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UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

OCCURRENCE OF *BABESIA* / *THEILERIA* AMONGST HUMANS, CATTLE, AND DOGS AT THE MIDDLE BELT OF GHANA

BY

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DECLARATION

I hereby declare that except for references to other people's work, which I have duly acknowledged, this work is a result of my own research under the able supervision of Dr. Patience Borkor Tetteh-Quarcoo and Rev. Prof. Patrick Ferdinand Ayeh-Kumi, both of the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana. This work neither in whole nor in part had been submitted for another degree elsewhere.

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Signed: ………………………….

Date: …………………………….

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DEDICATION

This thesis is first dedicated to God Almighty for His divine protection, guidance, and divine miraculous favors. I also dedicate it to my church for their prayers and spiritual support.

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ACKNOWLEDGEMENT

I will first of all thank Almighty God for His divine protection, guidance, and divine miraculous favors. He protected me throughout this course, despite all the uncertainties that sometimes come my way. Secondly, I thank my entire family for their understanding and prayers.

I am very grateful to the entire staffs of the Medical Microbiology Department for providing us with a supportive environment to learn. I am full of gratitude to my supervisors: Rev. Prof. Patrick Ferdinand Ayeh-Kumi and Dr. Patience Borkor Tetteh-Quarcoo, who were like my God-sent angels. I did not only learn academically from them. I specially thank all our knowledgeable God-fearing, loving, and understanding lecturers for the knowledge and all that they have impacted in us. Special thank goes to the Departmental Head, Prof. Kwamena W. C. Sagoe; Dr. Nicholas Dzifa Dayie; The Secretaries, especially Mad. Evelyn Omane; and the D - Block hostel managerees, Mad. Gloria Lariba Akan-Yidi. I express my gratitude to Rev. Dr. Charles Antwi - Boasiako for allowing his thermocycler to be used for the PCR part of this work. I am also grateful to Mr. Robert Aryeeh for teaching me how to practically extract DNA from my samples and do the PCR. Thank you also, Mr. Emmanuel Afutu and Mr. Ernest Akomeah for helping with editing of the thesis.

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ABSTRACT

Background: *Babesia/Theileria species* are intra-erythrocytic protozoa of the phylum apicomplexa. The merozoite stage of *Babesia/Theileria* have diagnostic significance and are found as intracellular inclusions of infected red blood cells. The trophozoite stages appear as ring forms which measure about 1.0 to 5.0µm. These parasites are transmitted by hard ticks and can cause a zoonotic disease known as babesiosis/theileriosis. Human babesiosis/theileriosis are usually asymptomatic except in immuno-compromised people in whom symptoms present like malaria, yet treatment for these diseases can be different. These similarities can increase the possibility of misdiagnosing a patient with malaria when he or she is really suffering from babesiosis/theileriosis or vice versa leading to an inappropriate treatment choice. Ghana is a malaria endemic country; thus, general malaise is usually treated as malaria.

Aim: The aim of this study was to investigate the occurrence of *Babesia/Theileria species* in cattle, dogs and humans at the Middle Belt of Ghana.

Methodology: A cross-sectional study involving humans, sick dogs and cattle was undertaken at communities within the Techiman and Kintampo municipalities. Microscopy (Giemsa stained thin smears), serology (RDT) and polymerase chain reaction (PCR) were techniques employed in the study. Whole blood samples were taken from sick cattle, sick dogs and human malaria positive cases (that were negative for rapid diagnostic test). Blood samples of all study subjects were microscopically screened to suspect *Babesia/Theileria* infection. Suspected samples were subsequently subjected to PCR amplification and sequenced for specific piroplasm and strain identification.

Results: Out of seventy-one (71) sick dogs, 30 (42.3%) were suspected by microscopy whiles 14 out of the suspected cases (14/30, 46.7%) were amplified by PCR. For sick cattle, 33 (15.9%) were suspected out of the two hundred and seven (207), out of which 20 (20/33, 60.6%) were amplified and subsequently sequenced. The piroplasm identified in the cattle after sequencing was *Theileria velifera*. Twenty (10.7%) out of the one hundred and eighty-seven (187) *Plasmodium*-like smear positive cases, were suspected (smear positive but RDT negative) of Babesia/Theileria infection, with 6 (30.0%) amplified and identified as *Theileria velifera* after successful sequencing.

Conclusion: *Babesia/Theileria* has been found in all the study groups (dogs, cattle and humans). This is the first report of human theileriosis (caused by *Theileria velifera*) in Ghana.

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ABBREVIATIONS

ACTs: …………. Artemisinine-based combination therapies

AFI: …………… Acute Febrile Illness

ANOVA: ………. Analysis of Variants

AIDS: …………. Acquired Immunodeficiency Syndrome

ARDS: …………. Acute Respiratory Distress Syndrome

BBC; …………... British Broadcasting Corporation

BUN: …………... Blood urea nitrogen

CDC: …………... Centers for Disease Control

CHS: …………… College of Health Sciences

CFSPH: ………... Center for Food Security and Public Health

DIC: ……………. Disseminated Intravascular Coagulopathy

ECF: ……………. East Coast Fever

EDTA: …………. Ethylenediaminetraacetic Acid

FAO: …………… Food and Agriculture Organization

FDA: …………… Food and Drug Administration

FISH: …………… Fluorescence in situ hybridization

GSS: ……………. Ghana Statistical Service

HIV: …………….. Human Immune Virus

HLH: ……………. Hemophagocytic Lymphohistocytosis

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IFA: ……………… Indirect Fluorescent Antibody

LFTs: ……………..... Liver Function Tests

MMWR: ………..... Morbidity and Mortality Week Report

mRDTs: ………….. Malaria Rapid Diagnostic Test

PCR: ……………… Polymerase Chain Reaction

Pf HRP-2: ………… *Plasmodium falciparum* Histidine Rich Protein-Two

qPCR: …………….. Quantitative Polymerase Chain Reaction

RBCs: ……………. Red Blood Cells

RDT: ……………...... Rapid Diagnostic Test

RLB: ……………… Reverse Line Blotting

rRNA: …………… . ribosomal Ribonucleic Acid

SBAHS: ………….. School of Biomedical and Allied Health Sciences

SSA: ………….....… Sub-Saharan Africa

TAE: ……………… Tris-acetate-EDTA

TBPs: …………….. Tick-Borne Pathogens

TTB: ……………… Transfusion-Transmitted Babesiosis

TTBDs: …………… Ticks and Tick-Borne Diseases

UNDP: ……………. United Nations Development Programme

UN SDGs: …….…... United Nations Sustainable Development Goals

US: ……………...... United States

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Babesiosis/Theileriosis is a tick-borne disease caused by intraerythrocytic protozoan parasites known as *Babesia/Theileria species*. Domestic animals that serve as reservoir hosts are cattle, horses, dogs, cats, and mice (Hasheminasab *et al*., 2018; Ascencio *et al*., 2018; Vannier & Krause, 2012) whiles in humans, it is considered zoonotic. Humans are accidental/opportunistic hosts for *Babesia/Theileria species* who get infected when an infected nymph or adult tick bites them. It starts by infecting their RBCs then the parasite invades the RBCs of the infected person and lyses them, similar to *Plasmodium species*. This results in febrile hemolytic anemia (Hunfeld *et al*., 2008; Uilenberg, 2006; Diuk-Wsser *et al*., 2014; Roshni *et al*., 2017; Safar, 2017; Akel & Mobarakai, 2017). *Babesia* and *Theileria* infections are widespread, especially in the Tropical and sub-tropical regions (Wagner *et al*., 2002).

There are a number of *Babesia* and *Theileria species* with species-specific characteristics in terms of disease severity, transmission, epidemiology and susceptibility to drugs. Therefore, accurate ways of identifying these causative agents is very crucial (Lempereur *et al*., 2017). Even among *Babesia species*, drug susceptibility is different (Solano-Gallego & Baneth, 2011; Mosqueda *et al*., 2012). *Babesia divergens, Babesia bigemina, Babesia bovis,* and *Babesia major* have been associated with Babesiosis in cattle whiles *Babesia equi* is the main specie that causes Babesiosis in horses. *Babesia canis, Babesia felis* and *Babesia microti* are the main species in dogs, cats and mice, respectively (Vannier & Krause, 2009; Jane & Adam, 2014; Abdela *et al*., 2018). *Babesia*

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microti, B. duncani, B. divergens, B. bovis and *B. venatorum* are the species that are known to mostly infect human beings (Vannier & Krause, 2009; CDC, 2012; Knapp & Rice, 2015).

For theileriosis, *Theileria microti* is the causative agent associated with human theileriosis. *T. annulata* and *T. parva* are responsible for Bovine Theileriosis; *T. annulata* also causes tropical theileriosis, whilst; T. parva is the causative agent for East Coast Fever. *Babesia rossi* is the main causative agent of canine Babesiosis in sub-Saharan Africa. *Babesia* and *Theileria species* infect a wide range of both domestic and wild animals (Bishop *et al*., 2004; Gupta *et al*., 2017). Canine Babesiosis is very common in South Africa but it was first isolated or discovered in Kenya (Penzhorn, 2011). *Babesiosis* has become an emerging health problem in humans, attracting worldwide attention (Akel & Mobarakai, 2017).

Babesia/Theileria infection can occur in everyone, though some are more at risk. Infection with Babesiosis can be severe/complicated at old age, in splenectomized patients, and in the immunocompromised (Babesiosis Fact Sheet, 2017; Linden *et al*., 2018). Example, it can be severe in cancer, HIV/AIDS, or transplanted patients because they mostly have weak immunities. Severe cases of *Babesia* can lead to blood clots, multi organ failure, unstable blood pressure, and sometimes death (Babesiosis Fact Sheet, 2017; Linden *et al*., 2018).

1.2 Problem statement

The symptoms of *Babesia/Theileria* infection are similar to that of malaria, and the morphology of *Plasmodium* is similar to that of *Babesia/Theileria sp* (Hunfeld *et al*., 2008). Therefore, accurate diagnosis of babesiosis/theileriosis and malaria continues to be a challenge. If *Babesia/Theileria* infection is misdiagnosed as malaria, or in the case of mixed infection (*Plasmodium* and *Babesia/Theileria*), the patient is likely to suffer adversely because, babesiosis/Theileriosis cannot be

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treated with the current combination of anti-malarial drugs for uncomplicated malaria (Quick *et al*., 1993). Furthermore, immuno-competent asymptomatic carriers of human *Babesia/Theileria sp.* are likely to donate blood to possibly already immuno-compromised patients. These individuals are at higher risk of being infected with *Babesia/Theileria*, which may lead to severe disease. Ghana is a malaria endemic region and there is the possibility that babesiosis/theileriosis might be misdiagnosed as malaria even after laboratory investigations.

Routine diagnosis of malaria in Ghana mainly employs microscopic examination of Giemsa-stained thick blood smears as well as rapid diagnostic test kits. These thick smears may not reveal the features (such as the tetrad and classical Maltese cross or paired pear-shaped morphology), which differentiate *Plasmodium* from *Babesia/Theileria*. Some cases of babesiosis/theileriosis found in other parts of the world were not confirmed until there was a review of slides initially misdiagnosed as malaria in patients' blood smears (Nagano *et al*., 2013; Quick *et al*., 1993; Skrabalo & Deanovic, 1957; Smeenk *et al*., 2000). A negative result from the rapid test kit (especially types that are made to detect all four major *Plasmodia* species that infect man) is usually assumed to mean no *Plasmodium* infection, even though there have been cases where smears have been positive (Ramharter *et al*., 2010; Rollend *et al*., 2013). This discrepancy has brought suspicion as to what those intracellular parasites (piroplasms) may be, if not *Plasmodium.* Further investigation about *Babesia/Theileria* in those samples (that are negative for rapid test kit but show intracellular parasites in smear) will be helpful.

Since babesiosis/theileriosis is a zoonotic infection transmitted by ticks, screening of cattle and dogs could help give an idea of the *Babesia/Theileria* species infecting them, and whether those species have been known to infect humans. Knowing this can help hypothesize possible zoonotic

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infection since animals could be found living in close proximity with humans in most parts of Ghana.

Apart from the five reported zoonotic *Babesia* species that infect man (*Babesia microti, B. duncani, B. divergens, B. bovis* and *B. venatorum*), a novel type of *Babesia* (KO1), similar to ovine *Babesia* was found infecting man in Korea (Kim *et al*., 2007). This suggests that there may be more species types of *Babesia* zoonotically infecting man, but are yet to be identified. There is little documented information on the presence or absence of *Babesia/Theileria* species infecting humans at the middle belt of Ghana, although some reports have been made on animals (Krause *et al*., 2000; Kunimoto *et al*., 1998), and the southern belt of Ghana (Owusu, 2015). Contrastingly, infections in humans have been established and documented elsewhere, such as the United States of America, Europe and some other parts of the world (Chen *et al*., 2014; Leiby, 2011; Marathe *et al*, 2005; Nagano *et al*., 2013; Persing *et al*., 1995). Therefore, this study will provide information about the state of babesiosis/theileriosis in the Middle Belt of Ghana.

1.3 Justification

At many locations (Skrabalo & Deanovic, 1957; Fang & McCullough, 2016; Scott, 2017; Huang *et al*., 2018) including Africa (Vermeil *et al*., 1983; Bush *et al*., 1990), Babesia/Theileria infection which has mostly been associated with animals (especially cattle and dogs) have now been detected in humans. Most cases of babesiosis/theileriosis were not confirmed until a review of slides, initially diagnosed as *Plasmodium falciparum* malaria in blood smears of patients due to

the morphological similarities between the two parasites under the microscope (Kunimoto *et al*., 1998; Scott, 2017; Huang *et al*., 2018). Therefore, knowledge of the state of babesiosis/theileriosis in different parts of Ghana will enable medical laboratory personnel to critically examine intra-

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erythrocytic parasites that look like malaria parasites before reporting. Rather than assuming that any intra-erythrocytic Giemsa-stained parasite is possibly *Plasmodium* spp since Ghana is a malaria endemic country.

Most human cases of babesiosis has been identified to occur in the summer (hot weather) and in areas where the vectors (tick, rodents, and deer) are in close proximity to humans (Spielman *et al*., 1985) of which Ghana is not an exception of such factors. Therefore, it is important to carry out studies aimed at searching for *Babesia/Theileria species* in animals (especially dogs and cattle) and in humans.

The outcome of this study could also inform health personnel on the relevance of continued use of antimalarial drugs in "persistent malaria" cases as well as extending investigations of unexplained febrile illnesses to *Babesia/Theileria sp.* (Stoler & Awandare, 2016). This study could also raise concerns about the need to include babesiosis/theileriosis screening in criteria to declare a person fit to donate blood. This is because, recipients of blood are mostly immune-compromised and will be at a higher risk of being infected if the blood they receive is infected with human *Babesia/Theileria sp.*

1.4 Main Aim

To determine the occurrence of *Babesia/Theileria species* in humans, cattle, and dogs at the middle belt of Ghana.

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1.5 Specific objectives

1. To investigate *Babesia/Theileria* species in blood samples of humans, sick cattle, and dogs; especially persons who would be negative for malaria rapid test kit but show intracellular parasites in their smears.
2. To determine the specific *Babesia/Theileria species* in the sick cattle, dogs and humans.

1.6 Hypothesis

Cattle and dogs, as well as humans in the Middle Belt of Ghana, might have been infected with *Babesia/Theileria*.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview Babesia/Theileria species

Babesia/Theileria species are tick-borne intraerythrocytic protozoan parasites that cause a disease known as Babesiosis/Theileriosis (Lempereur *et al*., 2017; Huang *et al*., 2018; Abdela *et al.,* 2018*)*. The genus, *Babesia*, is named after a Hungarian Pathologist and Microbiologist called Victor Babes who first discovered them in cattle, 1888 (Babes, 1888). He identified over 100 species of *Babesia*, describing them as intraerythrocytic "bacteria" that killed 30,000 to 50,000 heads of Romanian cattle with febrile hemoglobinuria. His discovery was later identified by Smith and Kilborne as protozoan parasites transmitted by blood-sucking ticks (Babes, 1888; Smith & Kilborne, 1893; Mosqueda *et al*., 2012). Arnald Theiler also first discovered *Theileria species* but the disease, "Theileriosis" was first identified by Dschunkowsky in 1904 (Jithendran *et al*., 1997). Safar, (2017), described *Babesia* as part of "Laboratory-acquired blood-borne parasites from accidental exposure" which are found worldwide.

Tick-borne diseases (caused by bacteria, viruses, and parasites), like Babesiosis, have been increasing for the past two decades, particularly in North America and Europe (Paddock *et al*., 2016). The intermediate host for *Babesia* is the same hard-bodied tick that transmits *B. burdorferi* causing lyme disease (Dunn *et al*., 2014), a single tick bite is reported to transmit multiple pathogens (Dunn *et al*., 2014; Rizzoli *et al*., 2014; Moutailler *et al*., 2016). Ticks transmit babesiosis attributed to *B. microti* in the US, *Babesia duncani* confirmed in patients in Western States, *B. divergens* is mostly responsible for Europe's *babesiosis (*Abdela *et al.,* 2018; CDC, 2012). Currently, *B. microti* is

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mostly reported to the US Food and Drug Administration as the common RBC transfusion-transmitted pathogen. Blood transfusion from an infected donor with *B. microti* (FDA, 2015) is common. Mothers who might be infected may also transplacentally transmits it to the fetus (Adaszek *et al*., 2016).

It is then recommended that, blood donors should be screened for *Babesia species* before blood donation (US Food and Drug Administration, 2014; Vannier & Krause, 2012; Herwaldt *et al*., 2011; Leiby, 2011). Undiagnosed immunocompetent donors remain *Babesia/Theileria* carriers, infecting immunocompromised persons, which can result in high morbidity and mortality (Cushing & Shaz, 2012; Owusu, 2015; Kitt *et al*., 2016).

2.1.1 Classification, Similarities, and Differences between *Babesia* and *Theileria species*

Even though *Babesia* and *Theileria species* are similar, there are some differences between them. These differences could be grouped into: Morphological differences, differences from molecular investigations, difference found in their life cycle, differences in their treatments, differences in the tick species that transmit the parasites and difference in the family, genus, and species of the parasite (Sivakumar *et al*., 2014; Uilenberg, 2006; Solano-Gallego *et al*., 2016; Solano-Gallego *et al*., 2011; Gupta *et al*., 2004; Ganga, *et al*., 2010). In this study morphological and molecular differences were mainly focused on.

Babesia and *Theileria* can be differentiated morphologically using microscopical examination of their stained smears. Piroplasms are obligate intracellular hemotropic protozoan parasites of vertebrates with a global distribution caused by the genera *Babesia, Theileria*, and *Cytauxozoon* (Alvarado-Rybak *et al*., 2016; Allsopp & Allsopp, 2006). The name piroplasm describes the pear-

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shaped appearance of the parasites often seen within erythrocytes on Romanowsky-stained cytological slides during microcopy (Uilenberg, 2006). Microscopy was the central point for taxonomically classifying parasites earlier in the genus *Babesia;* grouping *Babesia species* as large and small, using how they appear in terms of shape and size during their intra-erythrocytic stages (Solano-Gallego *et al*., 2016; Solano-Gallego *et al*., 2011). With this classification, large *Babesia* species included *B. bigemina, B. canis,* and *B. divergens,* whiles *B. microti* was regarded as the small *Babesia* (Mandal *et al.,* 2014; Gray *et al.*, 2010). These species can be differentiated from each other, based on their sizes and trans-ovarian transmission from female adult ticks to their offsprings, which occurs in large *Babesia species* but not in small *Babesia species* (Uilenberg, 2006). Molecular methods have also been used for classifications (Akoolo *et al*., 2017), where a phylogenetic tree can sometimes be used to differentiate large *Babesia species* from small ones based on the 18S rRNA gene sequence as seen in Figure 1 (Criado-Fornelio *et al.*, 2003; Mandal *et al.,* 2014; Gray *et al*., 2010).

Theileria is also categorized as schizont "transforming" and "non-transforming" species where the transforming species are all grouped as *T. taurotraqi* clade (Sivakumar *et al*., 2014). Proliferation of schizonts without control leads to pathologies found in corridor disease and East Coast fever caused by *Theileria parva* as well as tropical theileriosis by *T. annulata*. All the transforming species have a monophyletic origin because; they are able to cause schizont associated pathology in which the schizonts can be cultured (Bishop *et al*., 2004, Pienaar *et al*., 2014, Sivakumar *et al*., 2014). Though the non-transforming *Theileria* are noted as benign, they still have the ability of causing diseases since the piroplasmid stage induces anemia (Sivakumar *et al*., 2014).

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Many *Babesia species* that have been informally classified as 'small' *Babesia*, in particular, are more closely related to *Theileria* (Beck *et al*., 2009). Example*, B. equi* is now called *Theileria equi* because of its pre-erythrocytic development found in other *Theileria species* (Kumar *et al*., 2009). Without molecular investigation, the differences and similarities between these organisms is mostly undistinguishable (Figure 1).

