus* sensu lato, the brown dog

tick, most probably originated in Africa, but currently has a world-wide distribution and is an important parasite of dogs and other domestic animals (Dantas-Torres, 2010).

The report of this study concurred with the earlier report of Amuta *et al.*, (2010), in Makurdi metropolis of Benue state, who reported *Rhipicephalus sanguineus* as the most prevalent ticks of dog, but observed only a single (tropical bont tick), *Amblyomma variegatum*. Opara *et al.*, (2017), recently documented in his findings, that *Rhipicephalus sanguineus* was the only tick species collected from local dogs examined in Gwagwalada Area Council of the FCT. Our report differs from that of Konto *et al.*, (2014), who recovered four tick genera; *Boophilus* spp. (88%), *Rhipicephalus sanguineus* (10.8%), *Hyalomma* spp. (0.9%) and *Amblyomma variegatum* (0.3%) on stray dogs sampled in Maiduguri metropolis.

In the present study, the genus specific primers used successfully amplified partial sequence of the ribosomal 18S rRNA gene of *Rhipicephalus* spp. tick from the study area at the expected range of 173 bp in agarose electrophoresis gel. The nucleotide sequence analysis results showed that the *Rhipicephalus* spp. from the studied area, was 96 -100% similar to the sequences of *Rhipicephalus sanguineus* in the GenBank. The query sequences aligned fully with the sequences of the subject in six out of the nine tick amplicons sequenced. However, there were presence of some genetic variations in the nucleotide sequence showing as mismatch and deletions in the sequence alignment of Blast results. Our results of molecular characterization of *R. sanguineus* from our study area showed 96 -100% similar to the partial sequences of 18rRNA gene of *R. sanguineus* in the GenBank, signifying that the data in the present study have provided further confirmation that what was commonly refers to as *R. sanguineus* may actually not be *R. sanguineus* (sensu

stricto)but *R. sanguineus* (sensu lato).This finding agrees with the report of Kamani *et al.*, (2013) who recently documented evidence of occurrence of the agent of Mediterranean Spotted Fever and *A. platys* in *R sanguineus* and *R. turanicus* of dogs in Nigeria.

The present study showed that the genus specific primers used in the PCR, successfully amplified partial sequence of the ribosomal 18S rRNA gene of *Babesia* spp. from the study area at the expected range of 612 bp in agarose gel electrophoresis. The nucleotide sequence Blast analysis results for the *Babesia* spp. of dog in the study area showed 100% similarity to partial sequences of 18rRNA gene of *Babesiacanis vogeli* in the GenBank. The sequences of the query and the subject in the NCBI database in the Blast analysis results showed complete alignment. The primers used and the gene target successfully identified *Babesia canis vogeli* as the subspecies of *Babesia canis* infecting dogs in the studied area. Our work is in partial agreement with that of Sasaki *et al.* (2007), who first reported the occurrence of *B. canis rossi* and *B. canis vogeli* in dogs in Nigeria dogs, using PCR-sequence analysis. Adamu *et al.* (2014), using Reverse Line Blot molecular method, reported as high as 72% prevalence of different haemoparasites of dogs presented to a Veterinary clinic in Plateau state. His findings revealed that 53% were infected with *B. rossi*; 13% with *Theileria* sp., 7% with either *Ehrlichia canis* or *Anaplasma* sp. Omatjenne, respectively; 4% with *Theileria equi*; and 1.1% with *B. vogeli* and *E. ruminantium*, respectively, with 18% co-infections recorded. Other reports of occurrences of *B. canis vogeli* infection in other parts of the country include; Kamani *et al.* (2013), using the combinations of conventional PCR, Real Time PCR and sequencing analysis documented the DNA of *Hepatozoon canis* (41.4%), *Ehrlichia canis* (12.7%), *Rickettsia* spp. (8.8%), *Babesia rossi* (6.6%), *Anaplasma platys* (6.6%), *Babesia vogeli* (0.6%) and *Theileria* sp.