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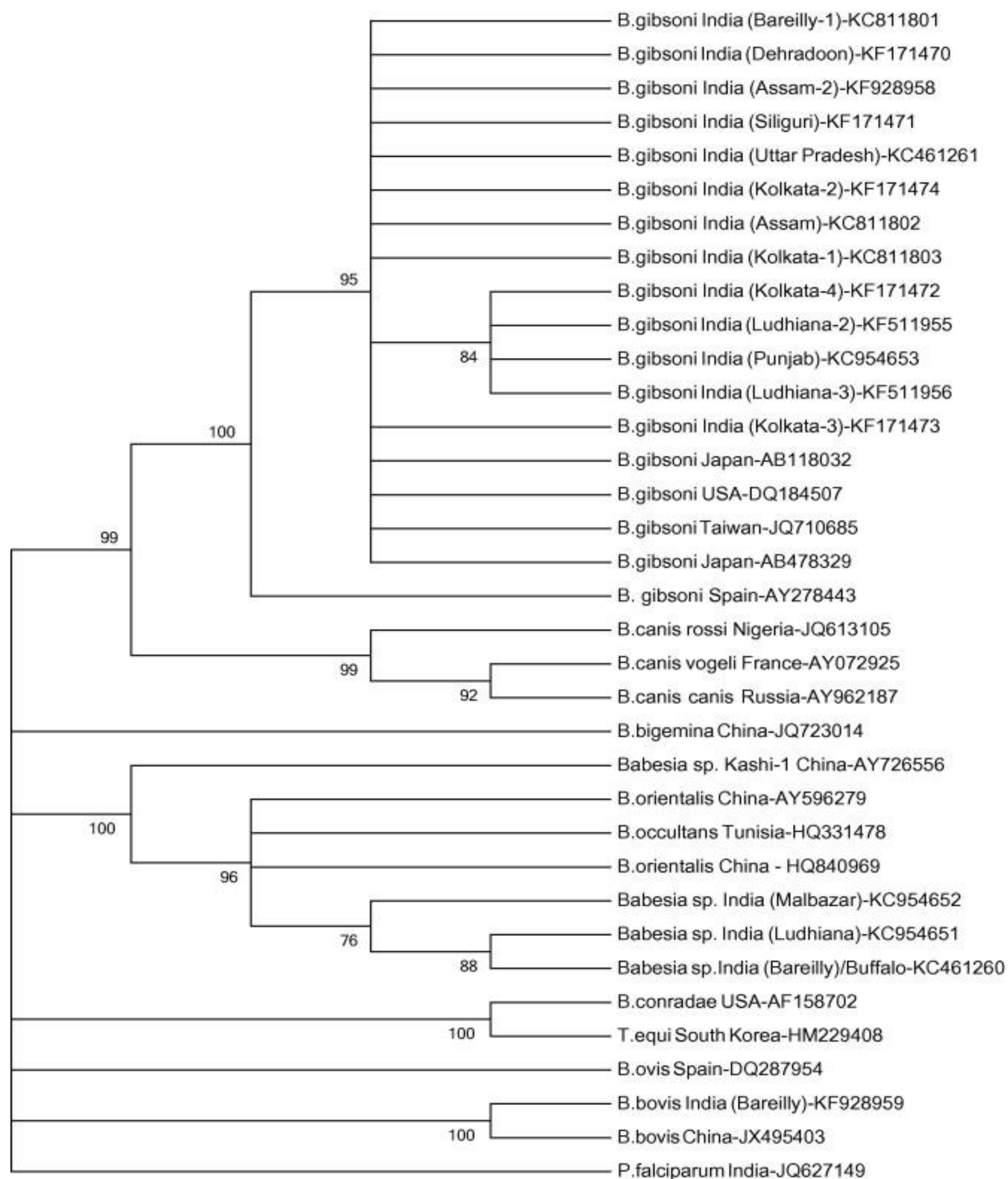

Figure 1: Phylogenetic tree based on 18S rRNA gene of Babesia isolates using Maximum Likelihood method (Adapted from Mandal *et al*., 2014).

In the case of *B. equi*, it was reclassified as *T. equi* after molecular investigations and 18S rDNA sequencing was done to bring out the differences and similarities (Mehlhorn & Schein, 1998; Bilgic *et al*., 2012). Western US *Theileria* – like group is closely related to *Babesia conradae* (Bock *et al*., 2004; Hunfeld *et al*., 2008). *B. microti*, the causative agent of human babesiosis in

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Wisconsin and Minnesota, US, was named again as *T. microti* a few years ago (Vannier *et al*., 2008). Some scientist also use both names at the same time as *Theileria/Babesia microti* and *Babesia* (*Theileria*) (Clancey *et al*., 2010; El-Ashker *et al*., 2015; Zanet *et al*., 2014).

Theileria annae was previously detected in a Spanish sick dog and is shown to be most closely related to *B. microti* in terms of phylogenetic analysis. Therefore, it was first named as *Babesia microti*-like (Zahler *et al*., 2000; Ascencio *et al*., 2018). Later, when it was observed to show different segregation from that of *Babesia species* known as *Babesia sensu stricto* group, it was then concluded that it belongs to the genus, *Theileria* (Schnittger *et al*., 2012; Zahler *et al*., 2000).

The placement of *T. annae* into the genus, *Theileria*, proved confusing (Criado-Fomelio *et al*., 2003) and was therefore given different names in several publications (Zahler *et al*., 2000; Birkenheuer *et al*., 2010; Camacho *et al*., 2005; Camacho, 2005; Clancey *et al*., 2010). Names such as *Babesia* Spanish dog isolate (Yeagley *et al*., 2009), *Babesia-microti*-like (Zahler *et al*., 2000; Birkenheuer *et al*., 2010), *Babesia annae* (Camacho *et al*., 2005; Camacho, 2005), *Babesia* (*Theileria*) *annae* (Clancey *et al*., 2010), and *Babesia cf. microti* (Karbowiak *et al*., 2010) were used as synonyms to avoid the confusion. To overcome these disagreements, phylogenetic investigation was done that confirmed that *T. annae* is not part of the genus, *Theileria* (Baneth *et al*., 2015; Ascencio *et al*., 2018).

Babesia/Theileria has a taxonomic classification. They belong to the same kingdom *Protoctista*; Phylum, *Alveolata*; Subphylum, *Apicomplexa*; and Order, *Priroplasmida. Babesia*, however, belongs to the Family, *Babesiidae*; and Genus, *Babesia*. (Solano-Gallego & Baneth, 2011; Lempe-

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reur *et al*., 2017; Abdella & Jilo, 2016; Pohl, 2013). *Theileria* also belongs to the family, *Theileriidae* and the genus, *Theileria*. Unlike *Babesia*, the genus, *Theileria* is differentiated by sporozoites' infection of leukocytes. Schizonts mature into merozoites, subsequently infecting RBCs to produce piroplasms (Uilenberg, 2006). Figure 1 shows the Phylogenetic or molecular identification of *Babesia species* as an example. Most *Babesia species* that were first detected in cattle and dogs are same/similar species that are found to infect humans, hence they are described in human infection as zoonosis (Diuk-Wasser *et al*., 2014; Gupta *et al*., 2017).

The same Ixodidae's ticks can transmit *Babesia/Theileria species* but the same/different genera of Ixodid's ticks are involved in transmitting Babesiosis/Theileriosis caused by the agents above. Example; Ixodidae's ticks of the genera *Hyalomma* and *Rhipicephalus* transmit tropical theileriosis (Mediterranean coast fever) and East Coast Fever in cattle respectively (Nejash & Bekele, 2016; Gul *et al*., 2015). Major vectors that transmit *B. bigemina* are *Rhipicephalus microplus* (originally, *Boophilus microplus*) and *R. annulatus* (previously, *Boophilus annulatus*). *Haemaphysalis punctata, Haemaphysalis longicornis,* and *Hyalomma marginatum* transmit *B. major, B. ovata,* and *B. occultans* respectively in cattle (CFSPH, 2008). Bovine theileriosis is caused by tick-borne blood protozoan, *Theileria orientalis*, in Australia, 2006 (Kamau *et al*., 2011; Eamens *et al*., 2013; Pulford *et al*., 2016; Gupta *et al*., 2017).

2.1.2 Life Cycle and Transmission of *Babesia/Theileria species*

Babesia/Theileria species have an indirect life cycle consisting of two hosts; the vertebrate (as the intermediate) host and an Ixodes' tick as the definitive host (Moustafa *et al*., 2016). The hosts are involved in developmental stages and the transmission of *Babesia species*. Different species of

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ticks in the ixodidae family are involved in the transmission of different *Babesia* species amongst humans and animals (Figure 2).

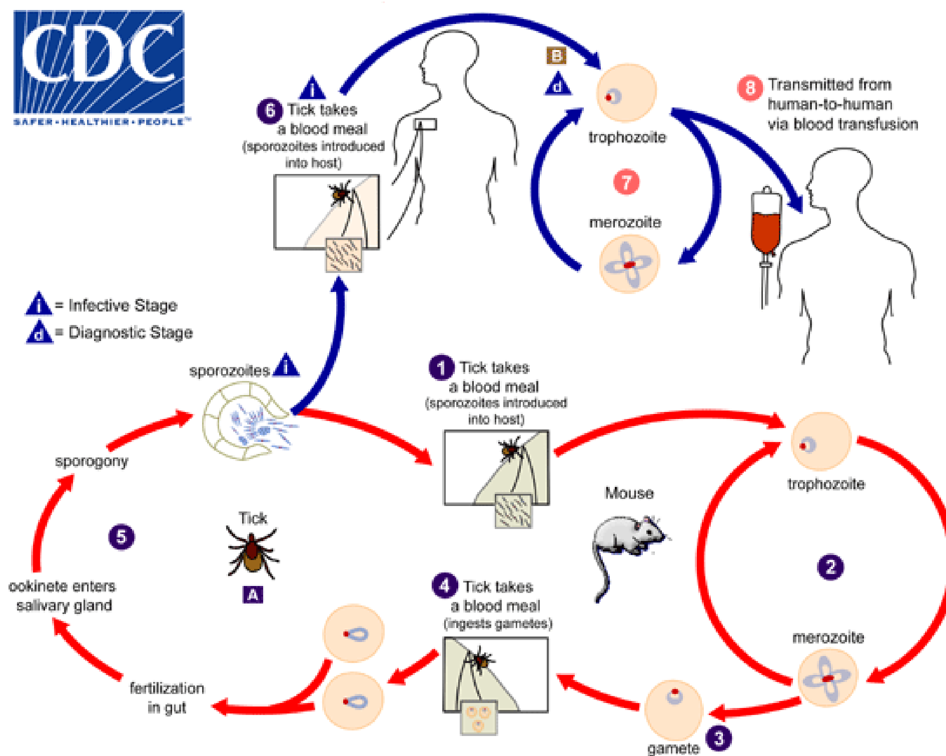

Figure 2: Life Cycle of Babesia species, *B. microti* (Adapted from CDC, 2015)

The piroplasms (*Babesia* and *Theileria*) are normally differentiated using the absence of pre-erythrocytic cycle in *Babesia* (Rudzinska *et al*., 1976) and no transovarian's transmission in *Theileria* (Mehlhorn *et al*., 1993). *Babesia* and *Theileria spp* go through both sexual and asexual part of their lives where sporogony follows the sexual part of their life cycle (Lempereur *et al*., 2017). *Babesia's* sporozoites are transmitted in the saliva of the tick when feeding on blood, directly infecting erythrocytes and multiplying asexually to form pyriform merozoites during merogony. However, as seen in Figure 3, a pre-erythrocytic schizogonic life form in WBCs is present in *Theileria* before merogony (Lempereur *et al*., 2017; Uilenberg 2006; Hunfeld *et al*., 2008).

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The life cycle of *Babesia microti*, for example, involves two hosts. These are a rodent, primarily the white – footed mouse, *Peromyscus leucopus* (same for *Theileria*, Figure 3), and a tick from the genus Ixodes. During a blood meal, a *Babesia*-infected tick injects sporozoites into the mouse host (Figure 3). The sporozoites enter into the erythrocytes and at this stage, asexual reproduction takes place through budding (Figure 3). In the blood, some of the parasites divide into male and female gametes but show no difference when observed under the light microscope (Figure 3). Transmission to man is possible, the moment the gametes are introduced into the definitive host - tick (Figure 3). However, transmission to man is accidental (just as in *Theileria*), humans are seen in the cycle when they are bitten by infected ticks (Vannier & Krause, 2012). The gametes become fused (united) and undergo a sporogonic cycle leading to the production of new sporozoites in the tick (Figure 3).

Babesia - infected tick injects sporozoites into the human host (Figure 3) when it goes for a blood meal. The sporozoites go straight into the erythrocytes and, from there, they undergo asexual replication (budding) like what happened in the first intermediate host (mouse). When the blood stage parasites are multiplying, the clinical manifestations of Babesiosis are noticed. Humans serve as the dead-end hosts for both *Babesia* and *Theileria species* (Figure 3). However, human-to-human transmission is well noted to occur through transfusion with contaminated *Babesia species*' blood and from mother to child (CDC). Transovarian transmission which is also called “vertical or hereditary transmission” is documented for “large” *Babesia species*. This occurs at low rate in *Theileria species* (Swilks *et al*., 2017). The protozoan parasites initially named as Piroplasma *parva* by Theiler, (1904), established as the genus *Theileria* in 1907 (Bettencourt *et al*., 1907) were first identified by Koch, (1898) as immature *Babesias*.

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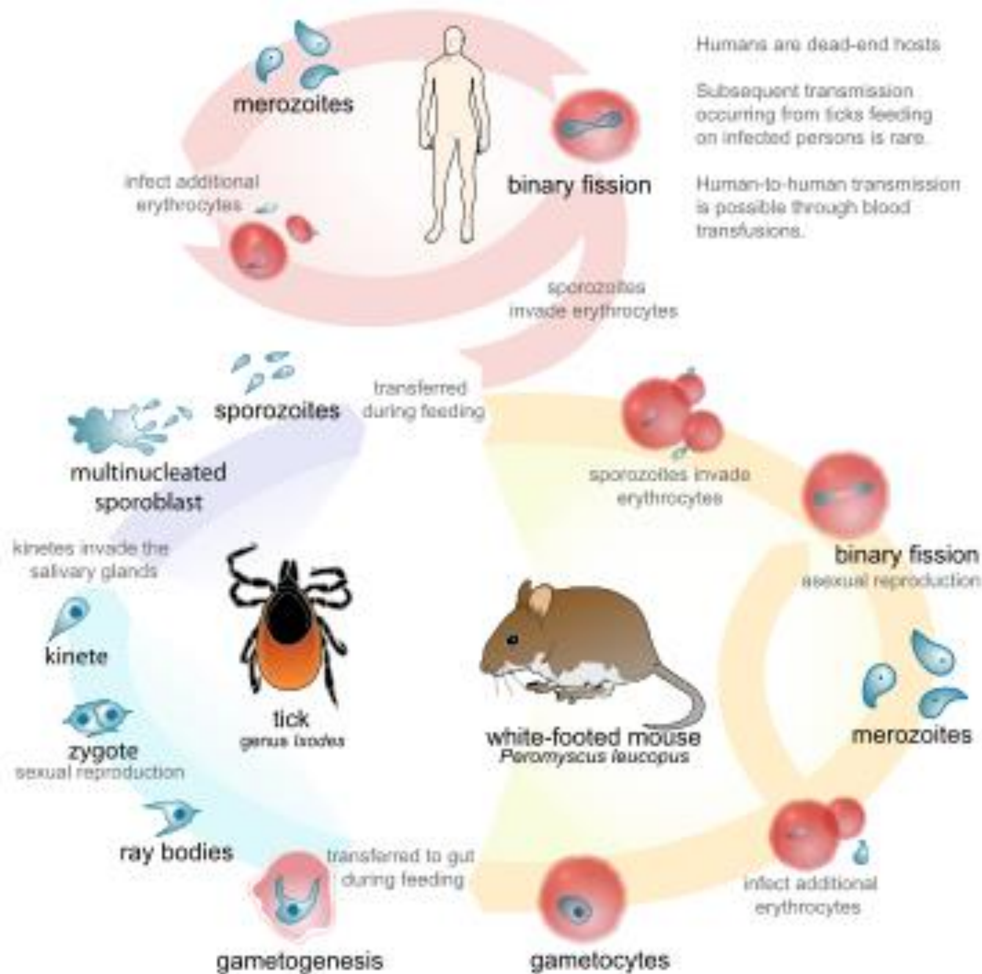

Figure 3: Life cycle Theileria species, T. microti (Adapted form https://en.wikipedia.org/wiki/Theileria_microti#/media/File:*Babesia*_life_cycle_human_en.svg. Date retrieved: 26/07/2018).

Babesia/Theileria species infection is transmitted through tick bites (example, *Ixodes scapularis* ticks; in the case of *B. microti*), blood transfusion, and vertical transmission (Vannier *et al*., 2015; Jane & Adam, 2014; Fang & McCullough, 2016; Tolkacz *et al*., 2017). Babesiosis can also be transmitted through organ transplantation (Meghan *et al*., 2016; Jane & Adam, 2014). The disease is called Human Babesiosis when the susceptible host is a human being (Moustafa *et al*., 2016; Vannier & Krause, 2012; Vannier *et al*., 2008). Generally, in animals, Babesiosis is normally called Tick Fever. In cattle, it is sometimes referred to as Bovine Babesiosis, Cattle Fever or Texas

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fever (Abdella & Jilo, 2016; Owusu, 2015). It is also specifically known as Canine Babesiosis when the *Babesia species* infect dogs (Baneth, 2018; Ćoralić *et al.*, 2018). In small ruminants, Ovine Babesiosis, is amongst the serious haemoparasitic tick-borne diseases (Gholamreza *et al.*, 2017). The same applies to *Theileria species* too.

2.2 Infection of Humans with Babesia/Theileria Species (Human Babesiosis/Theileriosis)

Human babesiosis occurs when a *Babesia/Theileria* infected tick bites a susceptible human during a blood meal by injecting the parasites into him/her (Human Babesiosis, 2018; Lempereur *et al.*, 2017; Meghan *et al.*, 2016). Human babesiosis was first detected in 1957 (Skrabalo & Deanovic, 1957). *B. microti, B. duncani, B. divergens, B. bovis, and B. venatorum* are humans' pathogens (Vannier & Krause, 2009).

In a study that examined 256 transfusion cases in search of possible Transfusion-Transmitted Babesiosis (TTB) in the USA, it was observed that, *Babesia* parasites were present in 165 of the blood samples (Fang & McCullough, 2016) which emphasized an increasing trend in Transfusion-Transmitted Babesiosis cases, in United States. In 2011, another study reported 159 TTB cases attributed to *B. microti* from 1979 to 2009. *Theileria microti* is also transmitted through blood transfusion. It has caused 27% (4/15) of transfusion-transmitted microbial infections. This has resulted in the US FDA approving an antibody-based screening test for blood and organ donors in 2018 (FDA, 2014). *Babesia* can be transmitted from mother to child. *Babesia microti*, the causative agent of zoonotic babesiosis, is confirmed to be transmitted vertically in laboratory mice, dogs, and humans (Tolkacz *et al*., 2017; Fox *et al*., 2006). *Babesia microti* has been demonstrated clearly to be vertically transmitted recently in BALB/c mice with a 100% success (Bednarska *et al*., 2015). Congenital human babesiosis are also seen in newborn human babies in the USA (Esernio-Jenssen

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et al., 1987; New *et al*., 1997). Congenital cerebral theileriosis was confirmed in eight (8) days newborn female twin Holstein (black-and-white) calves (Kaleibar *et al*., 2014).

2.2.1 How *Babesia/Theileria* complicates human malaria diagnosis and treatment

Plasmodium which causes malaria has similar morphology to *Babesia/Theileria* and uncomplicated malaria has clinical presentation similar to babesiosis/theileriosis, although treatment procedure varies (Whegang Youdom *et al*., 2017; Tivura *et al*., 2016; Quick *et al*., 1993).
Microscopic morphological features of *Babesia/Theileria* and *Plasmodium* include intra-erythrocytic rings which can sometimes look like oval, round, pear-shaped, tetrads and Maltese cross (Conrad *et al*., 2006), which can sometimes be confusing. This situation could complicate the diagnosis and treatment of *Babesia/Theileria*, especially in Africa, where malaria is endemic (Huang *et al*., 2018; Hunfeld *et al*., 2008).

Misdiagnosing babesiosis/theileriosis as malaria and vice versa (though in rare cases) could lead to wrong or inappropriate treatment (Kunimoto *et al*., 1998; Scott, 2017; Huang *et al*., 2018), and subsequent development of complicated form of the disease, such as disseminated intravascular coagulopathy (DIC), acute respiratory distress syndrome (ARDS), kidney or congestive heart failure, and coma that can lead to death (Wormser *et al*., 2011).

According to Birkenheuer *et al* (2004b), no specific drug is the drug of choice for treating canine babesiosis but a combined azithromycin and atovaquone therapy can successfully treat *Babesia gibsoni* infections in dogs with no infected erythrocytes in blood smear. This was showed when dogs who have been on this combined therapy for about four (4) months continued to produce

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negative results as revealed by PCR tests (Birkenheuer *et al*., 2004b). Meanwhile, using a mono therapy of atovaquone, *B. gibsoni* were intermittently detected in DNA of experimental infected dogs, thirty-three (33) days when the last treatment was done (Matsu *et al*., 2004). This confirms the assertion of Choidioni *et al* (1995) and Wittner *et al* (1996) that, in humans or animals infected with protozoans such as *P. falciparum* and *B. microti*, when atovaquone is used as a mono therapeutic agent, it is effective but recrudescence rates are high and there is a decreased susceptibility of the parasite after treatment.

Increase in the discovery of different species of *Theileria* in dogs (Dixit *et al*., 2010) has raised the concerns of researchers about mixed *Babesia/Theileria* infection in domestic animals (Criado-Fornelio *et al*., 2004, Sivakumar *et al*., 2014). Similarly, in humans, multiple infections as a result of different pathogens in the same host might cause nonspecific effects on each other caused by host immune responses (Homer *et al*., 2000). Researchers have reported on the begrudging or synergistical consequence of mixed infectious agents (Homer *et al*., 2000). Other unexplained febrile illnesses which are mostly misdiagnosed as malaria, might wrongly be treated as malaria though the patient might be suffering from a different disease (Stoler & Awandare, 2016; Hildenwall *et al*., 2016; Feachem *et al*., 2010; Reyburn *et al*., 2004) like *Babesia/Theileria.* Only those with strong immunity may be able to withstand this condition (Djokic *et al*., 2018).

In most situations, patients have been previously misdiagnosed as malaria and this resulted in inappropriate treatment and delaying of appropriate treatment, this for serious cases like *B. divergens*' infections may be too late (Homer *et al*., 2000). Using antigenic-based malaria RDTs (mRDTs) in Tanzania, it was confirmed that some patients who were initially treated for malaria

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were rather having non-malaria diseases (Hildenwall *et al*., 2016; Feachem *et al*., 2010; Reyburn *et al*., 2004). Other investigators have indicated that malaria has traditionally been the de facto presumptive diagnosis for patients experiencing acute febrile illness (AFI), a category that encompasses half of clinical visits in many Sub-Saharan Africa's (SSA) nations (O'Meara *et al*., 2010; Stoler & Awandare, 2016). Evidently, how malaria complicates the diagnosis and treatment of other fibrile illness such as Babesiosis/Theileriosis and vice versa should be considered a very important discussion for both scientific researchers and practitioners.

2.3 Babesia/Theileria species In Cattle (Bovine Babesiosis/Theileriosis)

Among animals and humans, *Babesia species* in cattle were the first to be detected and identified, as early as the 19th Century. A Romanian physician, Dr. Victor Babes detected the microorganism in the RBCs of cattle and sheep that have hemoglobinuria, which were later identified and named as *Babesia bovis* and *Babesia ovis* (Uilenberg, 2006). Up till now, Babesiosis is a very important haemoparasitic diseases in cattle, since it is responsible for significant morbidity and mortality among them (Abdella & Jilo, 2016; Mans *et al*., 2015; Tarimo, 2013) whiles it can be considered emerging in human beings (Abdella & Jilo, 2016). A study done (in central and southern regions of Portugal) among cattle randomly taken in four different Portuguese districts detected *Babesia* and *Theileria spp.* using PCR-reverse line blotting (RLB). That investigation was able to confirm 74.7% of the bovines as positive for *Babesia/Theileria spp*. Five apicomplexan spp; *Theileria buffeli*, *Theileria annulata, Babesia divergens, B. bovis*, and *B. bigemina* were identified with RLB in the bovines tested (Silva *et al*., 2010).

In Ghana, out of 397 cattle (4 breeds) sampled at three ecological zones, co-infection of *Theileria spp*., *Anaplasma marginale*, and *Babesia bigemina* was observed. In another project done at the

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Southern part of Ghana, Greater Accra region, it was observed that, out of 10 cattle from a total of 30 samples that were microscopically suspected, 9 of them were confirmed with PCR as *Babesia spp*. (Owusu, 2015). This indicated the presence of bovine babesiosis in the southern belt of the country (Ghana), meanwhile, there is little or probably no information on the disease at the Middle Belt of the country. Bovine babesiosis/theileriosis can be detected, wherever the tick vectors are present, but it is mostly identified in the tropics and subtropics. *B. bigemina* and *B. bovis* which are of importance to cattle are widespread in the tropics and subtropical areas (Abdella & Jilo, 2016; CFSPH, 2008).

Babesia bovis is normally infective between 2-3 days after attachment of larval tick, and it can live in cattle for years. In *R. microplus*, however, *B. bovis* cannot survive after the larval period. For *B. bigemina*, it matures almost 9 days after the hatching of larval tick; and is therefore only transmitted by nymphs and adults. It can survive in cattle for only few months. Interestingly for *R. ricinus*, all the three stages are able to transmit *B. divergens* (CFSPH, 2008). Vertical transmission reported in humans (Joseph *et al*., 2012) and dogs (Adaszek *et al*., 2016; Fukumoto, *et al*., 2005) is also found here because these species which are vectored by the same tick host, *Rhipicephalus species*, are mostly transmitted transovarially (Abdella & Jilo, 2016; CFSPH, 2008). *B. divergens, B. venatorum,* and *B. microti* first detected in cattle were later observed to also cause human and canine babesiosis respectively (Vannier & Krause, 2009; Abdullah *et al*., 2018) *B. bovis* infecting humans as well.

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2.4 Babesia/Theileria species in Dogs (Canine Babesiosis/Theileriosis)

The first and early description of *Babesia* infection in dogs was done in Africa, 1893 (Hutcheon, 1893). Two years later, infection with *Babesia species* in Dogs was also described in Italy, (Roncalli, 2001) and then later (1934) in Florida, United States (Eaton, 1934). Protozoan diseases like babesiosis/theileriosis are major tick-borne infections in dogs, they are found worldwide (Irwin, 2009; Boozer & Macintire, 2003; Solano-Gallego & Baneth, 2011). Dogs served as one of the reservoirs from which infected ticks infect majority of domestic animals, wild animals, and human beings (Schnittger *et al*., 2012). Several *Babesia spp* that causes canine babesiosis have different susceptibility to anti-protozoal drugs (Baneth, 2018). *Theileria species* which are pathogenic to dogs are: *T. annae* (Simõe *et al*., 2011; Garcia, 2006), *Theileria*-like group (*Babesia conradae*), *T. annulata* (Criado *et al*., 2006), and *T. equi* (Criado-Fornelio *et al*., 2003).

The full geographical range of canine piroplasms has been found in dogs in the Middle East, parts of Africa, North America, and Europe. *Theileria annulata* was detected in two herd dogs in Iran using their 18S rRNA gene sequence (Bigdeli *et al*., 2012). Two PCR positive dogs from Shiraz were infected with *T. annulata* (first reported case in Southern Iran, and the second reported case of *T. annulata* in dogs worldwide) and *B. canis* (Bigdeli *et al*., 2012). *Theileria annae, Theileria equi*, and *Theileria annulata* were identified in sick dogs in Spain (Garcia, 2006), South Africa (Matjila *et al*., 2008), Croatia (Beck *et al*., 2009), France (Fritz, 2010) and Portugal (Simões *et al*., 2011). Beck *et al*., 2009 in Croatia detected *T. annae* in sick dogs using PCR. *T. equi* too was amplified in three (3) asymptomatic dogs and one sick dog (Criado-Fornelio *et al*., 2003). Species like B. *divergens, B. venatorum*, and *B. microti* that infect dogs were also found to be associated with zoonotic human babesiosis (Vannier & Krause, 2009). Since just a proportion of some dogs

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some show clinical signs and symptoms of the infection, there are more questions on the clinical importance of theileriosis in dogs.

2.5 Pathogenesis, Signs and Symptoms of *Babesia/Theileria* Infection

The signs and symptoms associated with Babesiosis and theileriosis are similar to that of malaria. In most cases, the parasites involved in these disease conditions causes haemolysis, which results in anaemia and jaundice (Gray *et al*., 2010; Kappmeyer *et al*., 2012). The disease condition progresses to anoxia and increased cell toxicity, leadig to death in some cases. Although haemolysis is linked with infected cells, it can also occur in uninfected erythrocytes due to excessive production of pro-inflammatory cytokines. These cytokines cause symptoms including fever, myalgia, renal insufficiency, coagulopathy and hypotension (Clark and Jacobson, 1998; Krause *et al*., 2007).

The clinical manifestations of *Babesia*/*Theileria* progresses from mild anaemia to severe pancytopenia, rapture of the spleen, disseminated intravascular coagulation (DIC) as well as hemophagocytic lymphohistocytosis (HLH) (Akel & Mobarakai, 2017; James, 2015). Although immunocompetent individuals are usually asymptomatic, the persistence of these parasites could be deleterious, and in some cases, life threatening. However, in immunocompromised persons, the disease could lead to multiple organ failure (Genda *et al*., 2016; Akel & Mobarakai, 2017; Roshni *et al*., 2017; Linden *et al*., 2018). Human Babesiosis symptoms can be from subclinical to severe (Leibly, 2011; James, 2015; Human Babesiosis, 2018).

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2.6 Laboratory Diagnosis of *Babesia/Theileria*

The first method of diagnosis of *Babesia/Theileria* species was by microscopy (Babes, 1888; Theiler, 1904). The parasites are usually identified in Giemsa stained blood smears (Gunes *et al*., 2017). The diagnostic tests include microscopy, serology and molecular techniques, chest radiography, bone marrow aspirate (biopsy) (Mosqueda *et al*., 2012; Gupta *et al*., 2017; Hassan *et al*., 2018). Diagnosis using an indirect fluorescent antibody (IFA) test has much higher specificity compared to staining of blood smears. Molecular methods of detection are by far, the most sensitive and reliable tool for detecting infecting parasites (Mosqueda *et al*., 2012; Hassan *et al*., 2018). PCR targeted at the V4 hyper-variable region of 18S rDNA has the ability to identify wide varieties of *Babesia/Theileria* species (Bastian *et al*., 2012).

2.7 Epidemiology of Babesiosis/Theileriosis

The geographical distribution of Babesiosis/Theileriosis causing organisms varies from country to country. In the United States where Babesiosis is endemic, most of the cases are caused by *B. microti* (Vannier *et al.*, 2008). In Europe, most reported cases have been attributed to *B. divergens*. Results obtained from a previous study revealed that, *Theileria parva* is most abundant in sub-Saharan Africa (Tarimo, 2013). In recent times, there are several species of *Babesia* and *Theileria* which affect humans and animals in the sub-Saharan countries, and this has necessitated further research into other infecting species besides *B. microti* (Akel & Mobarakai, 2017; Solano-Gallego and Baneth, 2011; Dixit *et al*., 2010). The infecting species of Theileriosis is also widely distributed, among domestic and wild animals, as well as humans (Mans *et al*., 2015). A new *Babesia* strain (KO1 strain) has been identified in South Korea (Kim *et al*., 2007). Other cases caused by uncharacterized *Babesia* species has been documented in Egypt, Mozambique, and South Africa (Kjemtrup & Conrad, 2006). A case of *B. divergens*–like infection was reported on the Canary

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Islands, off the coast of West Africa (Gray *et al*., 2010). Bovine and canine babesiosis has also be reported in Ghana (Beckley, 2013).

2.7.1 Babesiosis/Theileriosis in Ghana

There is little information about *Babesia/Theileria* infection in Ghana, especially in the Middle Belt. Even though there has not been report of *Babesia/Theileria* in humans, there had been few reports in animals. Bell-Sakyi *et al*., (2004), in addition to detecting *Theileria species* in sheep and *Anaplasma species* in goats, found *Babesia bigemina, Theileria mutans,* and *Theileria velifera* in cattle. In that study, *T. mutans* was found to be the most prevalent tick-borne pathogen in cattle (Bell-Sakyi *et al*., 2004). Nine years later, Beckley (2013) detected *Babesia bigemina, Theileria species*, as well as *Anaplasma marginale* amongst local breeds of cattle (Sanga, Gudali, West African Shorthorn and White Fulani) in three agro-ecological areas of Ghana (Coastal Savana, Guinea Savannah and Transitional zone).

Adzigbe (2017) also investigated hemoparasites among cattle in Southern part of Ghana (Accra and Adidome). Two genera of hemoparasites caused by ticks were detected by the research – *Theileria* and *Babesia species* with *Theileria velifera* as the predominant one (Adzigbe, 2017). The study also detected T. *mutans* and B. *bovis* in the two study areas. Meanwhile, there is little information on the status of *Babesia/Theileria* in the Middle Belt of Ghana.

Blood samples of one hundred and fifty malaria positive patients, thirty sick cattle and thirty-three sick dogs were screened for the detection of *Babesia* species in the Greater Accra Region of Ghana

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(Owusu, 2015). After Giemsa stain microscopy and PCR, nine out of the thirty sick cattle, and one of the dogs was found to be infected with *Babesia/Theileria*, but none in human.

2.8 Prevention and Control of *Babesia/Theileria* species

The best way to eradicate *Babesia/Theileria* species that cause babesiosis/theileriosis, is to eliminate the definite host, tick(s). So many methods are available to control theileriosis, likewise babesiosis. Practically, chemical control with acaricides and vaccinations is mostly used worldwide (Morrison, 2015). Acaricides have been tried in U. S., aimed at eliminating ticks where all cattle were treated every 2 or 3 weeks with it (CFSPH, 2008). Despite this effort, cases of babesiosis are still reported in some parts of the world. There is a steady increase of diseases caused by ticks for the past two decades in North America and Europe (Dunn *et al*., 2014; Rizzoli *et al*., 2014; Moutailler *et al*., 2016).

In some nations cattle are prevented from the disease through vaccination with live attenuated strains of *B. bovis* and *B. bigemina* or *B. divergens.* In locations where these diseases are endemic, it is encouraged that sick animals are given antiparasitic drugs to treat them as quickly as possible. Treatment is most likely to succeed when they are diagnosed early. Example, Atovaquone/Buparvaquone plus azithromycin can be used to treat babesiosis in both human beings and animals (Tanyel *et al*., 2015; Plumb, 2015; Checa *et al*., 2017). Buparvaquone along with other supportive therapies like azithromycin can be used to treat both Babesiosis/Theileriosis (Ganga *et al*., 2010; Plumb, 2015; Checa *et al*., 2017). Treatment may also fail if the sickness makes the animal anemic and weak. Disinfectants and environmental cleanliness, one of the best preventive methods for most diseases like malaria, are generally not effective against them. However, if care is taken not

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to transfuse unscreened blood from one animal to another, it can reduce the spread of babesiosis (CFSPH, 2008).

Recently, genetically resistant cattle, known as *B. indicus*, is a proposed sustainable approach that can help decrease the incidence of Babesiosis (Abdella & Jilo, 2016). However, there is still the need of continue searching for new innovative methods that can serve as better alternative preventive and control methods. Prevention of *B. divergens* infection is possible, if persons with weak immunity are careful when travelling to babesiosis endemic regions, particularly within the seasons that ticks are prevalent. Putting on particular clothing like; long-sleeved shirts, long pants, and using tick repellents can help prevent *B. divergens* infection. After outdoors' activities, the skin and clothing should be checked for ticks and removed if found (CFSPH, 2008).

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

A cross sectional study was used in this research, where blood samples were collected at a point in time, from a population.

3.2 Study sites

The project was done among selected people and animals in the Middle Belt of Ghana using two municipalities: Techiman South and Kintampo North Municipalities (Figure 4).

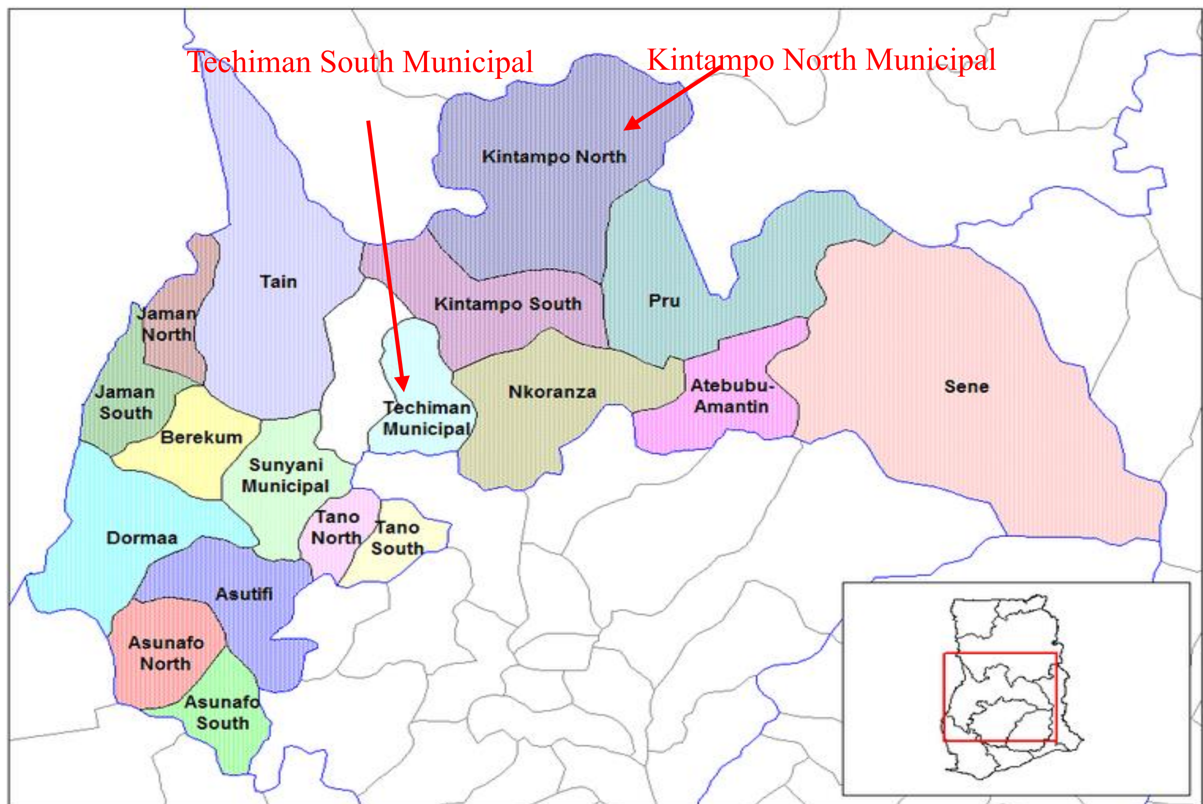

Figure 4: Map of Ghana Showing Study Sites of the Project at the Middle Belt (Adapted from; wikipedia.or./wiki/File:Brong_Ahafo_districts.png. Date retrived: 02/06/2018)

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3.2.1 Techiman South Municipality

The Techiman Municipality is found at the central part of the Brong-Ahafo Region, and shares common boundaries with four districts/municipalities: Techiman North, Wenchi, and Nkronza Municipalities of the Brong-Ahafo Region, as well as Offinso-North District in the Ashanti Region (Figure 5A). The municipality had a population of 147,788 in the most recent census (Ghana Statistical Service, 2014). This represented 6.4% of the total population of the Brong-Ahafo Region. According to the Ghana Statistical Service (2014), the second most important agricultural activity in the Techiman Municipality is rearing of livestock; practiced in both rural and urban areas within the municipality.

Figure 5A. Map of Techiman South Municipality (Adapted from Ghana Statistical Service, 2014. Date retrived: 02/06/2018).

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According to the Ghana statistical service (2014), cattle represents the most reared livestock in the municipality in terms of “average animal per keeper. Techiman Market (where variety of agricultural products are sold) is one of the largest markets in Ghana, that brings people from various parts of the country (especially the three Northern regions) and other neighboring countries to the municipality for business purposes. This market can also be classified as a trans-national market that attracts people from other West African countries such as Mali, Burkina Faso, Nigeria and Niger. Humans, dogs and cattle samples were collected from a number of areas within the municipality.

3.2.2 Kintampo North Municipality

The Kintampo North Municipality shares boundaries with five Municipalities: Central Gonja Municipal to the North; Bole Municipal to the West; East Gonja Municipal to the North-East, Kintampo South Municipal to the South; and Pru Municipal to the South- East, respectively (Figure 5B). The municipality had a population of 95,480 in the most recent census by the Ghana statistical service (2014). Geographically, the Municipality is strategically located at the Centre of Ghana, serving as a transit point between the northern and southern sectors of the country (Figure 3.2B). In the Municipality, 60.2% of households are engaged in agricultural activities (GSS, 2014). Similar to the Techiman South municipality, humans, dogs and cattle samples were collected from a number of areas within the Kintampo South municipality.

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Figure 5B: Map of Kintampo North Municipal (Adapted from Ghana Statistical Service, 2014, Date retrived: 02/06/2018)

3.3 Sample size determination

Sample size for the humans, cattle and dogs used in the current study was calculated using the formula: $n = [Z^2. P. (1\text{-}P)] / C^2$

Where: n= sample size; Z= standard value at a certain confidence level; P= estimated prevalence; *C*= margin of error. The proposed samples sizes for this study were calculated with the following parameters.

3.3.1 Sample size for Human Subjects

For the human samples, a confidence level of 95% with a corresponding z-value (Z) of 1.96; a prevalence (P) of 10.81% obtained from a study by Herrera *et al.,* 2017 was used. With a confidence interval (C) of 0.05, the sample size of human beings used for the project was calculated as:

$$\Rightarrow [Z^2. P. (1\text{-}P)] / C^2 = [1.96^2.0.1081. (1\text{-}0.1081)]/0.05^2 = [3.8416\text{x}0.1081\text{x} (0.8919)] / 0.0025$$

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$=> [Z^2. P. (1-P)] / C^2 = 0.3704/0.0025 = 148.2 \approx 148.0$.

Therefore, a minimum sample size of one hundred and forty-eight (148) people was used.

3.3.2 Sample size for Sick Cattle and Dogs

Using a prevalence rate of 5.5% also obtained from a study by Jirapattharasate *et al*., 2016; the sample size for cattle using the same calculation from the formula above was Eighty (80.0). That is $79.87 \approx 80$.

With the same prevalence of 5.5%, $79.87 \approx 80.0$ dogs were also sampled.

3.4 Selection of Study participants and sample collection

3.4.1 Human samples

Blood samples were collected from smear positive malaria patients from the Holy Family Hospital at Techiman and Kintampo Municipal Hospital. Cattle rearers from Techiman and Kintampo who consented (especially those that complain of not feeling well at the time of the study) were also sampled. At Techiman, the human samples were collected from three (3) areas within the municipality: Holy Family Hospital, Tanoso, and the Abattoir. The smear positive malaria samples were all collected at Holy Family Hospital whiles samples from the cattle rearers were taken from Tanoso and the Abattoir. At Kintampo, samples of smear positive malaria patients were taken at the Kintampo Municipal Hospital whiles samples from the Cattle rearers were collected from three (3) communities in the municipality, namely; Kaaka, Komadai and Kwabena Num. From each patient, two to three milliliters (2-3ml) of venous blood were collected for investigation.

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3.4.2 Animal samples

At Techiman, the cattle samples were collected from communities in Tanoso, James' Town, Abattoir (Slaughter house) and Sansama. Blood samples from dogs were collected from the two veterinary offices within the municipality. At Kintampo, cattle blood samples were collected from four (4) communities in the municipality, namely: Komadai, Techira Number 2, Mossi-Akura, and Suronuase. Blood samples form dogs were collected from the Kintampo Veterinary office. Whole blood samples were taken from the jugular vein of the sick cattle and the cardiac vein of sick dogs, according to Laboratory Standard Operation (Appendix IV). All blood samples were collected into well labeled EDTA tubes and kept in cold boxes for transportation to the parasitology laboratory of the Department of Medical Microbiology, SBAHS, University of Ghana. About 2 ml venous blood sample was taken from each of the selected animals for screening.

3.5 Inclusion/exclusion criteria

The following criteria were used for inclusion and exclusion of study subjects.

Inclusion Criteria

- Sick cattle and dogs whose owners have given their consents
- Cattle rearers who have given their consents were sampled
- People who were diagnosed as malaria patients through microscopy of stained smear

Exclusion Criteria

- Sick dogs and cattle who have been vaccinated / treated less than two weeks to sampling were excluded.

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3.6 Laboratory Procedures

A summary of laboratory procedures employed in the current study is presented in Figure 6. Questionnaires were used to gather demographic data for both humans and animals (Appendix III). Care takers of the animals provided the demographic data about the animals. Blood samples collected from humans were screened for *Babesia/Theileria* infection by microscopically examining Giemsa stained thin blood smears. Deoxyribonucleic acid (DNA) was extracted from the blood samples that were suspected to be infected with *Babesia/Theileria* for amplification. Samples that successfully amplified were sequenced. For samples from sick animals, those that showed intra-erythrocytic parasites microscopically were selected for DNA amplification. Similarly, samples that successfully amplified were chosen for sequencing (Figure 6).