(0.6%) in the blood samples of dogs, as well as the DNA of *E. canis* (23.7%), *H. canis* (21.1%), *Rickettsia* spp. (10.5%), *Candidatus Neoehrlichia mikurensis* (5.3%) and *A. platys* (1.9%) in *Rhipicephalus sanguineus* tick collected from dogs that were presented to veterinary clinics in four states: Rivers, Kwara, Kaduna and Plateau states of the country. *R. sanguineus* is a three-host tick of which adult, nymphal and larval stages feed on dogs. Matijala *et al.* (2004), confirmed the presence of the less virulent *Babesia vogeli*, transmitted by *Rhipicephalus sanguineus* in Africa. *Rhipicephalus sanguineus* has been shown to be a vector of *B. canis vogeli* in Nigeria (Kamani *et al.*, 2014; Adamu *et al.*, 2014). Indeed, *B. canis vogeli* is widespread, and believed to be consistent with the distribution of its vector *R. sanguineus* tick (Dantas-Torres *et al.* 2015). From this study, the occurrence of *B. canis vogeli* in the asymptomatic dogs is in agreement with previous studies that reported *B. vogeli* as the lowest pathogenic strain, causing a relatively mild disease, usually without evidence of clinical signs (Irwin, 2009). Indeed, no apparent clinical signs were observed in the all sampled dogs. This low percentage can be explained by the fact that *Babesia* parasites are usually visualized in blood smears only during the acute phase, and that percentage of infected cells is commonly low; the absence of parasites in blood smears does not exclude the possibility of *Babesia* infection. This study showed that *B. canis vogeli*, transmitted by *R. sanguineus* is the causative agent of canine babesiosis in the study area. The absence of *B. canis rossi* and *B. canis canis* in our study may be due to the absence of tick vectors on dogs in the study area. The *Dermacentor reticulatus* and *Haemaphysalis leachi* are known vectors of *B. canis canis* and *B. canis rossi* respectively. Nevertheless, the presence in the FCT of other canine *Babesia* transmitted by *R. sanguineus* such as *B. gibsoni* was not encountered in the present study. The phylogenetic tree of our study showed that the evolutionary relationship of *Babesia canis vogeli* from the

study area had *oneof* the isolate MF000389 showing minimal genetic diversity with subspecies from Taiwan, China and Brazil, while two isolate from our study area; MF000388 and MF000390 showed great divergent from the other isolate forming a different clades. This separation of our isolates into two different clades may be an indication that more than one genotype of *Babesia canis vogeli* may be in circulation among dog population in the study area.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results showed a relatively high prevalence of *Babesia* species infection in asymptomatic dogs investigated in the study area, showing 3.1% and 10.8% forthin and thick smears respectively. The typical large merozoites and trophozoites (size > 2.5 mm) were detected in blood smears from all infected dogs. The prevalence distributions of the *Babesia* spp. infections in the six Area Councils sampled was 6.3%, 12.5%, 10.0.%, 12.5%, 11.3%, and 12.5% in Abaji, Abuja Municipal, Bwari, Gwagwalada, kuje and Kwali Area Councils respectively, indicating constant challenge of the parasite and their vectors in the FCT.

The prevalence of the *Babesia* spp. infection was associated with age. The infection appeared to rise with age reaching its peak between the age 1 and 3 and then declined.

The prevalence of *Babesia* spp. infections in the study area was not breed dependent, although the infection rate was higher among exotic breed than the local or cross bred, demonstrating that all breeds of dogs are not uniformly at risk for developing babesiosis.

The prevalence of the infections was not sex dependent, however, our results showed an increased risk of canine babesiosis for both intact and neutered males and neutered females dogs than intact females.

This study demonstrated significantly, that *Babesia* spp. infections was dependent on the infestation rate of tick vector *Rhipicephalus sanguineus* on the canine host. Our results also showed that *Babesia* spp. infections was dependent on the purpose of the dogs, as hunting dogs was significantly more infected as compared to the guard or pet.

The prevalence of *Babesia* spp. infections was significantly higher in rainy seasons than dry season due to increase in activity of the tick vectors. The environmental conditions of our study area provide favourable and conducive climate for the survival and propagation of ticks throughout the year, resulting to occurrence of the infection all through the months of the year.

In the present study, anaemia, thrombocytopenia, and monocytosis were the most hematologic alterations observed. However, these changes may not be fully attributed to *Babesia canis* infection as the experimental design was not a case control study.

In the present study, ticks collected from the sampled dogs belonged to *Rhipicephalus sanguineus* and *Amblyomma variegatum* only as identified using morphological features.

In this study, the partial amplification of 18S rRNA gene successfully differentiate various *Babesia canis* subspecies and had confirmed *Babesia canis vogeli* as the aetiologic agent of canine babesiosis in the Federal Capital Territory and *Rhipicephalus sanguineus* as the main tick infesting them. The PCR amplified at 173bp and 612bp gene segments on gel electrophorogram for *Rhipicephalus* spp and *B. canis* respectively. The BLAST analysis of sequences of partial 18S rRNA gene amplicons revealed 96-100% and 100% similarity with *R. sanguineus* and *Babesia canis vogeli* in the GenBank respectively. The phylogenetic tree showed that tick samples (accession numbers: KY 799078 – KY 799086) formed a clade with *R. sanguineus* from Israel (KF958435.1) and Cuba (KP 830113.1) while *Babesia canis vogeli* (accession numbers: MF000388, MF 000389, MF000390), clades with isolates from South Africa (AF547387.1) and previous Nigeria isolate (AB 303076.1). This study represent the first confirmation of *Babesia canis vogelias* the *B. canis* subspecies present in the sampled asymptomatic dogs in the study area.

6.2 Recommendations

Based on the study carried out, the following recommendation are made:

1. A survey of a larger sample size that will involve neighbouring States should be carried out, for a better assessment of ticks and tick-borne pathogens of dogs in the FCT and its environ.
2. Future research should focus on the veterinary and zoonotic potentials of tick-borne pathogens, with the aim of developing anti-tick vaccines against *Rhipicephalus sanguineus*.

3. The complete amplification of 18S rRNA gene should be employed to re-confirm the presence of different genotypes of *B. c. volgeli* and ensure that their genetic variation is not overlooked.
4. The amplification of other gene regions such as; 16S rRNA, 28S rRNA, ITS 2 should be employed for the characterisation of *Rhipicephalussanguineus*, as means of re-confirmation.
5. The identification of ticks and their associated pathogens should not be limited to the use of morphological features only as they may not be conclusive, instead their use should be supported by more sensitive and specific molecular technique and bioinformatics tools.

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LIST OF APPENDICES

Appendix I: Tick(*R. sanguineus*) Samples from the Study Area used for DNA

Extraction

S/N	SAMPLE	SEX
1.	ABJ t1	Female
2.	ABJ t7	“
3.	ABJ t12	“
4.	ABJ t22	Male
5.	ABJ t56	“
6.	BWR t4	Female
7.	BWR t10	Male
8.	BWR t18	“
9.	BWR t30	“
10.	BWR t43	Female
11.	GWA t6	“
12.	GWA t19	“
13.	GWA t36	“
14.	GWA t44	Male
15.	GWA t60	“
16.	KUJ t4	“
17.	KUJ t7	“
18.	KUJ t14	Female
19.	KUJ t21	“
20.	KUJ t52	“
21.	KWA t5	“
22.	KWA t13	“
23.	KWA t38	“
24.	KWA t58	Male
25.	KWA t67	“
26.	MUN t11	“
27.	MUN t19	“
28.	MUN t34	Female
29.	MUN t48	“
30.	MUN t76	“

Appendix II: Blood Samples Positive for *Babesia* spp.in Thick Blood Smears from the Study

S/N	SAMPLE	EXTRACTED DNA
1.	ABJ b6	
2.	ABJ b22	✓
3.	ABJ b41	
4.	ABJ b56	✓
5.	ABJ b67	
6.	BWR b8	
7.	BWR b15	
8.	BWR b16	✓
9.	BWR b34	
10.	BWR b54	✓
11.	BWR b66	
12.	BWR b71	
13.	BWR b78	
14.	GWA b3	
15.	GWA b10	✓
16.	GWA b13	
17.	GWA b18	✓
18.	GWA b27	
19.	GWA b29	
20.	GWA b37	✓
21.	GWA b55	
22.	GWA b67	
23.	GWA b78	✓
24.	KUJ b1	
25.	KUJ b7	
26.	KUJ b19	
27.	KUJ b22	✓
28.	KUJ b27	

29	KUJ b38	
30.	KUJ b45	✓
31.	KUJ b58	
32.	KUJ b 66	
33.	KWA b1	
34.	KWA b5	✓
35.	KWA b11	
36.	KWA b20	
37.	KWA b34	
38.	KWA b36	✓
39.	KWA b45	
40.	KWA b 46	
41.	KWA b63	
42.	KWA b71	✓
43.	MUN b8	
44.	MUN b13	
45.	MUN b18	
46.	MUN b22	✓
47.	MUN b36	
48.	MUN b44	
49.	MUN b52	
50.	MUN b57	✓
51.	MUN b61	
52.	MUN b74	

Appendix III: Partial Sequences of 18S rRNA gene of *Babesia canis vogeli* from the Study Area in a Fasta Format.