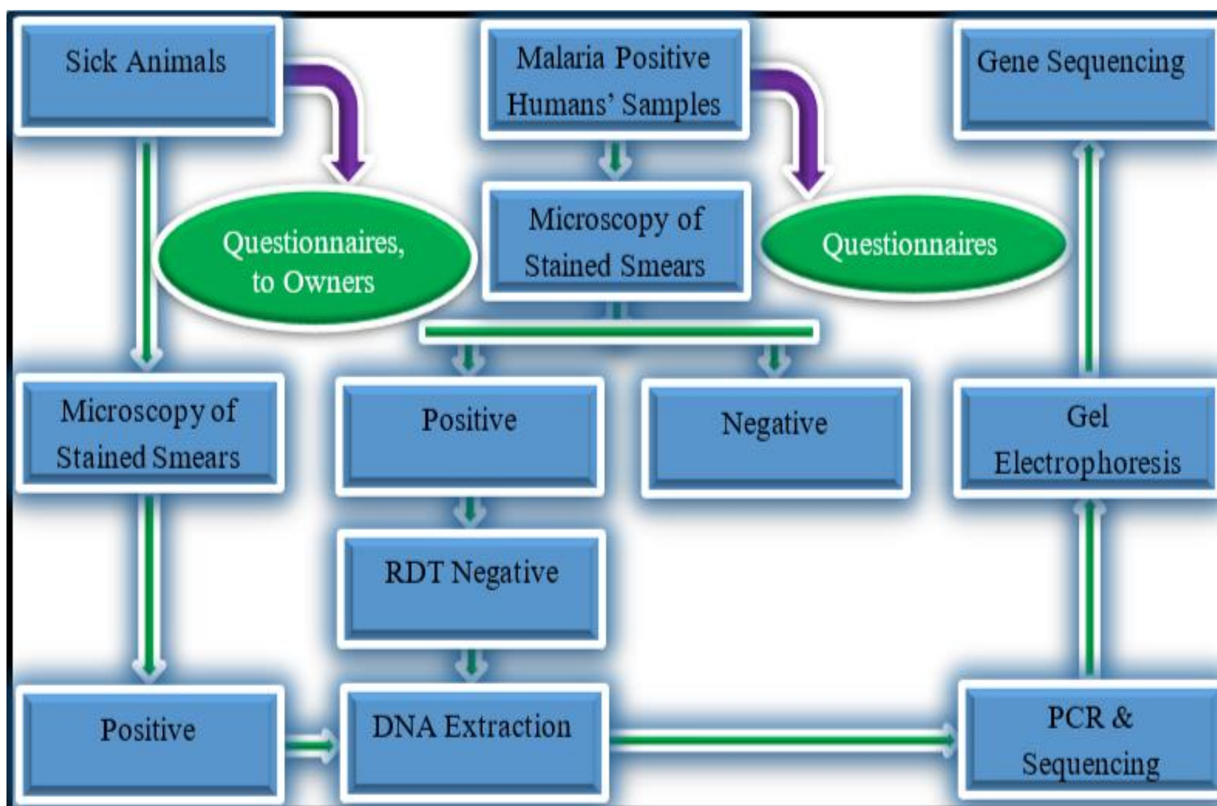

Figure 6: Diagrammatic Presentation of the Laboratory Procedures

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3.6.1 Microscopy (Thin and thick blood smears)

Thin and thick blood smears were made on clean labeled glass slides for each blood specimen. The smears on the slides were air-dried after which the thin smears were fixed in methanol. The slides were then flooded with 1 in 10 dilution of Giemsa stain and held for 10 minutes. Afterwards, the stain was gently washed off with clean water and the slides were air dried. Slides were viewed with oil emersion under high power lens using a light microscope (Olympus, UK). During the examination, samples with *Plasmodium*-like features in addition to characteristics (such as triad, tetrad, and classical Maltese cross or paired pear-shaped morphology) suggestive of possible *Babesia/Theileria* infection were selected, and after testing with rapid diagnostic test kit (Clinogen Diagnostics, Japan), those that were negative for the four human *Plasmodium sp* were labeled as "suspected" *Babesia/Theileria*-infected samples and selected for molecular analyses (Figure 6). The RDT kit contained a membrane strip pre-coated with two monoclonal antibodies as two distinct lines which detect antigens in whole blood. One line had antibodies specific for *P. falciparum* histidine rich protein-2 (Pf HRP-2) whiles the other line contained antibodies that were *pan* specific to the lactate dehydrogenase of *Plasmodium* species (*P. falciparum, P. vivax, P. ovale* and *P. malariae*). In the animals (cattle and dogs), the suspected samples included those whose thin smears showed intra-erythrocytic parasites.

3.6.3 DNA extraction

Extraction of total DNA from both blood samples and ticks were performed using the QI Amp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Twenty microlitres (20 µl) of protease was dispensed into the bottom of a well labeled 1.5 ml eppendorf tube. Two hundred microlitres (200 µl) of whole blood was added into the tube after which two hundred microlitres 200 µl of lyse buffer (Buffer AL) was added and mixed by pulse vortexing for

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15 seconds. The mixture was incubated at 56˚C for 10 minutes and briefly centrifuged to remove drops from the inside of the lid. Two hundred microlitres (200µl) of cold absolute ethanol was added to each sample and mixed by pulse vortexing for 15 seconds and briefly centrifuged to remove drops from the lid. The mixture was carefully transferred into a labeled QIAamp Mini spin column (in 2 ml collection tube) without wetting the rim. The cap of the column was closed and centrifuged at 6000 x gravity (8000 rpm) for a minute. The collection tube containing the filtrate was discarded and replaced with a clean tube.

Five hundred microlitres (500 µl) of wash buffer (Buffer AW1) was carefully added to each spin column and centrifuged at 6000 x gravity (8000 rpm) for 1 minute. The filtrate was again discarded with the tube and the spin column was placed in a clean collection tube. Five hundred microlitres (500 µl) of a second wash buffer (Buffer AW2) was added to each spin column and centrifuged at full speed (14,000 rpm or 20000 x gravity) for 3 minutes and the collection tube containing the filtrate was discarded. Each spin column was then placed in a labeled 1.5 ml eppendorf (microcentrifuge) tube. The spin column was carefully opened and two hundred microlitres (200 µl) of elution buffer (Buffer AE) was added and incubated at room temperature for 5 minutes. The sample was centrifuged at 6000 x gravity for 1 minute and the spin columns discarded. The extracted DNA samples were frozen at -20˚C for future use.

3.6.4 Detection and identification of *Babesia/Theileria species* from Extracted DNA

For the humans and cattle's samples, extracted DNA were amplified by PCR using primers that can detect either *Babesia* or *Theileria* DNA (Table 1)**.** The genus sets of primers that are specific for *Babesia/Theileria* in humans and cattle and were used in the running of the nested PCR are: Bab5 (5'-AATTACCCAATCCTGACACAGG-3') and Bab8 (5"-TTTGGCAGTAGT

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TCGTCTTTAACA-3') were used for the first round of amplification. Whilst an expected band sizes of 430bp after amplification, the primers: Bab6 (5'-GACACAGGGAGGTAGTGACAAGA-3') and Bab7 (5'-CCCAACTGCTCCTATTAACCATTAC-3') were also used for the second-round amplification (Wei *et al*., 2001). As seen in Table 1, highly conserved primers that can amplify many different *Babesia* and *Theileria species* were used (Gubbels *et al*., 1999; Centeno-Lima *et al*., 2003).

Table 1. Species specific primers that were used for amplification of PCR products positive after genus primer amplification

References	Species	Primers and Sequence (5´-3´)	Amplifiable Microbes
Wei et al., 2001	*B. microti-like*	Bab5 AATTACCCAATCCTGACACAGG Bab8 TTTCGCAGTAGTTCGTCTTTAACA Bab6 GACACAGGGAGGTAGTGACAAGA Bab7 CCCAACTGCTCCTATTAACCATTAC	Some *Babesia* and *Theileria spp.*
Sobczyk *et al*., 2005; 2000	*B. canis*	BcW-A: CATCTAAGGAAGGCAGCAGG BcW-B: TTAATGGAAACGTCCTTGGC	*Babesia canis*

T: thymine; A: adenine; C: cytosine; G: guanine

For the dogs' samples, primers specific for *Babesia canis* (BcW-A: CATCTAAGGAAGGAG-CAGG and BcW-B: TTAATGGAAACGTCCTTGGC) were used (Table 1). The reaction solution for PCR contained 12.5µl of One Taq 2X master mix with GC buffer (New England Bio labs Inc.), 0.5µl of 10µM of each primer, 3.5µl of One Taq high GC enhancer and 8µl of the DNA in a final volume of 25µl. PCR products positive for this primer were amplified using species specific primers (*B. canis*, for the dogs' samples). Thirty-five cycles were carried out for the dog samples

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which involved denaturing at 94°C for 120 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. For the human samples, a total of 30 cycles was carried out for nest one; consisting of denaturing at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 1 minute, with an initial pre-incubation at 94°C for 30 seconds and a final extension at 68°C for 10 minutes. Nest two was carried out for 40 cycles with the same cycling conditions as nest one except an annealing temperature of 58°C (Table 2).

Table 2. Cycling Conditions for Humans, Cattle and Dog Samples

Primer Used	Denaturation	Annealing	Extension	Cycles Used	Expected Bands' Size
First Round Amplification for Humans and Cattle					
Bab 5, Bab 8	94°C(30seconds)	55°C(30seconds)	68°C (1minute)	30	No Gel
Second Round Amplification for Humans and Cattle					
Bab 6, Bab7	94°C(30seconds)	58°C(30seconds)	68°C (1minute)	40	430bp
Amplification for Dog samples					
B. canis	94°C(2minutes)	60°C(30seconds)	72°C (30seconds)	35	509bp

Electrophoresis in 2% agarose was then carried out on the amplified DNA (5 µl) which was detected by ethidium bromide staining and UV trans-illumination. The expected target size was 509-bp and the band size was measured using 100bp and 1000bp DNA ladders (New England Biolabs Inc.).

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3.6.5 Gel electrophoresis

Briefly, agarose gel with wells was placed in the electroploresis gel tank ensuring that the wells were correctly positioned at the negative terminal of the tank. After, filling the tank with 1X TAE buffer until completely covering the gel with the buffer, a mixture (two microlitre volume of blue loading buffer mixed with ten microlitres of PCR products on a paraffin film) was carefully loaded into the gel wells submerged in buffer with the help of a micropipette. The first well contained the ladder whiles each of the subsequent wells were loaded with the PCR products were loaded after mixing with the loading buffer. A voltage of 100V was used to run the set-up until the dye line was approximately eighty percent of the way down the gel. The gel was subsequently viewed after the run on an ultra-violet light screen and the image of the gel was captured using a camera.

3.7 Statistical analysis

Data obtained from this study were stored using the Microsoft office Excel 2016 software (Microsoft® Office Professional 2016, Microsoft Corporation, USA) and analyzed using SPSS software version 16. Descriptive statistics was the main tool employed to analyze data collected. Tables and graphical displays were used where necessary and appropriate to summarize data. Frequency as well as mode was mainly used to analyze data. The frequencies of samples positive for *Babesia/Theileria* were used to determine which vertebrate species (humans, cattle and dogs) was more infected. Chi square (X^2) was used to find association between variables and a *P-value* less than 0.05 was considered statistically significant.

3.8 Research Clearance

This work received ethical clearance from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences, under the College of Health Sciences of the

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University of Ghana (Protocol Identification number: CHS-Et/M.6 – P 4.3/2016-2017) (Appendix IA). Permission to collect samples for the study were also sought from the Holy Family Hospital, Techiman (Appendix IB), the Kintampo Municipal Hospital (Appendix IC), the Techiman Veterinary Office (Appendix ID) and the Kintampo Veterinary Office (Appendix IE). Also, samples were collected with the consent of patients and owners of animals (cattle and dogs) (Appendix II). All subjects/patients who took part in the study were number coded for identification instead of being identified by name or personal data. This was done for security and confidentiality reasons.

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CHAPTER FOUR

4.0 RESULTS

4.1 Overview of Results

A total of 464 blood samples (comprising of 71 dog, 207 cattle and 187 humans) were used for the current study (Table 3). Thirty (representing 42.3%) out of the 71 dogs sampled were microscopically suspected for *Babesia*. Fourteen (46.7%, 14/30) out of the microscopically suspected samples, when subjected to PCR, were successfully amplified. For the cattle samples, 33 (representing 15.9%) were microscopically suspected of *Babesia/Theileria*, out of which 20 (66.6%) were amplified by PCR and subsequently sequenced. The human samples were made up of 95 smears positive for *Plasmodium-like* parasite and 92 cattle rearers from the two study sites. Meanwhile, 20 (10.7%) of these human samples which showed *Plasmodium*-like parasites microscopically, but RDT negative were suspected for *Babesia/Theileria* infection. When subjected to PCR, six (30.0%) out of the 20 suspected human samples were amplified (for *Babesia/Theileria*) and subsequently sequenced (Table 3).

Table 3. Summary of microscopic result of suspected *Babesia/Theileria* in the various sample populations

Samples	Number, N	Microscopy Suspected, n (%)	PCR Positive, n (%)
Humans	187	20 (10.7)	6 (30.0)
Cattle	207	33 (15.9)	20 (66.6)
Dogs	71	30 (42.3)	14 (46.7)

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4.1 Results on dog samples

4.1.1 Demographic Data of Dogs and *Babesia* Infection

Thirty-seven (representing 52%) of the dogs sampled for the current study were within the age of less than or equal to six months, followed by those within the age of 7-13 months (Table 4). Regarding age and microscopic suspicion of *Babesia* in dogs, half (50.0%, 15/30) of the positive cases were less than or equal to six (≤ 6) months, with 2 (6.7%) of the suspected cases above 27 months (Table 4). *Babesia canis* infection was confirmed in 35.7% of the doges within the age group of 7-13 months, followed by those within the age of ≤ 6 months with 28.6%, although 50% (15/30) of the suspected cases were within that age-group (≤ 6 months). Meanwhile, the age of one of the dogs could not be ascertained. Statistically, there was no association between age and *Babesia* infection by PCR (*P-value* = 0.1298)**.** With regards to gender, *Babesia* was microscopically suspected in 56.7% male dogs as compared to 43.3% females. Meanwhile, in terms of PCR confirmation of the microscopy suspected cases, the numbers were evenly distributed among the male and female dogs (Table 4). No significant association was observed between gender and *Babesia* in infection by PCR (*P-value* = 0.2833).

Fourty-five, (representing 63.4%) of the dogs sampled recorded temperature between the range of 35-40°C, with 2 (representing 2.8%) recording temperature above 45.1°C. Meanwhile, majority (53.3%) of the microscopically suspected dogs were within the temperature range of 40.1-45.1°C, and half (50.0%) of the PCR confirmed samples were also within that range (Table 4). Statistically, there was no significant association between temperature and *Babesia* infection by PCR (*P- value* = 0.4242).

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Table 4. Demographic data of dogs sampled and Babesia Infection

Parameter	Number sampled n (%)	Microscopically Suspected			PCR Positive		
		Tech.	K'po	Total n (%)	Tech.	K'po	Total n (%)
Ages(mths)							
≤ 6	37(52.1)	14(50.0)	1(50.0)	15(50.0)	4(30.8)	0(0.0)	4(28.6)
7-13	15(21.1)	5(17.9)	1(50.0)	6(20.0)	4(30.8)	1(100.0)	5(35.7)
14-20	7(9.9)	3(10.7)	0(0.0)	3(10.0)	1(7.7)	0(0.0)	1(7.1)
21-27	7(9.9)	4(14.3)	0(0.0)	4(13.3)	3(23.1)	0(0.0)	3(21.4)
Above 27	4(5.6)	2(7.1)	0(0.0)	2(6.7)	1(7.7)	0(0.0)	1(7.1)
Nil	1(1.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
						P –value	***0.1298***
Sexes							
Male	45(63.4)	17(60.7)	0(0.0)	17(56.7)	7(53.8)	0(0.0)	7(50.0)
Females	26(36.6)	11(39.3)	2(100.0)	13(43.4)	6(46.2)	1(100.0)	7(50.0)
						P –value	***0.2833***
Temp. °C							
35.0-40.0	45(63.4)	12(42.9)	1(50.0)	13(43.3)	6(46.2)	0(0.00	6(42.9)
40.1-45.1	24(33.8)	15(53.6)	1(50.0)	16(53.3)	6(46.2)	1(100.0)	7(50.0)
Above 45.1	2(2.8)	1(3.6)	0(0.0)	1(3.3)	1(7.7)	0(0.0)	1(7.1)
						P –value	***0.4242***
Symptoms							
Weakness	25(35.2)	14(50.0)	1(50.0)	15(50.0)	7(53.8)	0(0.0)	7(50.0)
Weight Loss	19(26.7)	7(25.0)	0(0.0)	7(23.3)	2(15.4)	0(0.0)	2(14.3)
Anorexia	17(23.9)	6(21.4)	1(50.0)	7(23.3)	3(23.1)	1(100.0)	4(28.6)
Others	10(14.1)	1(3.6)	0(0.0)	1(3.3)	1(7.7)	0(0.00	1(7.1)
						P –value	***0.1342***
Dog Types							
Mongrel	47(66.2)	16(57.1)	1(50.0)	17 (56.7)	7(53.8)	0(0.0)	7 (50.0)
G. Shepherd	8 (11.3)	4(14.3)	0(0.0)	4 (13.3)	1(7.7)	0(0.0)	1 (7.1)
Bullmastiff	7 (9.9)	3(10.7)	1(50.0)	4 (13.3)	2(15.4)	1(100.0)	3 (21.4)
Hybrid	3 (4.2)	1(3.6)	0(0.0)	1 (3.3)	0(0.0)	0(0.0)	0 (0.0)
Pit Bull	2 (2.8)	2(7.1)	0(0.0)	2 (6.7)	1(7.7)	0(0.0)	1 (7.1)
Poodle	2 (2.8)	1(3.6)	0(0.0)	1 (3.3)	1(7.7)	0(0.0)	1 (7.1)
Boerboel	1 (1.4)	1(3.6)	0(0.0)	1 (3.3)	1(7.7)	0(0.0)	1 (7.1)
Doberman	1 (1.4)	0(0.0)	0(0.0)	0 (0.0)	0(0.0)	0(0.0)	0 (0.0)
						P –value	***0.0004***

Tech. represents Techiman, **K'po** represents Kintampo, **Temp** represents Temperature, ***G.* Shepherd** represents German shepherd, **n** represents number, **%** represents percentage

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Regarding symptoms, 35.2% of the dogs sampled presented with weakness and half of the dogs that were microscopically suspected of *Babesia* showed signs of weakness, which all amplified as *Babesia canis* (Table 4). Some of the sick dogs laid helpless on the floor as seen in the case of a weak poodle dog in Figure 7F. Meanwhile, no significant association was observed between symptoms and *Babesia* in infection by PCR (*P-value* = 0.1342).

With type of dogs, more than half (66.2%) of the dogs sampled were mongrel, followed by German shepherd (8/71, 11.3%) and Bullmastiff with 9.9% (Table 4). Pitbull and Poodle (2.8%) apiece whiles Boerboel Dobermanwere 1.4% each. Most of the suspected *Babesia* cases were mongrel 17 (17/30, 56.7%) with 7 (7/14, 50.0%) of them amplified as *Babesia canis*. There was a significant association between type of dog and *Babesia* in infection by PCR (*P- value* = 0.0004).

Figure 7: Types and symptoms of some of the dogs sampled for the study.
(A) German shepherd dog that loss appetite and was not eating, (B&D) Sick older and puppy mongrel dogs (C) A sick Bullmastiff dog (E) A sick PitBull Puppy dog, (F) A weak Poodle dog lying helpless on the floor. (G) A sick Boerboel dog (H) Sick hybrid dog that has lost weight (emaciated). Except, B and H, blood samples from all the dogs in this figure were amplified by the *Babesia canis* specific primers.

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4.1.2 Microscopic Suspicion of Canine *Babesia* Infection

Microscopic examination of Giemsa stained thick and thin smears recorded thirty (42.3%, 30/71) suspected *Babesia* cases. Infection with two or more intra-erythrocytic parasites per Red Blood Cells (RBCs) was observed through microscopy (Figure 8A, E and F). One of the samples showed an intra-erythrocytic parasite that has a Maltese cross configuration (Figure 8F). More paired configurations were observed (Figure 8A, B, E, and F) but no tetrad formation was identified.

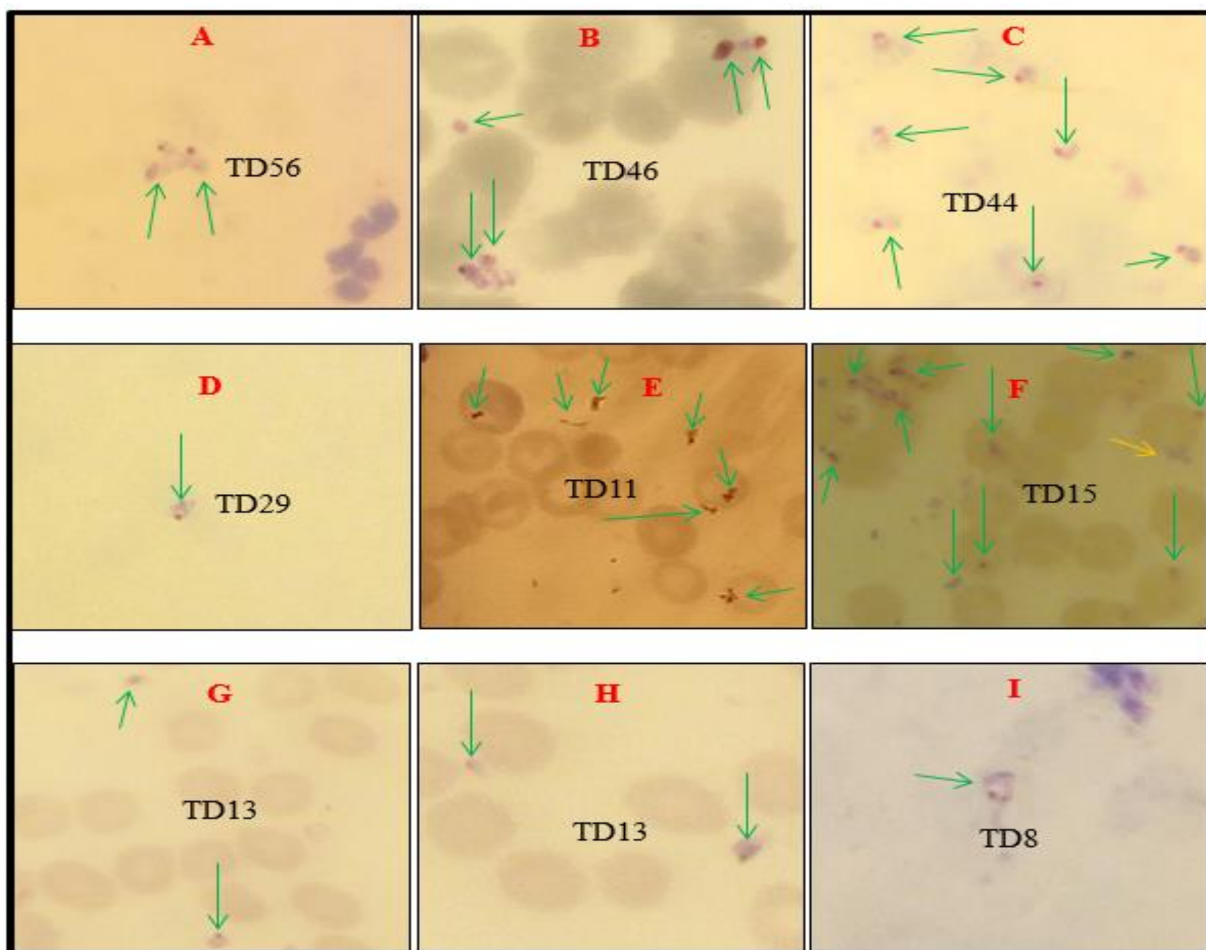

Figure 8: Thick and Thin Blood Films Revealing Intra-erythrocytic Parasites in Dogs.