>Seq1 [Organism=*Babesia canis vogeli*][Isolate=Abuja56][18SrRNA][partialCDS]

AGGGAAAACCCAAAAGCGAACTCGAAAAAGCCAAACGAATGGCAAACCTCGAA
CACGCTAAAATTCAACTACGAGCTTTTTAACTGCAACAAGTTTAATATACGCTA
TTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGCTAC
TCTGGTGAGGGTTTGGGTCACCATCATTCCAATTACAAGACATTAGCCCTGTAT
TGTTATTTCTTGTCACCTCCCTGTGTCAGGATTGGGTAATTTGCGCGCCTGC
TGCCTTCCTTAGATGTGGTAGCCGTCTCTCAGGCTCCCTCTCCGGAATCGAAC
CTAATTCCCCGTTACCCGTTGCTGCCTCGGTAGGCCAATACCCTACCGTCAAGC
TGATGGGTCAGAACTTGAATGGTCCATCGCTAAATGCGATTCGCCAGTTTATT
ATGAATCACCGAAAGCCAAGGCGGGTTTCAAATAATAAACGCGGTCAAAAGA
CCTCAAACGTGTATTAGCCCTACAATTAGCACGGTTATCCATTGAATCTGAATA
CCAAATAAACTATAACTGTTGTAATGAGCCAC

>Seq2 [Organism=*Babesia canis vogeli*][Isolate=Abuja17][18SrRNA][partialCDS]

GATTCATGGATAACCGTGCTAATTGTAGGGCTAATACACGTTTGAGGTCTTTTG
ACCGCGTTTATTAGTTTGAACCCGCCTTGGCTTTCGGTGATTCATAATAAACT
GGCGAATCGCATTAGCGATGGACCATTCAAGTTTCTGACCCATCAGCTTGACG
GTAGGGTATTGGCCTACCGAGGCAGCAACGGGTAACGGGGAATTAGGGTTCGA
TTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCTAAGGAAGGCAGCAGG
CGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAACAATA
CAGGGCTAATGTCTTGTAATTGGAATGATGGTGACCCAAACCCTCACCAGAGT
AGCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAAT
AGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTTAGCGTGT
TCGAGTTTGCCATTCGTTTGGCTTTTTTCGAGTTCGCTTTTGGGTTTTCCCTTTTA
CTTTGAGAAAATTAGAGTGTTTCAAGCAGACTTTTGTCTTGAATT

>Seq3 [Organism=Babesia canis vogeli][Abuja22[18SrRNA]][partialCDS]

GGGAAAACCCAAAAGCGAACTCGAAAAAGCCAAACGAATGGCAAACCTCGAAC
ACGCTAAAATTCAACTACGAGCTTTTTAACTGCAACAAGTTTAATATACGCTAT
TGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGCTACT
CTGGTGAGGGTTTGGGTCACCATCATTCCAATTACAAGACATTAGCCCTGTATT
GTTATTTCTTGTCACTACCTCCCTGTGTCAGGATTGGGTAATTTGCGCGCCTGCT
GCCTTCCTTAGATGTGGTAGCCGTCTCTCAGGCTCCCTCTCCGGAATCGAACCC
TAATTCCCGTTACCCGTTGCTGCCTCGGTAGGCCAATACCCTACCGTCAAGCT
GATGGGTCAGAACTTGAATGGTCCATCGCTAAATGCGATTCGCCAGTTTATTA
TGAATCACCGAAAGCCAAGGCGGGTTTCAAACATAAACGCGGTCAAAAGAC
CTCAAACGTGTATTAGCCCTACAATTAGCACGGTTATCCATTGAATCTGAATAC
CAAATAAACTATAACTGTTGTAATGAGCCAC

Appendix IV: Partial Sequences of 18S rRNA gene of *Rhipicephalus sanguineus* from the Study Area in Fasta Format

>KY799078

CGCGTGACCATGCCCGTTCTTCGCCCCCGGGTACTCCGTCCAAATCATTCCAA
AAGGCTGGCTCGTTAAATTATGTTAAGTTCCTTGGATCGTTTCTTCATACTTGG
TAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTGGGT
AACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGTGTGG
TGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTTTCTT
TCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGGTTGT
TACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACG
GGGAGGTAGTGACGAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAATT
GAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTG
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGC
GGTTAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACCCG
ATGCGACGGCTCGGACTGAACATCATGCCGGTCTTTCTTGGTGC ACTTCATTG
TGTGCCTCGAGATGGCCGGTGCTTTTACTTTGAAAAATTAGAGTGCTCAACGC
AGCGAGTCGCCTGAATAAACTTGCATGGAATATAGAACAAGACTCGTTTCTGT
CTGTGGTTTTTGGAT