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4.1.3 Molecular Detection of Babesia in Dogs

Polymerase Chain Reaction (PCR) amplified fourteen samples out of the thirty microscopically suspected *Babesia* cases as *Babesia canis*, representing 46.7%. Since the primers used were specifically for *Babesia canis* amplification, the number (14) amplified represented an overall *Babesia* canis positivity of 19.72% in the 71 dogs used for the current study. Visible DNA bands (which were in some instances not very clear) at size of 530bp were visualized for the *Babesia* positive samples after electrophoresis (Figure 9).

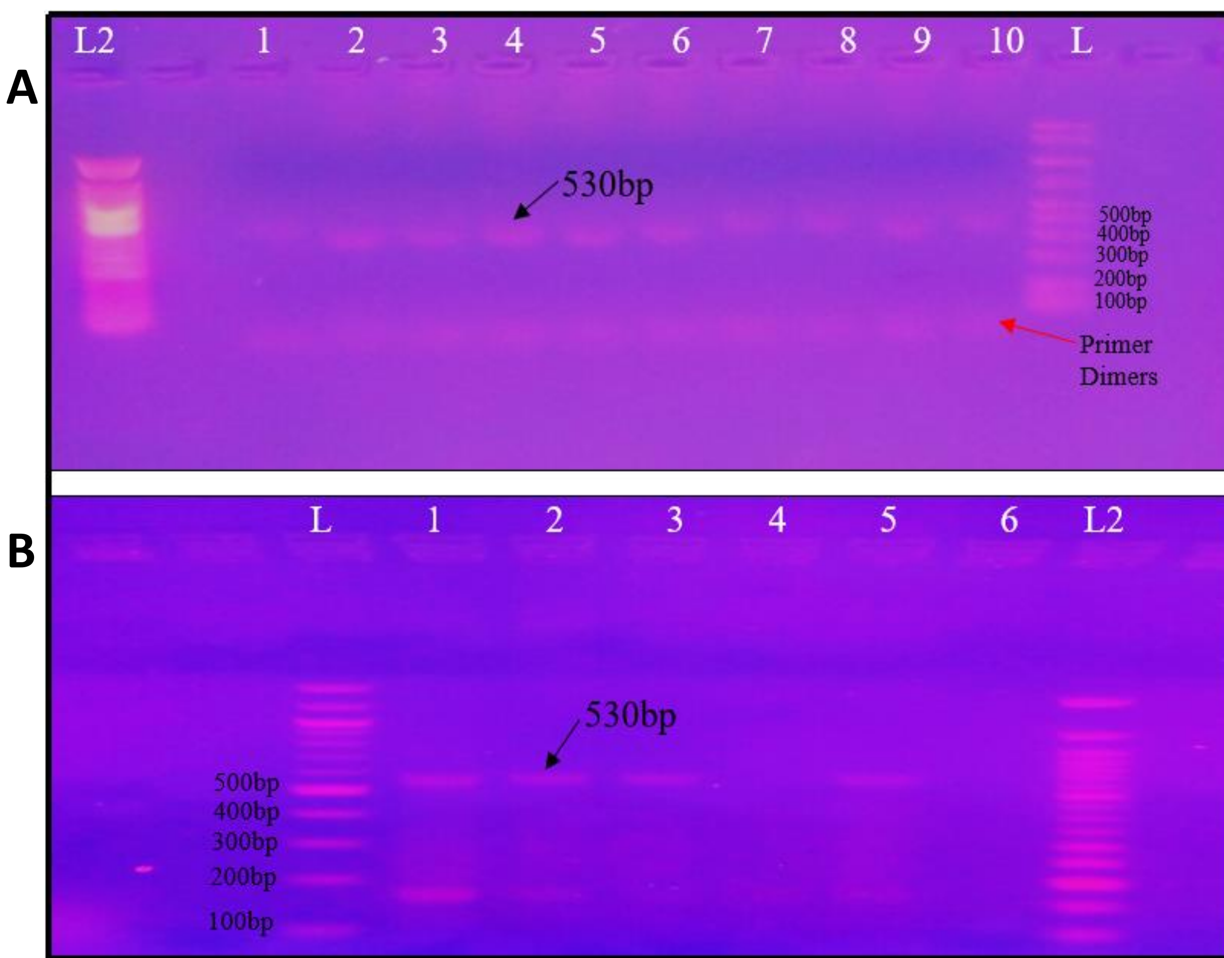

Figure 9: Samples of agarose gels showing PCR amplification for *Babesia canis* in Dogs. (**A**) Lanes 1 - 10 are dog samples amplified at a size of 530bp. (**B**) Lanes 1,2,3,5 are dog samples amplified at a size of 530bp whiles lanes 4 and 6 are samples that were not amplified. L and L2 represents 100bp and 50bp ladders respectively. Primer diamers were observed in the visualized gels.

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4.2 Results on Cattle samples

4.2.1 Demographic Data of Cattle and *Babesia/Theileria* Infection

Out of the total of 207 cattle used for the study, the number of adults (105) and juveniles (102) were almost evenly distributed with percentages of 50.7 and 49.3, respectively (Table 5**).** Microscopically, 18 (representing 54.5%) juvenile cattle were suspected of bovine Babesiosis/Theleiriosis compared to adults with 15 (45.5%). Statistically, there was significant association between age of cattle and *Babesia* infection by PCR (*P-value = 0.0355,* Table 5). Out of the thirty-three-microscopy suspected *Babesia/Theileria species*, twenty-one (64%, 21/33) were males whiles the rest were females (Table 5). PCR amplified 16 out of the 21 male cattle whiles 4 out of the 12 suspected female cattle were amplified. Meanwhile, there was no significant association between gender and *Babesia/Theileria* infection among the cattle (*P-value = 0.1368*).

In terms of colour description, 75% (15/20) of the samples what were amplified by PCR were from white-coloured cattle. Interestingly, there was a significant association between colour description and *Babesia/Theileria* infection among the cattle (*P-value = 0.004*).

It was noticed that, though most cattle showed signs and symptoms of weight loss 88 (43%, 88/207), those that were weak 75 (36%, 75/207) were more infected with *Babesia/Theileria species*. Fifteen (46%, 15/33) of cattle showing signs and symptoms of weakness were microscopically infected with *Babesia/Theileria species*, but 8 (24%, 8/33) of the emaciated (weight loss) cattle were found to also be infected (Table 5). Statistically, no significant association was observed between symptoms displayed and *Babesia/Theileria* infection (*P-value = 0.1935*).

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Table 5. Demographic characteristics of Cattle sampled and *Babesia/Theileria* infection

Parameter	No. Sampled, n (%)	Microscopically suspected			PCR Positive		
		Tech.	K'po	Total, n (%)	Tech.	K'po	Total, n (%)
Ages							
Adults	105 (50.7)	8	7	15 (45.5)	3	2	5 (25.0)
Juveniles	102 ((49.3)	9	9	18 (54.5)	11	4	15 (75.0)
						P-value	***0.0355***
Gender							
Males	123 (59.4)	13	8	21 (63.6)	11	5	16 (80.0)
Females	84 (40.6)	4	8	12 (36.4)	3	1	4 (20.0)
						P-value	***0.1368***
Description							
White	111 (53.6)	9	10	19 (57.6)	11	4	15 (75.0)
Black	19 (9.2)	0	2	2 (6.1)	1	0	1 (5.0)
Brown	23 (11.1)	1	2	3 (9.1)	1	1	2 (10.0)
Ash	5 (2.4)	1	0	1 (3.0)	0	0	0 (0.0)
Black/White	26(12.6)	2	0	2 (6.1)	0	0	0 (0.0)
Others	23 (11.1)	4	2	6 (18.2)	1	1	2 (10.0)
						P-value	***0.0004***
Symptoms							
Weight Loss	88 (42.5)	5	3	8 (24.2)	3	1	4 (20.0)
Weakness	75 (36.2)	7	8	15 45.5)	7	4	11 (55.0)
Anorexia	22 (10.6)	3	3	6 (18.2)	1	1	2 (10.0)
Dermatitis	16 (7.7)	1	2	3 (9.1)	1	0	1 (5.0)
Nil	6 (2.9)	1	0	7 (3.0)	2	0	2 (10.0)
						P-value	***0.1935***

4.2.2 Microscopic *Suspicion of Babesia/Theileria* Infection in Cattle

Microscopic examination revealed that 33 (15.9%) of the cattle were suspected of *Babesia/Theileria* infection. Trypanosoma species were also observed during the microscopic examination of cattle samples as shown by the blue arrows of Figure 10 I. Amongst the intra-erythrocytic parasites seen, more paired configurations were seen (Figure 10A, 10C, 10E, & 10H). No tetrad nor triad configurations were seen, but pyriform configuration was seen (Figure 10G).

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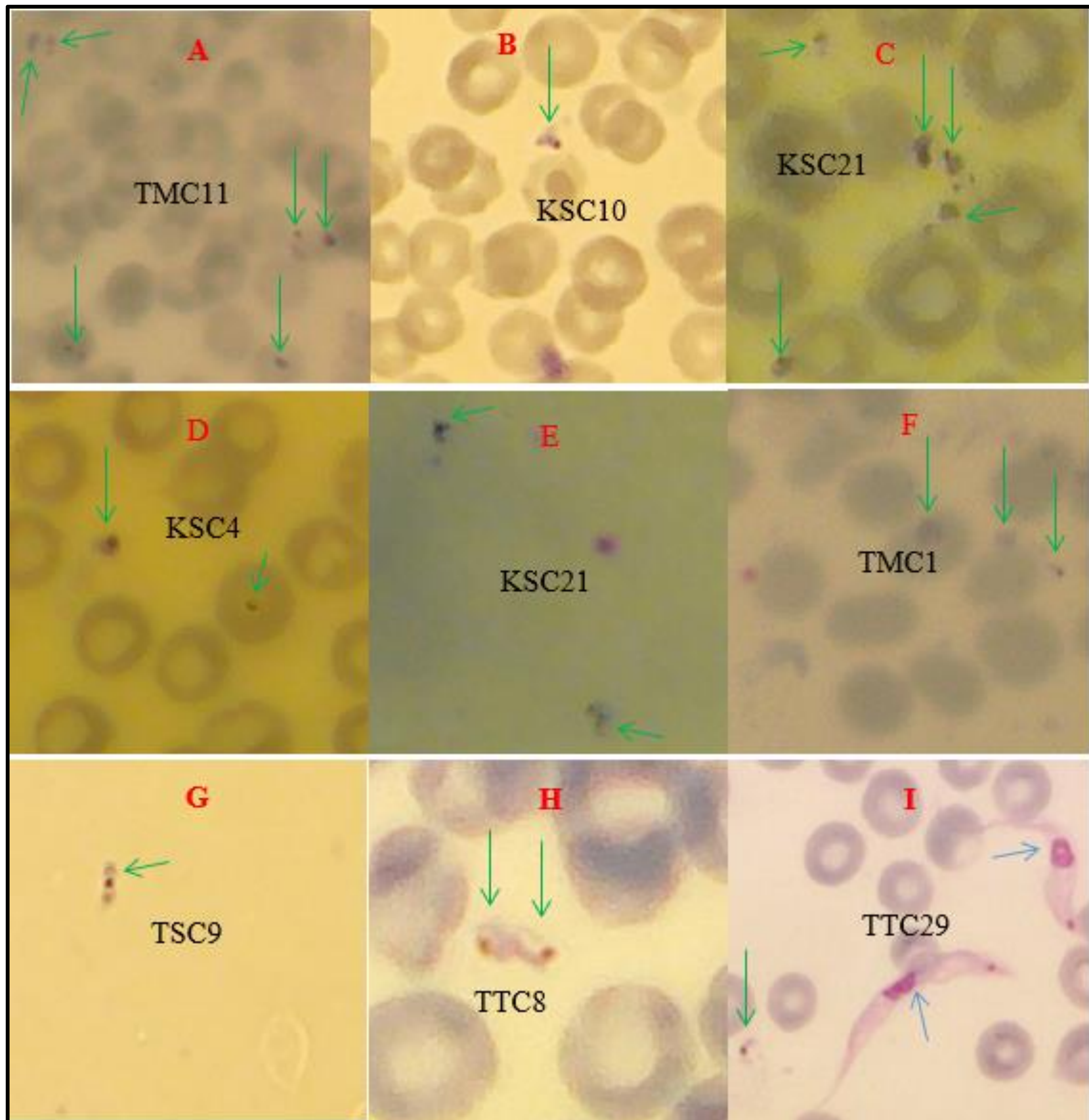

Figure 10: Stages of microscopic intra-erythrocytic parasites seen in thick and thin blood smears samples from Cattle. (Green Arrows show suspected *Babesia/Theileria* species, Sea Blue Arrows – *Trypanosoma* species)

4.2.5 Molecular Detection of Babesia/Theileria Infection in cattle

Twenty (20, 66.6%) out of the thirty-three (33) cattle samples that revealed intra-erythrocytic parasites and were suspected as *Babesia/Theileria* positives were found to be PCR positive. Very faint band of 430kb DNA size were observed in some cases, whiles in other cases, the bands were

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clearly visible (Figure 11 A). Sequencing results of the successfully amplified cattle samples revealed *Theileria velifera* isolates as the intra-erythrocytic parasites infecting the cattle (Figure 11 B).

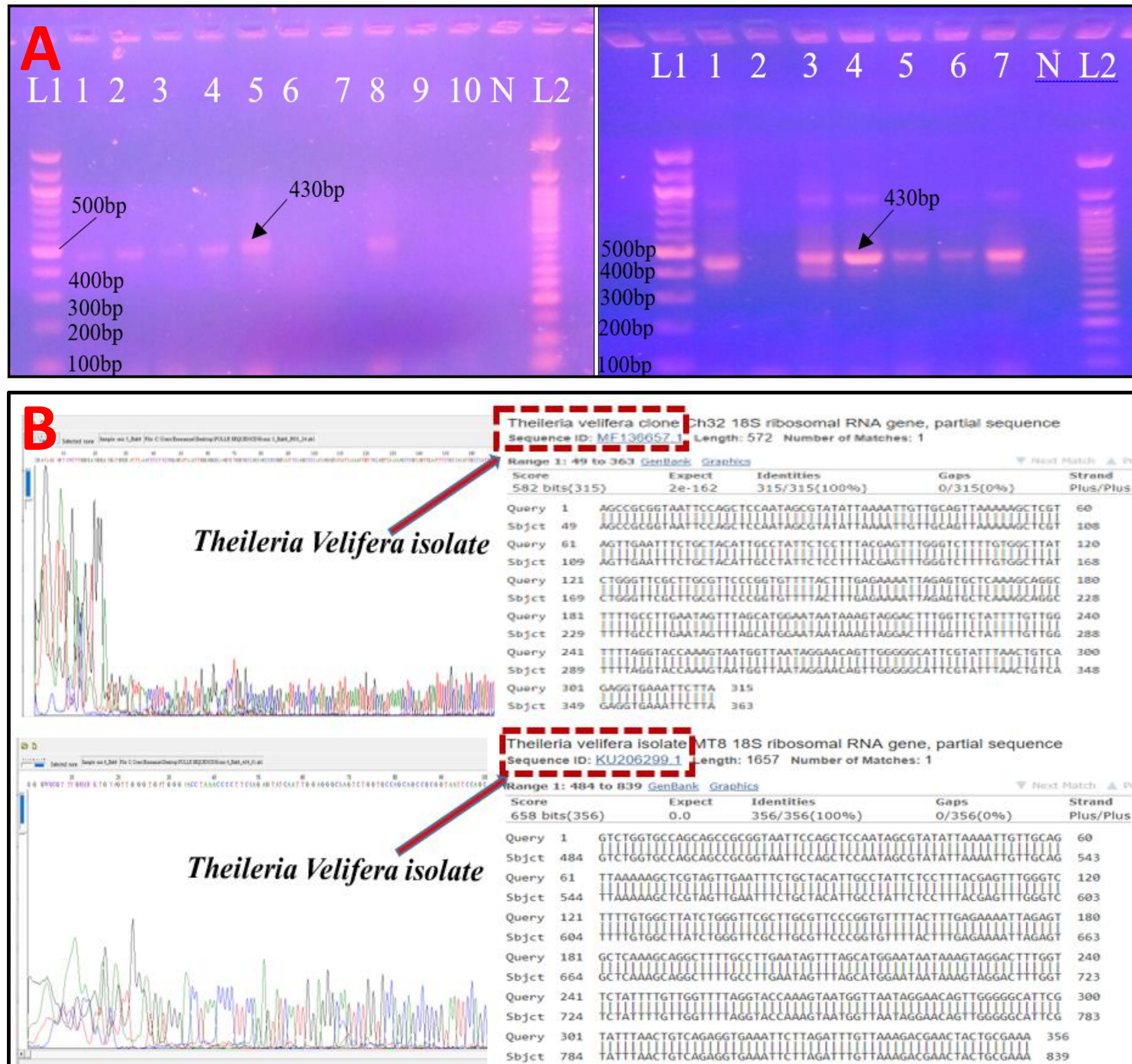

Figure 11: Molecular results of PCR and sequencing (A) Samples of agarose gels showing PCR amplification for *Babesia/Theieria* species in cattle. Lanes 1 - 10 are dog samples amplified at a size of 530bp. L and L2 represents 100bp and 50bp ladders respectively. Primer diamers were observed in the visualized gels

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4.3 Results on Human Samples

4.2.1 Demographic Data of Humans and *Babesia/Theileria* Infection

Most of the humans sampled from both the cases that showed *Plasmodium*-like parasites (34) and cattle rearers in the communities (31) were within the ages of below 10 years, followed by those in the age range of 11 – 20 years (Table 6). Meanwhile the number of microscopically suspected *Babesia/Theileria* cases was similar for age ranges of ≤10, 11-20 and 21-30 years, among the smear positives cases and the cattle rearers sampled (Table 6). There was a significant association between age and *Babesia/Theileria* infection (P-value = ***0.0124***)

Table 6: Demographic Data of Human samples and *Babesia/Theileria* infection

Parameters	SPMs n (%)	M. suspected n (%)	Cattle rearers n (%)	M. suspected n (%)	Total Humans n (%)	Total M. suspected n (%)
Age						
≤10 10	34 (35.8)	2(20.0)	31(33.7)	3(18.8)	65(34.8)	5(19.2)
11-20.	21(22.1)	2(20.0)	24(26.1)	3(18.8)	45(24.1)	5(19.2)
21-30	17(17.9)	2(20.0)	20(21.7)	3(18.8)	37(19.8)	5(19.2)
31-40	10(10.5)	1(10.0)	11(12.0)	3(18.8)	21(11.2)	4(15.3)
41-50	8(8.4)	3(30.0)	1(1.1)	0(0.0)	9(4.8)	3(11.5)
51-60	3(3.2)	0(0.0)	2(2.2)	2(12.5)	5(2.7)	2(7.7)
Above 60	2(2.1)	0(0.0)	3(3.3)	2(12.5)	5(2.7)	2(7.7)
					P-value	***0.0124***
Gender						
Males	31(32.6)	4(40.0)	63(68.5)	13(81.2)	94(50.3)	17(65.39)
Females	64(34.2)	6(60.0)	29(31.5)	3(18.8)	93(49.7)	9(34.62)
					P-value	***0.1455***
Study Sites						
Techiman	52(54.7)	8(80.0)	22(23.9)	5(31.2)	74(39.6)	13(50.0)
Kintampo	43(45.3)	2(20.0)	70(76.1)	11(68.8)	113(60.4)	13(50.0)
					P-value	***0.2228***

SPMs represents Smear Positive Malaria patients, % represent percentage, n represents number

In terms of gender, the total number of sampled male (50.3%) to female (49.7%) proportion was almost evenly distributed (**Table 6).** Meanwhile, 65.4% of the microscopically suspected *Babesia/Theileria* cases were males. Meanwhile, the association between gender and Babesia/Theileria

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infection in the humans was not statistically significant (P-value = ***0.1455*****).** Also, regarding location, the number of positivity at the two study sites (Techiman and Kintampo) was evenly distributed (Table 6). There was no significant association between location and *Babesia/Theileria* infection (P-value = ***0.2228*****)**

4.3.2 Microscopic Results of *Babesia species* in Humans

Twenty (20) representing 10.7% (20/187) of the humans were microscopically suspected (smear positive but *Plasmodium*- RDT negative) as *Babesia/Theileria* cases (Table 6). Stages of intra-erythrocytic parasites were found in thick and thin smears showing features such as tetrad configuration, triad configuration/Maltese-cross and paired Shaped Parasites (Figure 12).

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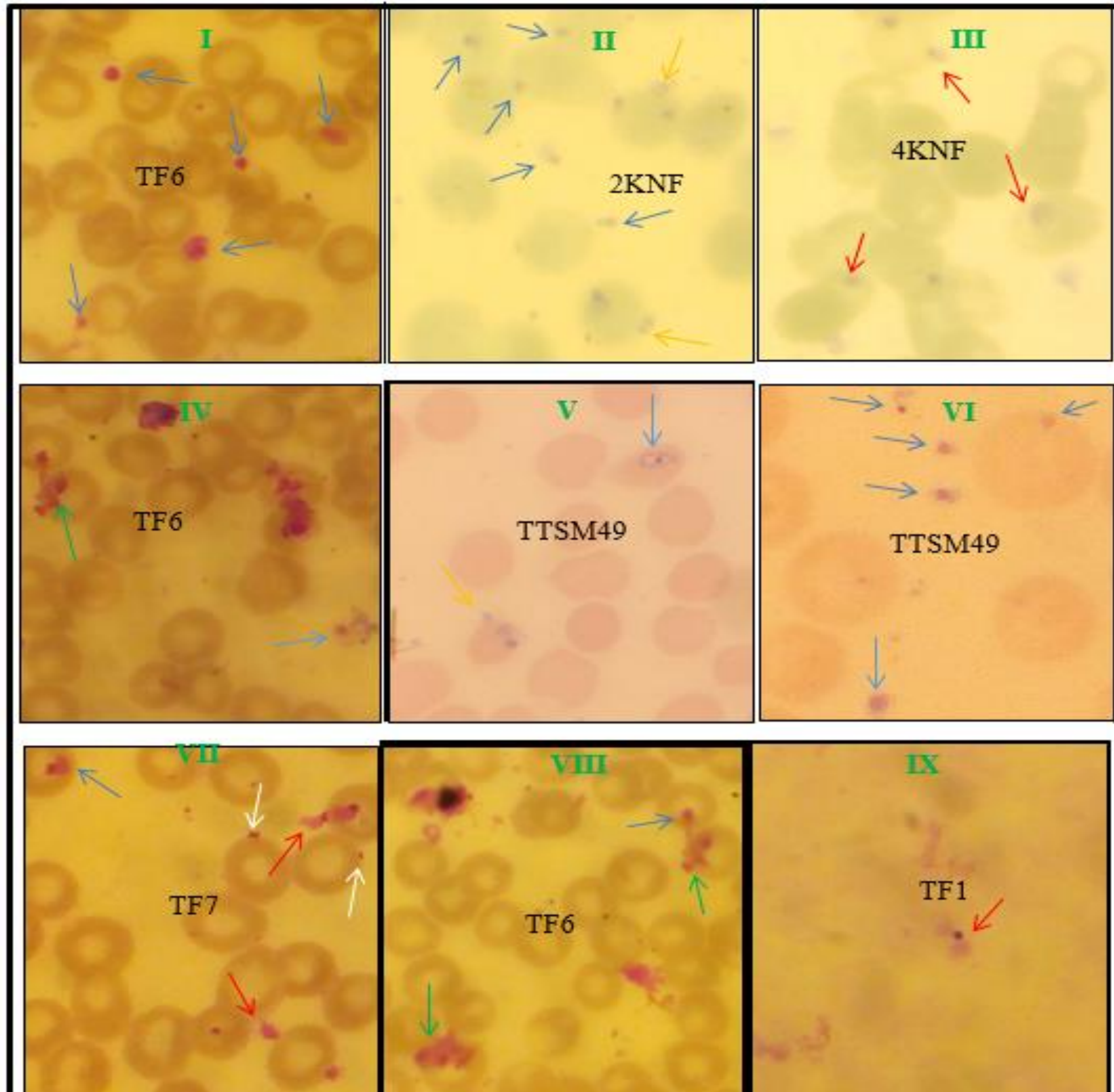

Figure 12. Blood Stages of Intra-erythrocytic parasites found in thick and thin smears of humans. (Green Arrow–Tetrad Parasites Configuration, Golden Arrow–Triad Configuration/Maltese-cross, Red Arrow–Pyriform/Paired Shaped Parasites, Sea Blue Arrows–Single Parasites, and White Arrow-Maltese-cross.)

4.3.3 Molecular Detection of Babesia/Theileria in Humans

Six (6) of out of the microscopically suspected *Babesia/Theileria* cases displayed a band size around 430pb after amplification, electrophoresis and visualization, hence they were considered PCR positive (Figure 13A). Four of the 6 positive samples qualified for sequencing and one was

successfully sequenced. Sequencing results confirmed that sample as *Theileria velifera* isolate (Figure 13B), same as what was observed from the sequencing results of the cattle,

Figure 13: Molecular results of humans. (A) Agarose gel showng amplified band and (B) Sequencing Results of *Babesia/Theileria* specie in Humans at the Middle Belt of Ghana. L1-Hundered base pair (100bp) ladder, L2-Fifty base pair (50bp) ladder.

4.4 The interaction between Humans and Animals (Dogs and cattle)

A close interaction was observed between humans and the animals (dogs and cattle). In the case of the dogs which mostly serve as the pets of the humans, they held them in their arms (Figure 14A) whiles in the case of the cattle the humans usually get very close when milking them (Figure 14B). Meanwhile, ticks that serve as a vector for transmitting the parasites were observed on the

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breast of some of the cattle (Figure 14B). Some of the cattle, especially the bulls, were used for farming whilst other times the humans ride them for fun (Figure 14B). It was also observed in the study that the cattle were mostly reared under poor conditions (Figure 14C).

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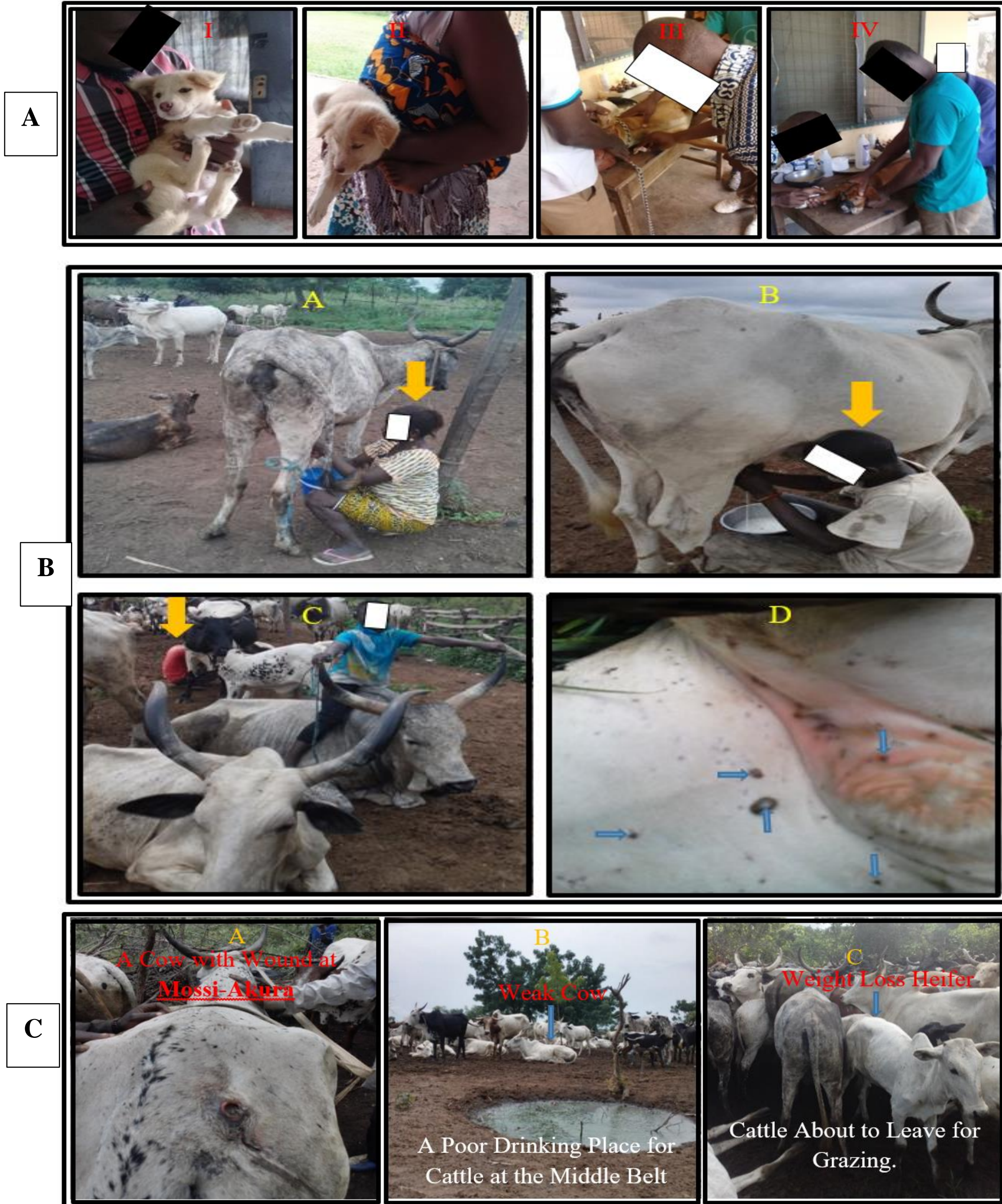

Figure 14: Close interaction between humans and anmals (A) owners carrying their dogs and veterinarians treating the dogs without wearing gloves (B) Cattle, golden arrows showing people milking cattle, Blue Arrows showing ticks around breasts of cattle. (C) Poor condition under which cattle were reared

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CHAPTER FIVE

5.0 DISCUSSION

5.1 Canine Babesiosis

The 42.3% microscopically suspected *Babesia* infection with PCR detection of 46.6% suggests the presence of Canine *Babesia* infection in the middle belt of Ghana; emphasizing its global distribution (Irwin, 2009; Solano - Gallego & Baneth, 2011). This observation agrees with Ćoralić *et al*., (2018) and Cannon *et al*., (2016) who through thin smears suspected *B. canis* in dogs using microscopy which was later confirmed by PCR. The microscopically suspected samples which were not amplified by PCR could be attributed to two reasons; firstly, the intra-erythrocytic parasites seen in those cases could be other piroplamsms (suchs as *Cytauxzoon*) which could not be amplified with the *Babesia canis* primers. Secondly it could be other *Babesia* species (such as *Babesia gibsoni*) which cannot be amplified by the primers specific for *Babesia canis* (Anderson *et al*., 1979; Apanaskevich *et al*., 2007).

Meanwhile, Babesiosis in dogs have mostly been observed by microscopy (Schetters *et al*., 1997; Zahler *et al*., 1998; Irwin, 2009; Solano-Gallego & Baneth, 2011) based on the morphological appearances of the parasites in the RBCs. Microscopic observation of intraerythrocytic forms have earlier led to the description of *B. canis* as "large *Babesia*" (Uilenberg *et al*., 1989; Uilenberg, 2006) compared to smaller ones such as *B. gibsoni* (Anderson *et al*., 1979; Apanaskevich *et al*., 2007). Therefore, microscopy examination of a typical pear-shaped large intraerythrocytic parasite inside of the RBCs led to the suspicion of canine babesiosis in the dog samples, similar to what was done by Salem & Farag, (2014).

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The current study therefore emphasizes the suggestion that the infection is in Ghana and could be transmitted from animals (such as dogs) to humans. El-Bahnasawy *et al.*, (2011) reported on a case of Babesiosis in an Egyptian boy which was suspected to have been from his pet dog. Therefore, it was very necessary that dogs were included in the current study, particularly because of the close interaction between humans and dogs as compared to cattle.

Most of the *Babesia* detected among the sampled dogs falling within the age group of ≤6 months could be attributed to the reason that, more dogs in that age group were sick and have been brought to the veterinary clinics at the period of collecting the dog samples. This could be because, the older dogs might have developed some immunity or most of them were asymptomatic due to their immuno-competence, therefore they were not at the veterinary clinics during the sampling. In a study which looked at occurrence of *Babesia canis vogeli* in Egyptian dogs, in terms of age, the dogs were grouped into < 3 years, 3 – 5 years and > than 5 years. Most of the positive cases were within the age group of 3–5 yrs, followed by the age group of less than 3 years. Therefore, similar to the current study, the lower age groups recorded high number of positivity. On the contrary, it was observed in a study which used mice to specifically find the link between age and ones being infected with *Babesia* that, older mice were more susceptible to being infected with *B. microti* (Vannier *et al*., 2004). All the same, age has been established as a risk factor for most infections, and canine Babesiosis is no exception (Krause, 2002).

Chauvin *et al*., (2009) realized that, there were low parasitemia in older dogs that had *Babesia* for a longer period of months or years. This normally results in the *Babesia species* adapting to the host's environment, thereby helping it to survive for a longer period; in this case they become asymptomatic carriers to transmit *Babesia species* to immunocompromised individuals

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Regarding gender, more females were infected with *Babesia* than male dogs in the current study (although not statistically significant at a P-value of 0.2833); a finding that contradicts what was reported by Salem & Farag, (2014) and Nalubamba *et al*., (2015), where more *Babesia* were found in the male dogs than in the females. Meanwhile, Martinod *et al*., (1986) found no difference in sex susceptibility between males and females. In the study by Salem & Farag (2014), the ratio of positivity in terms of females and males was 1:3. The aggressiveness and hormonal status of male dogs may be a contributory factor for this observation which was contrary to what was found out in this study (van Zyl, 1995).

From this project, higher temperature was shown to have a link with *Babesia infection* in the dogs, and this agrees with many projects in which changes in body's temperature was revealed to be associated with morbidities like Babesiosis/Theileriosis. Even though there was not statistically significant association between *Babesia* infection and temperature in the current study, more dogs were within the temperature of 35.0 - 40.0°C whiles more positive cases were observed in the temperature range of 40.1-45.1°C, agreeing with 107°F (41.67°C) observed by El-Deeb & Younis, (2009). Similarly, Cunha *et al* (2015) reported that five out of the ten hospitalized adults with babesiosis had temperatures of greater than 39°C. This means that high body temperature may be indicative of *Babesia/Theileria* infection as in the case of malaria. This agrees with literature where high body or too cold body temperatures are considered among the sign and symptom of most diseases like babesiosis/theileriosis and malaria (James, 2015).

In the current study, fever was the most common symptom observed among the dogs which were found to be infected with *Babesia* with a percentage of fifty-six (56%), which is lower, in comparion with the 87.3% observed by Nalubamba *et al*., (2015). Similarly, the percentage of infected dogs showing symptoms of anorexia was lower (23.3%) when compared to the 65.3% identified

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in the infected dogs from the study by Nalubamba *et al*., 2015. Anorexia has been reported as a frequently found symptom caused by *B. canis* (Taboada & Merchant, 1991).

As in Cook *et al.*, (2018), PCR detection of *Babesia canis* in 46.6% of the microscopically suspected samples was a confirmatory procedure in the current study since *Babesia canis* specific primers were used, unlike the cattle and human samples where the primers used could amplify some *Babesia* and *Theileria* species. Same primers have been used to amplify *Babesia canis* earlier in Accra, Ghana (Owusu, 2015). Elsewhere, various researchers have confirmed canine *Babesia*osis with the use of *Babesia* specific primers such as what was used in this study (Sobczyk *et al*., 2000; 2005; Ionita *et al*., 2012; Andersson *et al*., 2017).

A close interaction between sick dogs and their owners or veterinarians observed during the study suggests that dog owners and caretakers would need to put in measures to control ticks on the dogs, which could go a long way to help both the dogs and the owners/caretakers. Direct contact with dogs (especially ticks' infested ones) should be discouraged to some extent in order to reduce chances of being bitten by ticks, which serve as vectors of human pathogens that cause *Babesiosis*, spotted fever and Q-fever. This possibility has been established in Mexico, by Osorno *et al*., (1976) where they identified 38 asymptomatic humans out of 101 carry antibodies against *Babesia canis*, indicative of an infection at one time or the other. A possible reason could be that, these individuals were immuno-competent, however, the story might not be same for immuno-compromised persons.

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5.2 Bovine Babesiosis/Theileriosis

The housing system of cattle in Ghana (where several cattle share the same kraal) could explain the observation of microscopically suspected *Babesia*/*Theileria spp*. in 15.9% of the cattle sampled. Therefore, until a cow among the group is carrying an infected tick, it is difficult for the other cattle to get bitten and get infected.

In this study, a high percentage (60.61%, 20/33) of the microscopically suspected *Babesia*/*Theileria* positive samples were amplified by PCR, implying that the "catch-all" primers (primers that amplify some *Babesia* and *Theileria*) increases the probability of picking a suspected *Babesia* or *Theileria* than a *Babesia* specific primer (as used for the dog samples in the current study). This ability of the "catch-all" primers was demonstrated by Silva *et al*., (2010) where 11.5% of their samples were PCR positive for *Babesia/Theileria* when "catch-all" primers were used; however, all the samples were negative when *Babesia* genus-specific primers were used. A similar study conducted by Owusu (2015) in Accra, using the same "catch-all" primers had a high (90%) percentage of microscopically suspected *Babesia* cattle being confirmed as PCR positives.

Among the PCR positive samples, sequenced result of those that qualified showed that the amplified samples were *Theileria velifera* and that is not surprising since T. velifera is among the species that can be amplified by the "catch-all" primers. Also, *Theileria velifera* has earlier been reported in Ghana by Bell-Sakyi *et al*., (2004) in a study that examined Giemsa-stained thin blood smears prepared monthly from cattle, sheep and goats in the Greater Accra region of Ghana between the period of May 1994 and December 1996 for presence of tick-borne hemoparasites. Among cattle, mixed infection of *Theileria, Babesia* species as well as Anaplasma marginale has also been reported by Beckley, (2013) in Ghana. Tanzania is among the few African countries that have reported Theileriosis caused by *T. velifera* in cattle (Uilenberg and Schreuder, 1976). The other cattle

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samples that were negative for *Babesia/Theileria* with PCR using the "catch-all" primers, though showed intra-erythrocytic parasites might have been infected with other piroplasms that are not able to be amplified by the primers used.

Theileria velifera is mainly located in Africa and its association with cattle has earlier been reported and categorized among the species of *Theileria* that causes benign theileriosis (Gubbels *et al*., 1999; Adjou Moumouni *et al*., 2015). All the same this species need to be given attention since *T. orientalis* which was normally associated with benign theileriosis in some parts of the world is now causing outbreaks and has now been considered to be pathogenic (Hammer *et al*., 2015; Kolte *et al*., 2017; Aparna *et al*., 2011; George *et al*., 2015; Kakati *et al*., 2015; Vinodkumar *et al*., 2016).

The most widespread and malignant *Theileria* specie is *Theileria annulata*, causing tropical theileriosis, which occurs around the Mediterranean basin, in the Middle East, and in Southern Asia. The other malignant *Theileria* specie is *T. parva*, which occurs in East and Southern Africa and causes East Coast fever. Bovine babesiosis is caused by *Babesia bovis* and *B. bigemina*, both of which occur worldwide in tropical and subtropical regions. *B. divergens* occurs in cattle in Europe and extends into North Africa (Bouattour and Darghouth, 1996). Evidently, tick-borne protozoan diseases (e.g., theileriosis and babesiosis) pose important problems for the health and management of domestic cattle in the tropics and subtropics (Jongejan and Uilenberg, 1994).

Microscopically, more male cattle were suspected with *Babesia/Theileria species* than the females; an observation which agrees with the findings from a study by Choramo & Ibrahim, (2017) who identified more (16.6%) *Babesia* spp in male cattle than females (10.5%).

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Age has been shown to be linked with *Babesia* infection (Behera, 2016; Owusu, 2015; Chauvin *et al*., 2009) and this was emphasized in the current study where more juveniles (young) cattle were microscopically suspected of *Babesia/Theileria species*. This observation could be attributed to the reason that the older cattle might have stronger immune system compared to the younger ones or might have attained adaptive immunity due to previous exposure to the infection, as in the case of the T-helper immune response in the mice study (Djokic *et al*., 2018). In an attempt to explain how age is a contributory factor to immunity, Giefing-Kröll *et al* (2015) suggested that, during aging, interventions that specifically use the changing levels of individual's hormones normally provide strong alternatives to maintain ideal immune responses.

In terms of signs and symptoms, similar to Taboada & Merchant (1991), the current study identified anorexia as the most prevalent symptom among the *Babesia/Theileria* positive cattle, followed by weakness and weight loss. This finding was also in accordance with Choramo & Ibrahim (2017). Taboada & Merchant (1991) revealed an association between *Babesia* infection and signs and symptoms like pallor (anemia), fever, anorexia, depression, and splenomegaly. Also, Behera (2016) noticed anorexia as one of the signs and symptoms of babesiosis in cattle.

The significant association between color description and *Babesia/Theileria* infection, where *Babesia* infection was detected in more of the white-colored cattle supports the report by Stjernberg & Berglund (2005) that, ticks are more attracted to light colored clothing compared do dark ones.

For the cattle samples, *Trypanosoma species* were microscopically detected, mostly in the adult cattle emphasizing that Trypanosomosis is one of the most important diseases restricting livestock development in Africa (Panin & Mahabile, 1997). Bovine Trypanosomiasis have been reported in

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other African countries including Nigeria (Ameen *et al*., 2008; Ahmed & Agbede, 1993) which share a lot of activities with Ghana, including animal agriculture. Earlier in Ghana, Nakayima *et al*., (2012), showed that there is a high prevalence of *Trypanosoma* parasites in Ghana, when they conducted molecular epidemiological studies on animal trypanosomiases, using tsetse flies, pigs and cattle blood samples collected from Adidome and Koforidua environs in Ghana in the year 2010. Also, the occurrence of bovine Trypanosomosis has been reported in two districts (Savelugu and West Mamprusi) of Northern Ghana with different land use and environmental characteristics (Mahama *et al*., 2004). Therefore, the environmental characteristics of the current study might have made it possible for the survival of vectors of the *Trypanosoma* parasites which in turn infect the cattle.

5.3 Human Babesiosis/Theileriosis

Human Babesiosis has been mainly associated with *B. microti* (Abdullah *et al*., 2018; Abdella & Jilo, 2016; Vannier & Krause, 2009). *Babesia* microti has sometimes been referred to as *Theileria microti* because of its close relatedness to *Theileria microti* (even more than other *Babesia* species). Some schoolers have therefore sometimes joined the two names as *Babesia*/*Theileria* (El-Ashker *et al*., 2015; Zanet *et al*., 2014) and this approach was adapted in the current study.

The amplification of 30% of the suspected *Babesia*/*Theileria* by PCR is a very important outcome since human Babesiosis/Theileriosis can be considered an important emerging disease capable of posing a public health problem, especially in connection with malaria diagnosis and management (Akel & Mobarakai, 2017; Demessie & Derso, 2015; Yabsley & Shock, 2013). Unlike Theileriosis, Babesiosis continue to emerge in various countries (Scott, 2017; Huang *et al*., 2018). In 2017, *B. duncani* was detected in a patient in Canada for the first time using serology and molecular tests

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(Scott, 2017). A female patient, aged 60, from a province in Southeast China was also confirmed for *Babesia species* in May 2017 at Yuying Children's Hospital of Wenzhou Medical University (Huang *et al*., 2018). Meanwhile, there is scanty report on human Theileriosis worldwide. Therefore, outcome of the current study is notable since it confirmed human theileriosis for the first time in Ghana. Another worth mentioning fact is that, human theileriosis caused by *Theileria velifera* has never been reported, thus this is the first report of human theileriosis caused by *Theileria velifera*, worldwide. Interestingly the same species of *Theileria* (velifera) was confirmed in the cattle.

The human *Theileria* case in this study was that of a 34 years old male smear positive malaria case. He confirmed living in a cattle rearing community where he has at some point in time been involved in taking care of the cattle. He remembered being bitten by ticks from the cattle on a number of occasions.

This case highlights the zoonotic nature of *Babesia*/*Theileria* (Lempereur *et al*., 2017; Homer *et al*., 2000), thus he might have gotten infected through a thick bite during the period when he took part in taking care of the cattle. Although it has been a long time since that happened, this is so a possibility since *Theileria* has been identified to mostly present asymptomatically (Cushing & Shaz, 2012; Owusu, 2015; Kitt *et al*., 2016). PCR for *Babesia/Theileria* which was positive in three (3) of the cattle rearers who were not sick during sampling in this study also confirms the assertion that healthy people can be asymptomatic carriers of these piroplasms without knowing (Krause *et al*., 1996; Allred, 2003).

Also, infection with *Babesia/Theileria* normally results in disease with signs and symptoms (clinical manifestations) which might vary with respect to the different *Babesia/Theileria species* and strains responsible for the infection, as well as their specific virulence. The variations in these

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manifestations may also be due to factors which are determinants of the host's response to infection like age and the person's state of immunity (Birkenheuer *et al*., 1999; Jacobson, 2006; Irwin, 2009). This makes the identification of human infected with *Theileria* in the study more worrying, since the infected person could end up infecting others in case he happens to donate blood for those individuals. If they happen to be immune compromised, it could be detrimental to them.