>KY799079

CTGGATGATCTGCCGATAGTCTGTAAGACTGCGGAAGGATCCATCATCATTCAA
AGTGCCTGGCTCTTTATCATATTTAAGGTTCTTAGATCGTTTCTTCATACTTGG
ATAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTGG
GTAACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGTGT
GGTGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTTTCT
TTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGGTT
GTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC
GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCAC
GGGGAGGTAGTGACGAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCT
GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTG
CGTTAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACCC
GATGCGACGGCTCGGACTGAACATCATGCCGGTCTTTCTTGGTGC ACTTCATT
GTGTGCCTCGAGATGGCCGGTGCTTTTACTTTGAAAAATTAGAGTGCTCAACGC
AGCGAGTCGCCTGAATAAACTTGCATGGAATAATAGAACAAGACTCGTTTCTG
TTCTGTGGTTTTTGA

>KY799080

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ACGCGAATGGCTCATTATATCAGTTATGGTTCCTTAGATCGTTTCTTCCTACTTG
GATAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTG
GGTAACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGTG
TGGTGACTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTTT
CTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGGT
TGTTACGGGTAACGGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA
CGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCA
CGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCCGTAA
TTGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTC
TGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCT
GCGGTTAAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACC
CGATGCGACGGCTCGGACTGAACATCATGCCGGTTCCTTCTTGGTGCACTTCAT
TGTGTGCCTCGAGATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAAC
GCAGCGAGTCGCCTGAATAAACTTGCATGGGAATAATAGAACAAGACCTCGTT
TCTGTCTGTGGTTTTT

>KY799081

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AAGGGGGAGGCTCCCTGTTATATTTAAGGTTCCGTACATCGTTTCTTCCTACTT
GGATAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTT
GGGTAACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGT
GTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTT
TCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGG
TTGTTACGGGTAACGGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA
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TGTGTGCCTCGAGATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACG
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>KY799082

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TGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACTACCCGGCGACGTTTCTT
TCAAGTGTCTGCCTTATCAACTTTCAATGGTAGGTTACTTGTCTTACCATGGTTGT
TACGGGTAACGGAAAATCAGGGTTCGATTCCGGAAAGGGAGCCTGAGAAACG
GCTACCACATCCAAGGAAGGCAGCACGCGCGCAAATTACCCACTCCCGGCACG
GGGAGGTAGTGACGAAAATAACAATACGGGACTCTTTTGAGGCCCGTAATT
GAAATGATTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTG
GTGCCAGCATCCGCGGTAATTCCAGCTCCAATATCGTATACTAAAGCTGCTGCG
GTTAAAAAGCTCGTAGTTGGATCTCACTTCCAGACGAGTAGTGCATCTACCCGA
TGCGACGGCTCTGACTGAACATCATGCCGGTCTTTCTTGGTGCACCTCATTGT
GTGCTCGAGATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCA
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GATGGTTTTGGAC

>KY799083

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TAACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTGGGT
AACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGTGTGG
TGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGTTTCTT
TCAAGTGTCTGCCTTATCAACTTTTCGATGGTAGGTTACTTGTCTTACCATGGTTGT
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GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACG
GGGAGGTAGTGACGAAAATAACAATACGGGACTCTTTTGAGGCCCGTAATT
GAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTG
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGC
GGTAAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACCCG
ATGCGACGGCTCGGACTGAACATCATGCCGGTCTTTCTTGGTGCACCTCATTG
TGTGCTCGAGATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGC
AGCGAGTCGCCTGAATAAACTTGCATGGAATAATAGAACAGACCTCGTTTCTG
TTCTGTTGGTTTTTG

>KY799084

CTTATGATCTGCCGTAGTCATTCACACGGCGGCCGCTCCATCATCATTTAAAGC
GTGTGGCTCATTATTACAATTAAGGTTCTTAGATCGTTTCTTCTACTTGGATA
ACTGTGGCAATTCTAGAGCTAATACATGCAGTGAGCCTGGAGCCCTTTGGGTA
ACGGGTGCTTTTATTAGACCAAGATCGATCGGGTTTCGGCCCGTATTGTGTGGT
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CAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATGGTTGTT
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CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGG
GGAGGTGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCCGTAATTGA
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GTCTGTGTTTTGAA