Most of the humans infected with *Babesia*/*Theileria* being children agrees with others studies where it was noticed that majority of humans sampled and those infected were children (Birkenheuer *et al*., 1999; Jacobson, 2006; Irwin, 2009). Reason for this observation could be that younger persons are more prone to infections for which *Babesiosis/Theileriosis* is not an exemption. Vannier *et al*., (2008) also stated that although the majority of Babesiosis/Theileriosis cases are reported in adults, there is evidence that the disease is more common in children than is currently reported, and the current study has highlighted this assertion.

These non-malaria patients and cattle rearers who were suspected through microscopy (*Plasmodium*-like parasites but *Plasmodium* RDT negative) could also be carriers of other piroplasms like *Anaplasma* or *Borrelia species*, some of which could be a co-infection. It also agrees with the literature that, a single tick bite can transmit multiple pathogens (Dunn *et al*., 2014; Rizzoli *et al*., 2014; Moutailler *et al*., 2016). Though the study by Owusu in 2015 did not confirm human babesiosis in six (6) of them were suspected of having intra-erythrocytic parasites that were not *Plasmodium species* (Owusu, 2015). Likewise, even though the current study did not also confirm human Babesiosis, Human theileriosis caused by *Theileria* velifera has been confirmed.

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CHAPTER SIX

6.0 CONCULUSION AND RECOMMENDATIONS

6.1: CONCULUSION

In this study *B. canis* was detected in 14 (14/30, 46.6%) dogs by PCR, whiles *Babesia/Theileria species* were detected in 20 (20/33, 60.61%) cattle and 6 (30%, 6/20) humans. The amplified cattle and human samples were confirmed as *Theileria velifera* after sequencing. This imply that, *Babesia/Theileria* infection (*Babesia canis* in dogs, *T. velifera* in cattle and humans) is in circulation among both animals and humans in the middle belt of Ghana and must be given critical attention, in view of the connection with malaria misdiagnosis and inappropriate management of *Babesia/Theileria* infected individuals, who might have been taken as unresponsive malaria patients. This is the first report of human *Theileria* infection in Ghana, and human theileriosis caused by *Theileria velifera*, worldwide.

6.2: RECOMMENDATIONS

1. Samples should be collected at other locations of the country for the detection of *Babesia/Theileria species*.
2. Other tests that can confirm the presence of other piroplasms should be included in subsequent projects.
3. Clinicians should request further investigations (especially *Babesia/Theileria*) in malaria patients who may not respond to treatments.

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REFERENCES

Abdela, N., Ibrahim, N., Begna, F. (2018). Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia. *Acta Trop*. **177**: 9-18.

Abdella, N., & Jilo, K. (2016). Bovine Babesiosis and its Current Status in Ethiopia: A Systemic Review. Advances in Biological Research. 10 (3): 138-146. doi: 10.5829/idosi.abr.2016.10.3.10398.

Abdullah, S., Helps, C., Tasker, S., Newbury, H., & Wall, R. (2018). Prevalence and distribution of Borrelia and *Babesia* species in ticks feeding on dogs in the U.K. *Medical and Veterinary Entomology*. **32**: 14-22. doi: 10.1111/mve.12257.

Adaszek, L., Obara-Galek, J., Piech, T., Winiarczyk, M., Kalinowski, M., & Winiarczyk, S. (2016). Possible vertical transmission of *Babesia canis canis* from a bitch to her puppies: a case report. *Vet Med – Czech*. **61**: 263-266.

Adjou Moumouni, P., F., Aboge, G., O., Terkawi, M., A., Masatani, T., Cao, S., Kamyingkird, K., Jirapattharasate, C., Zhou, M., Wang, G., Liu, M., Iguchi, A., Vudriko, P., Ybanez, A., P., Inokuma, H., Shirafuji-Umemiya, R., Suzuki, H., & Xuan, X. (2015). Molecular detection and characterization of *Babesia* bovis, *Babesia* bigemina, *Theileria* species and Anaplasma marginale isolated from cattle in Kenya. *Parasites & Vectors*. **8**:496. Doi 10.1186/s13071-015-1106-9.

Adzigbe, J. (2017). Molecular Characterization of Tick-Borne Parasites in Naturally Infected Cattle in Accra and Adidome, Ghana. University of Ghana. Retrieved from https:// ugspace.ug.edu.gh on 25th May 2018 at 11:15AM.

Akel, T., & Mobarakai, N. (2017). Hematologic manifestations of babesiosis. *Ann Clin Microbiol Antimicrob*. **16**(1): 6. doi: 10.1186/s12941-017-0179-z.

Akoolo, L., Schlachter, S., Khan, R., Alter, L., Rojtman, A., D., Gedroic, K., *et al*., (2017). A novel quantitative PCR detects *Babesia* infection in patients not identified by currently

University of Ghana http://ugspace.ug.edu.gh

available non-nucleic acid amplification tests. *BMC Microbiol*. **17**:16. Doi:10.1186/s12866-017-0929-2.

Allred, D., R. (2003). Babesiosis: persistence in the face of adversity. *Trends Parasitol*. **19**(2): 51-55.

Allsopp, M., T., E., P., & Allsopp, B., A. (2006). Molecular sequence evidence for the reclassification of some *Babesia* species. *Ann N Y Acad Sci*. **1081**: 509-517.

Alvarado-Rybak, M., Solano-Gallego, L., & Millan, J., A. (2016). Review of piroplasmid infections in wild carnivores worldwide: importance for domestic animal health and wildlife conservation. *Parasit Vectors*. **9**: 538.

Ahmed, M., I., & Agbede, R., I., S. (1993). A three-year retrospective study of the prevalence of trypanosomes infection in cattle, sheep and goats in Zaria, Northern Nigeria. OAU/STRC Publication No. 116, Nairobi, Kenya.

Ameen, S., A., Joshua, R., A., Adedeji, O., S., Raheem, A., K., Akingbade, A., A., & Leigh, O., O. (2008). Preliminary studies on prevalence of Ruminant Trypanosomosis in Ogbomoso area of Oyo State, Nigeria. Middle East Journal of Scientific Research. 3(4): 214-218.

Anderson, J., F., Magnarelli, L., A., Donner, C., S., & Spielman, A. (1979). Canine Babesia new to North America. *Science*. **204**(4400): 1431-1432. doi: 10.1126/science.451574.

Andersson, M., O., Tolf, C., Tamba, P., Stefanache, M., Waldenström, J., Dobler, G., & Chitimia-Dobler, L. (2017). Canine tick-borne diseases in pet dogs from Romania. *Parasites & Vectors*. **10**:155. Doi 10.1186/s13071-017-2092-x.

Apanaskevich, D., A., Horak, I., G., & Gamica, J., L. (2007). Redescription of Haemaphysalis (Rhipistoma) elliptica (Koch, 1844), an old taxon of the Haemaphysalis (Rhipistoma) leachi group from East and Southern Africa, and of Haemaphysalis (Rhipistoma) leachi (Audouin, 1826) (Ixodida, Ixodidae). Onderstepoort *J vet Res*. **74**: 181-208.

Aparna, M., Ravindran, R., Vimalkumar, M., B., Lakshmanan, B., Rameshkumar, P., Kumar, K., G., Promod, K., Ajithkumar, S., Ravishankar, C., Devada, K., Subramanian, H.,

University of Ghana http://ugspace.ug.edu.gh

George, A., J., & Ghosh, S. (2011). Molecular characterization of *Theileria* orientalis causing fatal infection in crossbred adult bovines of South India. *Parasitol Int*. **60**(4): 524-529. doi: 10.1016/j.parint.2011.08.002.

Ascencio, M., E., Florin-Christensen, M., Mamoun, C., B., Weir, W., Shiels, B., & Schnittger, L. (2018). Cysteine Proteinase C1A Paralog Profiles Correspond with Phylogenetic Lineages of Pathogenic Piroplasmids. *Vet. Sci*. **5**:41. doi:10.3390/vetsci5020041.

Babes, V. (1888). Sur I'hemoglobinuric bacterienne boeuf. *Compt Rend Acad Sci*. **107**: 692-694.

Babesiosis Fact Sheet: Babesiosis. Last Reviewed: July, 2017. Retrieved from https://www.health.ny.gov/diseases/communicable/babesiosis/fact_sheet.htm. Dtae retrieved: 15th September 2017 at 3:18PM.

Baneth, G. (2018). Antiprotozoal treatment of canine babesiosis. *Veterinary Parasitology*. **254**: 58-63.

Baneth, G., Florin-Christensen, M., Cardoso, L., & Schnittger, L. (2015). Reclassification of *Theileria annae as Babesia vulpes* sp. nov. *Parasit Vectors*. **8**: 207.

Bastian, S., Jouglin, M., Brisseau, N., Malandrin, L., Klegou, G., L'Hostis, M., & Chauvin, A. (2012). Antibody prevalence and molecular identification of *Babesia* spp. in Roe deer in France. *J Wildl Dis*. **48**(2): 416-424.

Bednarska, M., Bajer, A., Drozdowska, A., Mierzejewska, EJ., Tolkacz, K., & Welc-Faleciak, R. (2015). Vertical transmission of *Babesia* microti in BALB/c mice: *preliminary report*. PLoS One. **10**(9): e0137731. doi.10.1371/journal.pone.0137731.

Beck, R., Vojta, L., Mrljak, V., Marinculic, A., Beck, A., Zivicnjak, T., & Cacciò, S., M. (2009). 'Diversity of *Babesia* and *Theileria* species in symptomatic and asymptomatic dogs in Croation'. *International Journal of Parasitology*. **39:** 843-848. Doi.org/10.1016/j.ijpara.2008.12.005.

University of Ghana http://ugspace.ug.edu.gh

Beckley, C., S., K. (2013). Susceptibility of indigenous cattle breeds to co-infection with multiple tick-borne pathogens. University of Ghana. p. XV. http://ugspace.ug.edu.gh.

Behera, S., C. (2016). Haematological and biochemical alteration in theileriosis affected cattle: A Thesis to The Orissa University of Agriculture and Technology iPartial Fulfilment of the Requirement for the Degree of Master of Veterinary Science in Veterinary Pathology. Bhubaneswar-751003.

Bell-Sakyi, L., Koney, E., B., M., Dogbey, O., & Walker, A., R. (2004). Incidence and prevalence of tick-borne hemoparasites in domestic ruminants in Ghana. *Vet. Parasitol*. **124**(1-2): 25-42.

Bettencourt, A., França, C., & Borges, I. (1907). Addendum, â nota sôbre piroplasmose dô gamo. *Revta Med. Vet*., Lisb. **6**, 37-40.

Bigdeli, M., Rafie, S., M., Namavari, M., M., *et al*., (2012). Report of *Theileria* annulata and *Babesia* canis in dogs. *Comp Clin Pathol*. **21**(3): 375-377. https://doi.org/10.1007/s005580-012-1505-7.

Bilgic, H., B., Karagenc, T., Simuunza, M., Shiels, B., Tait, A., Eren, H., & Weir. (2012). Development of a multiplex PCR assay for simultaneous detection of *Theileria annulata, Babesia bovis* and *Anaplasma marginale* in cattle. *Experimental Parasitology*. **133**: 222-229.

Birkenheuer, A., J., Correa, M., T., Levy, M., G., Breitschwerdt, E., B. (2005). Geographic distribution of babesiosis among dogs in the United State and Association with dog bites: 150 cases (2000-2003). *J Am Vet Med Assoc***. 227**: 942-947.

Birkenheuer, A., J., Horney, B., Bailey, M., Scott, M., Sherbert, B., Catto, V., Marr, H., S., Camacho, A., T., Ballman, A., E. (2010). *Babesia* microti-like infections are prevalent in North American foxes. *Vet Parasitol*. **172:** 179-182.

University of Ghana http://ugspace.ug.edu.gh

Birkenheuer, A., J., Levy, M., G., Savary, K., C., Gager, R., B., & Breitschwerd, E., B. (1999). Babesia gibsoni infections in dogs from North Carolina. *J Am Anim Hosp Assoc*. **35**(2): 125-128.

Birkenheuer, A., J., Neel, J., Ruslander, D., Levy, M., G., & Breitschwerdt, E., B. (2004). Detection and molecular characterization of a novel large *Babesia* species in a dog. Vet *Parasitol.* **124**: 151-160.

Birkenheuer, A., J., LEVY, M. G., & Breitschwerdt, E. B. (2004b): Efficacy of combined atavaquone and azithromycin for therapy of chronic *Babesia gibsoni* (Asian genotype) infections in dogs. *J. Vet. Intern. Med*. **18:** 494-498.

Bishop, R., Musoke, A., Morzaria, S., Gardner, M., Nene, V. (2004). Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitology*. **129:** S271-S283.

Bock, R., Jackson, L., de Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*. **129(**Suppl): S247–269.

Boozer, A., L., & Macintire, D., K. (2003). Canine babesiosis. *Vet Clin North Am Small Anim Pract*. **33**: 885-904.

Bouattour, A., Darghouth, M., A. (1996). First report of *Babesia divergens* in Tunisia. *Vet Parasitol*. **63**:161–165.

Bush JB, Isaäcson M, Mohamed AS, Potgieter FT, de Waal DT. (1990). Human babesiosis--a preliminary report of 2 suspected cases in South Africa. S Afr Med J. 78(11):699.

Camacho, A., T., Guitian, J., Pallas, E., & Gestal, J., J. (2005). Theileria (Babesia) equi and Babesia caballi Infections in Horses in Galicia, Spain. Trop Animal Health and Production. **37**(4): 293-302. doi: 10.1007/s11250-005-5691-z.

University of Ghana http://ugspace.ug.edu.gh

Cannon, S., H., Levy, J., K., Kirk, S., K., Crawford, P., C., Leutenegger, C., M., Shuster, J., J., Liu, J., & Chandrashekar, R. (2016). Infectious diseases in dogs recued during dog fighting investigations. *Vet J*. **211**: 64-69.

Centeno-Lima, S., Do Rosário, V., Parreira, R., Maia, A., J., Freudenthal, A., M., Nijhof, A., M., & Jongejan, F. (2003). A fata case of human babesiosis in Portugal: molecular and phylogenetic analysis. *Trop Med Int Health*. **8**(8): 760-764.

Centers for Disease Control and Prevention, CDC., (2012). Babesiosis – 2012 Data & Statistics. Retrieved from https://www.cdc.gov/parasites/babesiosis/data-statistics/2012.html on 25th July 2017 at 5:15PM.

Centers for Disease Control and Prevention, CDC., (2015). Life cycle of *Babesia* (*B. microti*). Retrieved from https://www.cdc.gov/parasite/babesiosis/biology.html on 19th December 2016 at 8:15PM.

Chauvin, A., Moreau, E., Bonnet, S., Plantard, O., & Malandrin, L. (2009). *Babesia* and its hosts: Adaptation to long-lasting interactions as a way to achieve efficient transmission. Veterinary Research. **40:**37. https://doi.org/10.0151/vetre/2009020.

Checa, R., Montoya, A., Ortega, N., González-Fraga, J., L., Bartolomé, A., Gálvez, R., Marino, V., Miró, G. (2017). Efficacy, safety and tolerance of imidocarb dipropionate versus atovaquone or buparvaquone plus azithromycin used to treat sick dogs naturally infected with *Babesia* microti-like piroplasm. *Parasit Vectors*. **10**: 145.

Chen, Z., Liu, Q., Liu, J., Q., Xu, B., L., Lv, S., Xia, S., & Zhou, X., N. (2014). Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. Parasite vectors. **22**: 1-8.

Choidioni, P. L., Conlon, C. P., Hutchinson, D. B., Farquhar, J. A., Hall, A. P., Peto, T. E., Birley, H., *Ogo et al: A review of Canine Babesiosis* and WARRELL, D. A. (1995): Evaluation of atovaquone in the treatment of patients with uncomplicated *Plasmodium falciparum* malaria. *J. Antimicrob. Chemother.* **36**: 1073-1078.

University of Ghana http://ugspace.ug.edu.gh

Chomel, B. (2011). Tickborne infections in dogs – an emerging infectious threat. *Veterinary Parasitology* 179: 294-301. https://dx.doi.org/10.1016/j.vetpar.2011.03.040.

Choramo, A., & Ibrahim, N. (2017). Prevalence of Babesiosis in Cattle in and Around Jimma Town, OromiaRegion, South Western Ethiopia. *Acta Parasitological Globalis*. **8**(2): 96-100.

Clancey, N., Horney, B., Burton, S., & Birkenheuer, A. (2010). Babesia (Theileria) annae in a red Fox (Vulpes vulpes) from Prince Edward Island, Canada. Journal of wildlife diseases. **46**(2): 615-621. doi: 10.7589/0090-3558-46.2.615.

Clark, I., A., & Jacobson, L., S. (1998). Do babesiosis and malaria share a common disease process? *Ann of Trop Med and Parasitol*. **92**(4): 483-488.

Cook, K., L., Frenzer, P., Tucker, S., J., Crawford, P., C., Kirk, S., K., & Levy, J., K. (2018). Rapid Diagnosis of *Babesia* gibsoni by Point-of-Need Testing by Insulated Isothermal PCR in Dogs at High Risk of Infection. *J Vet Intern Med*. **32**: 232-235.

Ćoralić, A., Gabrielli, S., Zahirović, A., Stojanović, N. M., Milardi, G., L., Jažić, A., Zuko, A., Čamo, D., & Otašević, Z. (2018). First molecular detection of *Babesia canis* in dogs from Bosnia and Herzegovina. Ticks and Tick-borne Diseases. **9**: 363-368.

Criado, A., Martinez, J., Buling, A., Barba, J., C., Merino, S., Jefferies, R., & Irwin, P., J. (2006). New data on epizootiology and genetics of piroplasms based on sequences of small ribosomal subunit and cytochrome b gene. Vet Parasitol. **142**: 238-247. doi: 10.1016/j.vetpar.2006.07.004.

Criado-Fornelio, A., Gónzalez-del-Río, M., A., Buling-Saraῇa, A., Barba-Carretero, J., C. (2003c). Molecular characterization of a *Babesia* gibsoni isolate from a Spanish dog. *Vet. Parasitol*. **117**: 123-129.

Criado-Fornelio, A., Gónzalez-del-Río, M., A., Buling-Saraña, A., & Barba-Carretero, J., C. (2004). The “expanding universe” of piroplasms. *Vet Parasitol*. **119**: 337-345.

University of Ghana http://ugspace.ug.edu.gh

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A., & Barba-Carretero, J., C. (2003). Molecular studies on *Babesia*, *Theileria* and *Hepatozoon* in southern Europe. Part II. Phylogenetic analysis and evolutionary history. *Vet Parasitol*. **114**: 173-194.

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraῆa, A., Barba-Carretero, J., C. (2003a). Molecular studies on *Babesia, Theileria* and *Hepatozoon* in southern Europe Part I. Epizootiological aspects. *Vet. Parasitol*. **113**: 189-201.

Cunha, B., A., Raza, M., & Schmidt, A. (2015). Highly Elevated Serum Ferritin Levels Are a Diagnostic Marker in Babesiosis. *Clinical Infectious Diseases*. **60(5)**:827-829. doi: 10.1093/cid/ciu960.

Cushing, M., & Shaz, B. (2012). Transfusion-transmitted babesiosis: achieving successful mitigation while balancing cost and donor loss. *Transfusion*. **52**: 1404-1407. doi: 10.1111/j.1537-2995.2012.03746.x.

Demessie, Y., & Derso, S. (2015). Tick Borne Hemoparasitic Disease of Ruminants: A review. *Advance in Biological Research***. 9**(4): 210-224.

Diuk-Wasser, M., A., Liu, Y., Steeves, T., K., Folson-O'Keefe, C., Dardick., K., R., Lepore, T., Bent, S., J., Usmani-Brown, S., Telford III, S., R., Fish, D., & Krause, P. (2014). Monitoring Human Babesiosis Emergence through Vector Surveillance, New England, USA. *Emerg. Infect. Dis*. **20**(2): 225-229. www.cdc.gov/eid...

Dixit, P., Dixit, A., K., & Varshney, J., P. (2010). Evidence of new pathogenic *Theileria* species in dogs. *J Parasit Dis*. 34(1): 29-32. doi: 10.1007/s12639-010-0009-0.

Djokic, V., Akoolo, L., & Parveen, N. (2018). *Babesia* microti Infection Changes Host Spleen Architecture and is Cleared by a Th1 Immune Response. *Frot. Microbiol***. 9**:85. doi: 10.3389/fmicb.2018.00085.

University of Ghana http://ugspace.ug.edu.gh

Dunn, J., M., Krause, P., J., Davis, S., Vannier, E., G., Fitzpatrick, M., C., Rollend, L., Belperron, A., A., States, S., L., Stacey, A., Bockenstedt, L., K., Fish, D., & Diuk-Wasser, M., A. (2014). Borrelia burgdorferi promotes the establishment of *Babesia* microti in the northern United States. PLOS ONE 9:e115494. doi: 10.1371/journal.pone.0115494.

Eamens, G., J., Gonsalves, J., R., Jenkins, C., & Bailey, G. (2013). *Theileria* orientalis MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. *Vet Parasitol*. **191**(3-4): 209-217.

Eaton, P. (1934). *Piroplasma canis* in Florida*. J Parasitol*. **20**: 312-313.

Ecija, Philippines using Nested Polymerase Chain Reaction. *Annals of Parasitol*. **63**(4): 309-316. Doi: 10.17420/ap6304.117.

El-Ashker, M., Hotzel, H., Gwida, M., El-Beskawy, M., Silaghi, C., & Tomaso, H. (2015). Molecular biological identification of *Babesia***,** *Theileria***,** and *Anaplasma* species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray *Vet. Parasitol*., **207**(3-4):329-334. doi: 10.1016/j.vetpar.2014.12.025.

El-Bahnasawy, M., M., Khalil, H., H., & Morsy, T., A. (2011). Babesiosis in an Egyptian boy acquired from pet dog, and a general review. *J Egypt Soc Parasitol*. **41**(1): 99-108.

El-Deeb, W., M., & Younis, E., E. (2009). Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular orientation to oxidative stress and ketosis relationship. *Vet Parasitol*. **164**: 301-305. doi: 10.1016/j.vetpar.2009.06.002.

El Moghazy, H., Ebied, M., H., Abdelwahab, M., G., & El-Sayed, A., A., A. (2014). Epidemiological studies on Bovine Babesiosis And Theileriosis in Qalubia Governorate. Benha Veterinary Medical Journal. **27**(1): 36-48.

Esernio-Jenssen, D., Scimeca, P., G., Benach, J., L., Tenenbaum, M., J. (1987). Transplacental/perinatal babesiosis. *J Pediatr*. **110**(4): 570-572.

University of Ghana http://ugspace.ug.edu.gh

Fang, D., C., & Jeffrey, M. (2016). Transfusion-Transmitted *Babesia microti*. *Transfusion Med. Rev*. **30**(3): 132 - 138.