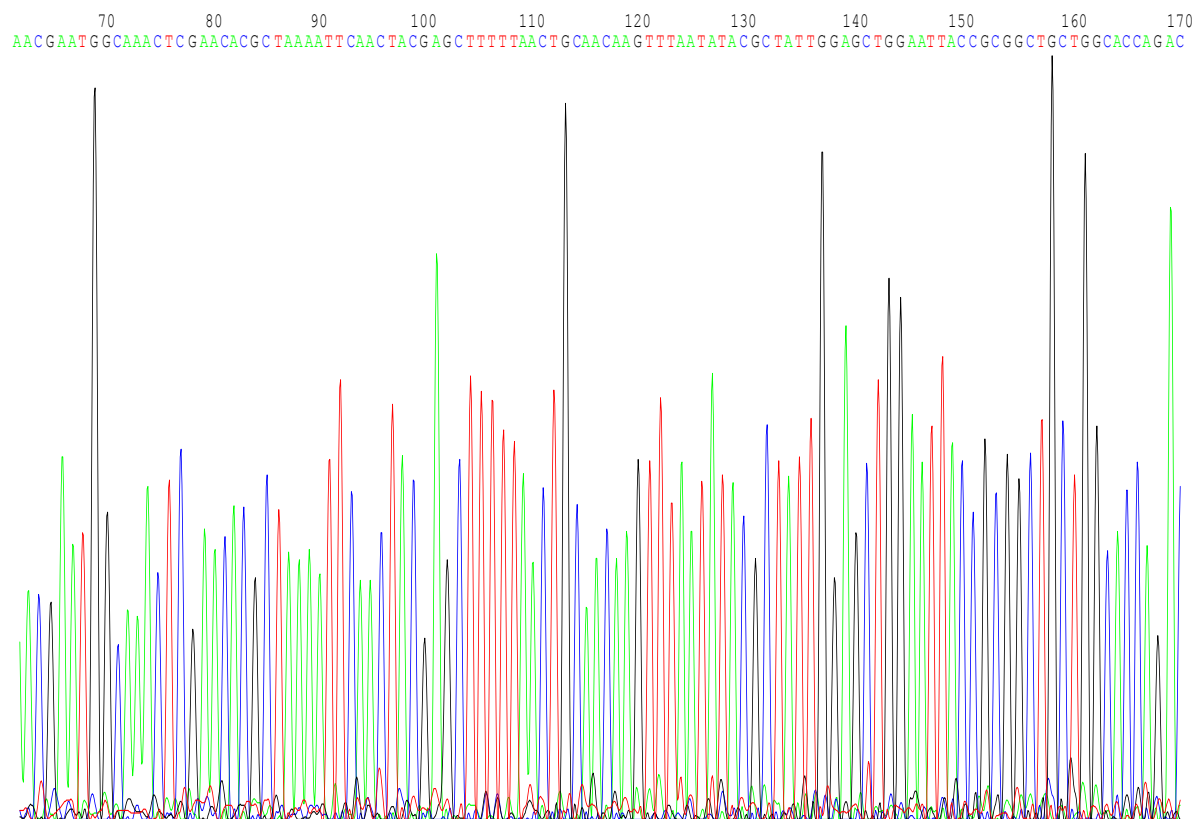
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GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCAC
GGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCT
GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTG
CGGTTAAAAAGCTCGTAGTTGGATCTCAGTTCAGACGAGTAGTGCATCTACCC
GATGCGACGGCTCGGACTGAACATCATGCCGGTTCTTTCTTGGTGCCTTCATT
GTGTGCCTCGAATGGCCGGTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGC
AGCGAGTCGCCTGAATAAACTTGCATGGGAATAATAGACAAGACTCGTTCTGTCT
GGTGTTTTTGGATAC

>**KY799086**

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AGGGGATGATCGGGCATTCTAGAGCTAATGGCTGCAGTGAGCCTGGAGCCCTT
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TGTGGTGA CTCTGGATAACTTTGTGCTGATCGCATGGCCACGAGCCGGCGACGT
TTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACTTGCTTACCATG
GTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
AACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGG
CACGGGGAGGTAGTGACGAAAATAACAATACGGGACTCTTTTGAGGCCCGT
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TCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGC
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CCGATGCGACAGCTCGGACTGAACATCATGCCGTTCTTTCTTGGTGCATTCA
TTGTGTGGCCTCAAGATGGGCCGGTGCTTTTTACTTTGAAAAAATTAGAAGTGC
TCAACGCAGGCGAGTCGCCCTGAATAAACCTTGCATGGAAATAATAAAACAAG
ACCTCGTTTCTGTTC

Appendix V: The Chromatograms of *B. canis vogeli* Sequence in a Plasmid Editor



The Chromatograms of 18S rRNA gene nucleotide sequences for *B. canis vogeli* from the Study Area, in ApE software. Representative colours for the nucleotides: Adenine (green), Cytosine(blue), Guanine (black) and Thymine (red).

Appendix VI: BLAST analysis result for *Babesia* spp sequence in NCBI database

Babesia canis vogeli isolate RJ32 18S ribosomal RNA gene, partial sequence

Sequence ID: [KU710803.1](#) Length: 677 Number of Matches: 1

Range 1: 12 to 361 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
647 bits(350)	0.0	350/350(100%)	0/350(0%)	Plus/Plus
Query 1	GTAGGGCTAATACACGTTTGAGGTCTTTTGACCGCGTTTATTAGTTTGAAACCCGCCTTG	60		
Sbjct 12	GTAGGGCTAATACACGTTTGAGGTCTTTTGACCGCGTTTATTAGTTTGAAACCCGCCTTG	71		
Query 61	GCTTTCGGTGATTTCATAATAAACTGGCGAATCGCATTTAGCGATGGACCATTCAAGTTTC	120		
Sbjct 72	GCTTTCGGTGATTTCATAATAAACTGGCGAATCGCATTTAGCGATGGACCATTCAAGTTTC	131		
Query 121	TGACCCATCAGCTTGACGGTAGGGTATTGGCCTACCGAGGCAGCAACGGGTAACGGGGAA	180		
Sbjct 132	TGACCCATCAGCTTGACGGTAGGGTATTGGCCTACCGAGGCAGCAACGGGTAACGGGGAA	191		
Query 181	TTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCTAAGGAAGGCAGC	240		
Sbjct 192	TTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCTAAGGAAGGCAGC	251		
Query 241	AGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAACAATACAGG	300		
Sbjct 252	AGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAACAATACAGG	311		
Query 301	GCTAATGTCTTGTAATTGGAATGATGGTGACCCAAACCCTCACCAGAGTA	350		
Sbjct 312	GCTAATGTCTTGTAATTGGAATGATGGTGACCCAAACCCTCACCAGAGTA	361		

BLAST analysis result for *Babesia* spp. Sample Query represents *B. c. vogeli* while subject represents *B. c. vogeli* in GenBank (AB 303076.1), nucleotide sequences fully aligned

Appendix VII: GenBank AccessionNumbers of *R. sanguineus* from the Study Area

GenBank: KY799079.1

[FASTA](#) [Graphics](#)

[Go to:](#)

LOCUS KY799079 820 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Abuja 2 18S ribosomal RNA gene,
partial sequence.
ACCESSION KY799079
VERSION KY799079.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Molecular identification and characterization of Rhipicephalus
sanguineus ticks in Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

GenBank: KY799080.1

[FASTA](#) [Graphics](#)

[Go to:](#)

LOCUS KY799080 748 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Abuja 3 18S ribosomal RNA gene,
partial sequence.
ACCESSION KY799080
VERSION KY799080.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 748)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Molecular identification and characterization of Rhipicephalus
sanguineus ticks in Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 748)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

GenBank: KY799081.1

[FASTA](#) [Graphics](#)

Go to:

LOCUS KY799081 820 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Abuja 4 18S ribosomal RNA gene,
partial sequence.
ACCESSION KY799081
VERSION KY799081.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Molecular identification and characterization of Rhipicephalus
sanguineus ticks in Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

GenBank: KY799082.1

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LOCUS KY799082 593 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Bwari 1 18S ribosomal RNA gene,
partial sequence.
ACCESSION KY799082
VERSION KY799082.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 593)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
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Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

[Go to:](#)

LOCUS KY799083 820 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Gwagwalada 1 18S ribosomal RNA
gene, partial sequence.
ACCESSION KY799083
VERSION KY799083.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Molecular identification and characterization of Rhipicephalus
sanguineus ticks in Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

Bank: KY799084.1

[STA](#) [Graphics](#)

to:

CUS KY799084 820 bp DNA linear INV 27-JUN-2017
FINITION Rhipicephalus sanguineus isolate Gwagwalada 2 18S ribosomal RNA
gene, partial sequence.
SESSION KY799084
RSION KY799084.1
YWORDS .
JRCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
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TITLE Molecular identification and characterization of Rhipicephalus
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JOURNAL Unpublished
REFERENCE 2 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

GenBank: KY799085.1

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LOCUS KY799085 819 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Gwagwalada 3 18S ribosomal RNA
gene, partial sequence.
ACCESSION KY799085
VERSION KY799085.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 819)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Molecular identification and characterization of Rhipicephalus
sanguineus ticks in Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 819)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

GenBank: KY799086.1

[FASTA](#) [Graphics](#)

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LOCUS KY799086 820 bp DNA linear INV 27-JUN-2017
DEFINITION Rhipicephalus sanguineus isolate Abuja 18S ribosomal RNA gene,
partial sequence.
ACCESSION KY799086
VERSION KY799086.1
KEYWORDS .
SOURCE Rhipicephalus sanguineus (brown dog tick)
ORGANISM [Rhipicephalus sanguineus](#)
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Chelicerata; Arachnida;
Acari; Parasitiformes; Ixodida; Ixodoidea; Ixodidae;
Rhipicephalinae; Rhipicephalus; Rhipicephalus.
REFERENCE 1 (bases 1 to 820)
AUTHORS Obeta,S.S., Lawal,I.A., Natala,J.A., Balogun,E.O., Ogo,N.I.,
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TITLE Molecular identification and characterization of Rhipicephalus
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REFERENCE 2 (bases 1 to 820)
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Jegade,O.C. and Opara,M.N.
TITLE Direct Submission

Appendix VIII: Materials used for DNA Extraction from Ticks and Blood samples

Lysis Buffer ATL

Proteinase K

Lysis Buffer AL

Buffer AW1

Buffer AW1

Buffer AE

Equipment

1.5 ml microcentrifuge tubes

0.2ml PCR tubes – Biosystems

Thermocycler Applied Biosystems verity 96 well thermal cycler

Amplification reaction mix

Reagent	Volume
10xBuffer for KOD Dash	2 μ l
2mM dNTPs	2 μ l
50 μ M forward primer	0.2 μ l
50 μ M reverse primer	0.2 μ l
Template DNA	2 μ l
KOD Dash (2.5u/ μ l)	0.2 μ l
H ₂ O – s	13.4 μ l
Total	20 μl

Appendix IX: Keys used for morphological identification of tick - Genera

Ornamented scutum and basis capitulum hexagonal shape from a dorsal view –
Rhipicephalus

Large mouth parts and festoon ornamented and large in size; legs with pale ring –
Amblyomma

Key to *Rhipicephalus* males

Rhipicephalus sanguineus

Dorsal region: Medium in size (2-3.2mm); fine to large punctuation of the scutum; regular
rows of large punctuation

Ventral region: triangulated and elongated adanal plates; with wide posterior

Key to *Rhipicephalus* females

Rhipicephalus sanguineus

Dorsal region: medium in size (3-3.5mm); fine and large sparse punctuation on the
conscutum

Ventral region: U-shaped genital aperture

Key to *Amblyomma* males

Dorsal region: Convex eyes; no enamel on festoons; narrow posteriomedian does not touch

Appendix X: Summarized Table of the Prevalence of *Babesia* spp. in the Study Area

Prevalence of <i>Babesia</i> spp. infection in the Federal Capital Territory						
Variables	Total no of dogs	Positive	Positive %	OR	LOWER CI	UPPER CI
Sex						
Male	227	31	9.3	1.24	0.73	2.11
Female	253	21	11.5	1.00	-	-
Age						
0->1	170	15	8.82	0.58	0.28	1.78
1-<3	194	33	17.0	1.00	-	-
3-<5	94	7	7.44	0.26	0.82	6.42
>5	22	2	9.01	1.70	0.60	3.49
Breed						
Exotic	62	8	12.9	1.16	0.50	2.69
Cross	170	16	9.4	0.82	0.43	1.56
Mongrel/local	248	28	11.3	1.00	-	-
Utility						
Guard	395	44	11.1	2.59	1.17	5.78
Hunting	71	8	11.3	2.57	0.98	3.74
Pet	14	0	0	1.00	-	-

Presence of ticks						
Yes	226	42	3.5	5.4	2.5	11.8
No	254	8	16.5	1.00	-	-
Coat colour						
Black	72	15	20.8	1.09	0.14	4.82
Brown	167	12	7.2	0.87	0.55	7.96
Mixed	176	10	5.7	1.26	0.71	10.3
White	65	1	1.5	1.00	-	-
Columns with no confidence interval for the odd ratio are reference group						

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Appendix XI: Sampling Form

Date of collection _____ Sample number _____

Specimen _____ Location _____

Breed _____ Sex _____ Age _____

Present/Absent of ticks _____ Utility _____