FDA. (2014). Transfusion/Donation Fatalities: Fatalities Reported to FDA Following Blood Collection and Transfusion: Annual Summary of Fiscal Year 2014 (Report). Archived from the original on 6 September 2015. Retrieved from https://www.fda.gov/biologicsbloodvaccines/safetyavailability/reportaproblem/transfusiondonationfatalities/default.htm on 16th July 2018 at 9:10PM.

FDA. (2015). Fatalities Reported to FDA Following Blood Collection and Transfusion: Annual Summary of Fiscal Year 2015. Retrieved from https://www.fda.gov/biologicsbloodvaccines/safetyavailability/reportaproblem/transfusiondonationfatalities/default.htm on 16th July 2018 at 9:10PM.

Feachem, R., G., Phillips, A., A., Hwang, J., Cotter, C., Wielgosz, B., Greenwood, B., M., Sabot, O., Rodriguez, M., H., Abeyasinghe, R., R., Ghebreyesus, T., A., & Snow, R., W. (2010). Shrinking the malaria map: progress and prospects. Lancet. **376**: 1566–1578. doi: 10.1016/S0140-6736(10)61270-6.

Fox, LM., Wingerter, S., Ahmed, A., Arnold, A., Chou, J., Rhein, L., & Lew, O. (2006). Neonatal babesiosis case report and review of literature. *Pediatr Infect Dis J*. **25**: 169-173.

Fritz, D. (2010). A PCR study of piroplasms in 166dogs and 111 horses in France (March 2006 to March 2008). *Parasitol Res*. **106**: 1339-1342.

Fukumoto, S., Suzuki, H., Igarashi, I., & Xuan, X. (2005). Fatal experimental transplacental *Babesia gibsoni* infections in dogs. *Int J Parasitol*. **35**(9): 1031-5.

Galuppi, R., Aureli, S., Bonoli, C., Caffara, M., & Tampieri, M., P. (2011). Detection and molecular characterization of *Theileria* sp. in fallow deer (Dama dama) and ticks from an Italian natural preserve. *Res Vet Sci***. 91**: 110-115.

University of Ghana http://ugspace.ug.edu.gh

Ganga, N., Ananda, K., J., & Kavitha Rani, B. (2010). Theileriosis in calves and its successful treatment. Veterinary World. **3**(4): 191. Retrieved from www.veterinaryworld.org/vol.3/April/Theileriosis...on 20th July 2018 at 5:20PM.

Garcia, A., T., C. (2006). 'Piroplasma infection in dogs in northern Spai'. *Vet Parasitol*. **138**: 97-102. http://dx.doi.org/10.1016/j.vetpar.2006.01.043.

George, N., Bhandari, V., Reddy, D., P., & Sharma, P. (2015). Emergence of new genotype and diversity of *Theileria* orientalis parasites from bovines in India. *Infect. Genet. Evol*. **1972**(36): 27-34.

Genda, J., Negron, E. A., Lotfipour, M., Balabhadra, S., Desai, D. S., Craft, D. W., & Katzman, M. (2016). Severe *Babesia* microti infection in an immunocompetent host in Pennsylvania. *J. Investig. Med. High Impact Case Rep*. **4**(3):2324709616663774. doi: 10.1177/2324709616663774.

Ghana Statistical Service (2014). Municipal Analytical Report, Techiman South and Kintampo Municipalities – Brong Ahafo Region, 2010 Population and Housing Census. Retrieved from Ghana Statistical Service, 2014. Date retrived: 02/06/2018 at 12:25PM.

Gholamreza, S., Somaieh, M., Roya, S., Alireza, B., Ghazale, A., & Yasin, B. (2017). First detection of Babesia ovis in Dermacentor spp. in Ardabil area, northwest of Iran. J Vector Borne Dis. **54**(3): 277-281.

Giefing-Kröll, C., Berger, P., Lepperdinger, G., & Grubeck-Loebenstein. (2015). Review: How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell*. **2015**(14): 309-321. Doi: 10.1111/acel.12326.

Gray, J., Zintl, A., Hildebrandt, A., Hunfeld, K., & Weiss, L. (2010). Ticks and Tickborne Diseases Zoonotic babesiosis: Overview of the disease and novel aspects of pathogen identity. *Ticks and Tick-Borne Diseases,* **1**(1), 3–10. http://doi.org/10.1016/j.ttbdis.2009.11.003.

University of Ghana http://ugspace.ug.edu.gh

Gubbels, J., M., de Vos, A., P., van der Weide, M., Viseras, J., Schouls, L., M., de Vries, E., & Jongejan, F. (1999). Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J. Clin. Microbiol*. **37**: 1782-1789.

Gul, N., Ayaz, S., Gul., I., Adnan, M., & ulakbar, N. (2015). Tropical Theileriosis and East Coast Fever in Cattle: Present, Past and Future Perspective. *International Journal Current Microbiol and Applied Science***. 4**(8): 1000-1018.

Gunes, V., Onmaz, A., C., Keles, I., Varol, K., & Ekinci, G. (2017). The diagnostic importance of coagulation parameters in cattle having natural theileriosis. *Polish Journal of Veterinary Sciences*. **20**(2): 369-376. doi: 10.1515/pjvs-2017-0045.

Gupta, A., Gupta, K., Leishangthem, G., D., Bal, M., S., Sood, N., K., & Singh, Amarjit. (2017). Molecular and pathological studies on natural cases of bovine theileriosis. *J Parait Dis*. **41**(1): 211-218. Doi 10.1007/s12639-016-0781-6.

Gupta, S., K., Yadav, A., Raina, A., K., & Rajiv, S. (2004). Theileriosis in a seven days old bovine calf – A case report. *Indian J. Vet*. Med. **24**: 55.

Hammer, J., F., Emery, D., Bogema, D., R., & Jenkins, C. (2015). Detection of Theileria orientalis genotypes in Haemaphysalis longicornis ticks from southern Australia. *Parasit Vectors*. **8**: 229. doi: 10.1186/s13071-015-0839-9.

Hassan, M., A., Liu, J., Sajid, M., S., Rashid, M., Mahmood, A., Abbas, Q., Guan, G., Yin, H., & Luo, J. (2018). Simultaneous detection of *Theileria annulata* and *Theileria orientalis* infections using recombinase polymerase amplification. *Ticks Tick Borne Dis*. **9**(4): 1002-1005. doi: 10.1016/j.ttbdis.2018.03.028.

Herrera, P., Ch., T., Viloria, V., V., Balbin, M., M., & Mingala, C., N. (2017). Prevalence of Babesiosis (*Babesia bovis* and *Babesia bigemina*) in cattle and water buffalo in Nueva

University of Ghana http://ugspace.ug.edu.gh

Ecija, Philippines using Nested Polymerase Chain Reaction. Annals of Parasitology. **63**(4): 309-316. doi: 10.17420/ap6304.117.

Herwaldt, B., L., & Alanta, G., A. (2012). Babesiosis Surveillance – 18 States, 2011. *Centers for Disease Control and Prevention*. **61**(27): 505-509.

Herwaldt, B., L., Linder, J., V., Bosserman, E., Young, C., Olkoweska, D., & Wilson, M. (2011). Transfusion-associated babesiosis in the United States: a description of case. *Ann Intern Med*. **55**: 509-519.

Hasheminasab, S., S., Moradi, P., & Wright, I. (2018). A four-year epidemiological and chemotherapy survey of babesiosis and theileriosis, and tick vectors in sheep, cattle and goats in Dehgolan, Iran. *Anns Parasitol*. **2018**(1): 1-1. https://www.researchgate.net/publication/...

Hildenwall, H., Amos, B., Mtove, G., Muro, F., Cederlund, K., & Reyburn, H. (2016). Causes of non-malarial febrile illness in outpatients in Tanzania. *Tropical Medicine and International Health*. **21**(1): 149-156. doi:10.1111/tmi.12635.

Homer, M., J. Aguilar-Delfin, I., Telford III, S., R., Krause, P., J., & Persing, D., H. (2000). Babesiosis. *Clinical Microbiology Review*. **13**(3): 451-469.

https://igenex.com/ticktalk/2018/01/11/babesiosis-... (2018). Babesiosis: Type of Tick Bite Diseases. IGeneX Tick Talk. Date retrieved: 11th January 2018 at 4:22PM.

Huang, S., Zhang, L., Yao, L., Li, J., Chen, H., Ni, Q., Pan, C., & Jin, L. (2018). Human babesiosis in Southeast China: A case report. *Int J Infect Dis*. **68**: 36-38. Doi: 10.1016/j.ijid.2018.01.004.

Human Babesiosis, 2018. Retrieved from labs.russell.wisc.edu/Wisconsin-ticks/babesiosis/… on 25th July 2018 at 10:05AM.

University of Ghana http://ugspace.ug.edu.gh

Hunfeld, K., Hildebrandt, A., & Gray, J. (2008). Babesiosis: Recent insights into an ancient disease. *Int J Parasitol*. **38**: 1219-1237.

Hutcheon, D. (1893). Diseases amongst dogs. Malignant jaundice or bilious fever of the dog. *Agric J Cape Good Hope*. **6**: 476-477.

Ionita, M., Mitrea, I., L., Pfister, K., Hamel, D., Buzatu, C., M., & Silaghi, C. Canine babesiosis in Romania due to *Babesia* canis and *Babesia* vogeli: a molecular approach. Parasitol Res. **110**: 1659-1664.

Irwin, P., J. (2009). Canine babesiosis: from molecular taxonomy to control. *Parasit Vectors*. **2**(1): S4.

Jacobson, L., S. (2006). The South African form of severe and complicated canine babesiosis: clinical advances 1994-2004. Vet. Parasitol. **138**: 126-139.

James, S. (2015). Advanced 2015 Babesia Care: Profound Testing Defects and Preventing Disability and Death. **239**: 263-0133. Retrieved from www.personalconsult.com/posts/advanced-2015-babesia-care.html, 25th May 2017, 3:15PM.

Jane, E., S., & Adam, J., B. (2014) Babesiosis: Canine and Feline Infectious Diseases. *Elsevier*. **1**: 727-738.

Jithendran, K., P. (1997). Blood Protista of cattle and buffaloes in Kangra valley, Himachal Pradesh. *Indian J Anim. Sci*. **67**: 207-208.

Jongejan, F., & Uilenberg G. (1994). Ticks and control methods. *Rev Sci Tech Off Int Epizoot*. **13**:1201–1226

Joseph, J., T., Purtill, K., Wong, S., J., Munoz, J., Teal, A., Madison-Antenucci, S., Horowitz, H., W., Aguero-Rosenfeld, M., E., Moore, J., M., Abramowsky, C., & Wormser, C., P. (2012). Vertical Transmission of *Babesia microti*, United States. Emerging Infectious Diseases. **18**(8).

University of Ghana http://ugspace.ug.edu.gh

Karbowiak, G., Majlathova, V., Hapunik, J., Petko, B., & Wita, I. (2010). Apicomplexan parasites of red foxes (Vulpes vulpes) in northeastern Poland. *Acta Parasitol*. **55**: 210-214.

Kakati, P., Sarmah, P., C., Ray, D., Bhattacharjee, K., Sharma, R., K., Barkalita, L., M., Sarma, D., K., Baishya, B., C., Borah, P., & Stanley, B. Emergence of oriental theileriosis in cattle and its transmission through Rhipicephalus (Boophilus) microplus in Assam, India. *Vet World.* **8**(9): 1099-1104.

Kaleibar, M., T., Helan, J., A., & Fathi, E. (2014). Occurrence of congenital cerebral theileriosis in a newborn twin Holstein calves in Iran: Case report. *Veterinary Research Forum*. **5**(3): 237-241.

Kappmeyer, L., S., Thiagarajan, M., Herndon, D., R., Ramsay, J., D., Caler, E., Djikeng, A., Gillespie, J., J., Lau, A., O., Roalson, E., H., Silva, J., C., Silva, M., G., Suarez, C., E., Ueti, M., W., Nene, V., M., Mealey, R., H., Knowles, D., P., & Brayton, K., A. (2012). Comparative genomic analysis and phylogenetic position of *Theileria* equi. *BMC Genomics*. **13**: 603. doi: 10.1186/1471-2164-13-603.

Kamau, J., de Vos, A., J., Playford, M., Salim, B., Kinyanjui, P., & Sugimoto, C. (2011). Emergence of new types of *Theileria* orientalis in Australian cattle and possible cause of theileriosis outbreak. *Parasit Vectors*. **4**: 22.

Kim, J., Cho, S., Joo, H., Tsuji, M., Cho, S., Park, I., Chung, G., Ju, J., Cheun, H., Lee, H., Lee, Y., & Kim, T. (2007). First Case of Human Babesiosis in Korea: Detection and Characterization of a Novel Type of *Babesia* sp. (KO1) Similar to Ovine *Babesia*. *Journal of Clinical Microbiology*, **45**(6), 2084–2087. http://doi.org/10.1128/JCM.01334-06.

Kitt, E., Keaton, A., A., & Graf, E., H. (2016). The brief case: probable transfusion-transmitted babesiosis in a transplant patient. *J. Clin. Microbiol*. **54**: 2632-2634. 10.1128/JCM.00981-16.

University of Ghana http://ugspace.ug.edu.gh

Kjemtrup, A., M., & Conrad, P., A. (2006). A review of the small canine piroplasms from California: *Babesia conradae* in the literature. *Vet Parasitol*. **138**: 112-117.

Kjemtrup, A., M., Wainwright, K., Miller, M., Penzhom, B., L., & Carreno, R., A. (2006). *Babesia conradae,* sp. Nov., a small canine *Babesia* identified in California. *Vet Parasitol*. **138**: 103-111.

Knapp, K. L., & Rice, N. A. (2015). Human coinfection with *Borrelia burgdoferi* and *Babesia microti* in the United States. *J. Parasitol. Res*. **2015**:587131. doi:10.1155/2015/587131.

Koch, R. (1898). Reisebericht tiber Rinderpest. Bubonenpest in Indien und Afrika, Tsetse-oder Surrakrankheit, Texasfieber, Tropische Malaria, Schwarzwasserfieber. Springer, Berlin.

Kohli, S., Atheya, U., K., & Tnapiiyai, A. (2014). Prevalence of theileriosis incross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun District, Uttarakhand, India. Veterinary World. EISSN: 2231-0916.

Kolte, S., W., Larcombe, S., D., Jadhao, S., G., Magar, S., P., Warthi, G., Kurkure, N., V., Glass, E., J., & Shiels, B., R. (2017). PCR diagnosis of tick-borne pathogens in Maharashtra state, India indicates fitness cost associated with carrier infections is greater for crossbreed than native cattle breeds. PLOS One. 12(3): e0174595. doi: 10.1371/journal.pone.0174595.

Krause, P. J., Leopore, T., Sikand, V. K., Gadbaw, J., Burke, G., Telford, S. R., Brassard, P., Pearl, D., Azlanzadeh, J., Christianson, D., McGrath, D., & Spielman, A. (2000). Atovaquone and Azithromycin for the Treatment of Babesiosis. *New England Journal of Medicine*, 343:1454-1458.

Krause, P., J. (2002). Babesiosis. *Med Clin North Am*. **86:**361-373.

University of Ghana http://ugspace.ug.edu.gh

Krause, P., J., Daily, J., Telford, S. R., Vannier, E., Lantos, P., Spielman, A. (2007). Shared features in the pathology of babesiosis and malaria. *Trends in Parasitol*. **23**(12): 605-610. http://doi.org/10.1016/j.pt.2007.09.005.

Krause, P. J., Telford III, S., Spielman, A., Ryan, R., Magera, J., Rajan, T. V., Christianson, D., Alberghini, T. V., Bow, L., & Persing, D. (1996). Comparison of PCR with Blood Smear and Inoculation of Small Animals for Diagnosis of *Babesia microti* Parasitemia. *Journal of Clinical Microbiology,* **34**(11), 2791–2794.

Krause, P., J., Telford, S., R., 3rd., Spielman, A., Sikand, V., Ryan, R., Christianson, D., D., Burke, G., Brassard, P., Pollack, R., Peck, J., & Persing, D., H. (1996). Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. **275**: 1657-1660.

Kumar, S., Kumar, R., & Sugimoto, C. (2009). A perspective on Theileria equi infections in donkeys. *Japanese Journal of Veterinary research*. **56** (4): 171-180.

Kubelová, M., Mazancová, J., & Široký, P. (2012). Theileria, *Babesia*, and Anaplasma detected by PCR in ruminant of herds at Bié Province, Angola. *Parasite*. **19**: 417-422.

Kunimoto, D., Krause, K. & Morrison, D. (1998). First Case of (imported) Babesiosis Diagnosed in Canada. *Canadian Journal of Infectious Disease***. 9**(6): 387-389.

Leiby, D., A. (2011). Transfusion-transmitted *Babesia* spp.: bull-eye on *Babesia* microti. *Clin Microbiol Rev*. **24:** 14-28.

Lempereur, L., Beck, R., Fonseca, I., Marques, C., Duarte, A., Santos, M., Zúquete, S., Gomes, J., Walder, G., Domingos, A., Antunes, S., Baneth, G., Silaghi, C., Holman, P., Zintl, A. (2017). Guidelines for the Detection of *Babesia* and *Theileria* Parasites. *Vector Borne Zoonotic Dis*. **17**(1): 51-65. doi: 10.1089/vbz.2016.1955.

University of Ghana http://ugspace.ug.edu.gh

Linden, J., V., Prusinski, M., A., Crowder, L., A., Tonnetti, L., Stramer, S., L., Kessler, D., A., White, J., Shaz, B. & Olkowska, D. (2018). Transfusion-transmitted and community acquired babesiosis in New York, 2004 to 2015. *Transfusion*. **58**(3): 660-668.

Lorusso, L., Wijnveld, M., Majekodunmi, A., O., Dongkum, C., Fajinmi, A., Dogo, A., G., Thrusfield, M., Mugenyi, A., Vaumourin, E., Igweh, A., C., Jongejan, F., Welburn, S., C., & Picozzi, K. (2016). Tick-borne pathogens of zoonotic and veterinary importance in Nigerian Cattle. *Parasites & Vectors*. **9**: 217. Doi 10.1186/s13071-016-1504-7.

Mahama, C., I., Desquesnes M, Dia ML, Losson B, De Deken R, Geerts S (2004). A cross-sectional epidemiological survey of bovine trypanosomosis and its vectors in the Savelugu and West Mamprusi districts of northern Ghana. *Vet Parasitol*. **122**: 1-13. 10.1016/j.vetpar.2004.03.009.

Mans, B., J., Pienaar, R., & Latif, A., A. (2015). A review of *Theileria* diagnostics and epidemiology. *International Journal of Parasitology: Parasites and Wildlife*. **4**(1): 104-118.

Mandal, M., Banerjee, P., S., Garg, R., Ram, H., Kundu, K., Kumar, S., Kumar, & G.V.P.P.S.R. (2014). Genetic characterization and phylogenetic relationships based on 18S rRNA and ITS1 region of small form of canine Babesia spp. from India. **27**: 325-331.

Marathe, A., Tripathi, J., Handa, V., & Date, V. (2005). Human Babesiosis – A Case Report. *Indian Journal of Medical Microbiology*, **23**(4), 267–269.

Maria, R., B., & Jeffrey, A., G. (1995). Babesiosis: State-of-the-Art Clinical Article. *Clinical Infectious Diseases*. **22**: 611-615.

Martinod, S., Laurent, N., Moreau, Y. Resistance and immunity of dogs against *Babesia canis* in an endemic area. *Veterinary Parasitology*. **19**(3-4):245–254.

Matjila, P., T., Leisewitz, A., L., Oosthuizen, M., C., Jongejan, F., & Penzhorn, B., L. (2008). 'Detection of *Theileria* species in dogs in south Africa'. Veterinary Parasitology. **157**: 34-40. http://dx.doi.org/10.1016/j.vetpar.2008.06.025.

University of Ghana http://ugspace.ug.edu.gh

Matsu, A., Koshida, Y., Kawahara, M., Inoue, K., Ikadai, H., Hikasa, Y., Okano, S., and Higuchi, S. (2004): Efficacy of atovaquone against *Babesia gibsoni* in vivo and in vitro. *Vet. Parasitol.* **124:** 9-18.

Matysiak, P., Dudko, K., Dudek, M., Dudek, A., Junkuszew, & Tryjanowski. (2016). The occurrence of pathogens in Rhipicephalus microplus ticks from cattle in Madagascar. Veterinarni Medicina. **61**(9): 516-523. Doi: 10.17221/59/2016-VETMED.

Mehlhorn, H., Shein, E., & Ahmed, J., S. (1993). *Theileria*. In: Kreier, J., P. (Ed). Parasitic protozoa. New York. N. Y: Academic Press. **7**: 217-304.

Mehlhorn, H., & Schein, E. (1984). The piroplasms: life cycle and sexual stages. *Adv Parasitol*. **23**: 37-103.

Mehlhorn, H., & Schein, E. (1987). The piroplasms: life cycle and sexual stages. *Adv Parasitol*. **23**:37-103.

Mehlhorn, H., Schein, E. (1998). Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1998. *Parasitol Res*. **84**:467–75.

Meghan, B. B., Barbara, L. H., James, J. K., John, W. W., Christina, L. K., Catherine, P. L., Rong H., Matthew, J. O., Laura, T., Patricia, P. W., & Gregory, M. G. (2016). Transmission of *Babesia* microti Parasites by Solid Organ Transplantation. *Emerg Infect Dis***.** **22**(11): 1869-1876.

Morrison, W., I. (2015). The etiology, pathogenesis and control of theileriosis in domestic animals. Revue scientifique et technique (International Office of Epizootics). **34**(2): 599-611.

University of Ghana http://ugspace.ug.edu.gh

Mosqueda, J., Olvera-Ramírez, A., Aguilar-Tipacamú, G., & Cantó, G., J. (2012). Current Advances in Detection and Treatment of Babesiosis. *Curr Med Chem*. **19**(10): 1504-1518.

Moustafa, M., A., Taylor, K., Nakao, R., Shimozuru, M., Sashika, M., Rosà, R., Thu, M., J., Rizzoli, A., & Tsubota, T. (2016). Dynamics, co-infections and characteristics of zoonotic tick-borne pathogens in Hokkaido small mammals, Japan. *Ticks Tick Borne Dis*. **7**(5): 922-928. doi. 10.1016/j.ttbdis.2016.04.014. Epub 2016 Apr 26.

Moutailler, S., Valiente, M., C., Vaumourin, E., Michelet, L., Tran, F., H., Devillers, E., Jean-François, C., Gasqui, P., Van, V., T., Mavingui, P., Vourc'h, G., & Vayssier-Taussat, M. (2016). Co-infection of ticks: the rule rather than the exception. PLOS *Negl Trop. Dis*. **10**:e0004539. doi: 10.1371/journal.pntd. 0004539.

Nagano, D., Sivakumar, T., De macedo, A., C., Inpankaew, T., Alhassan, A., Igarashi, I., & Yokoyama, N. (2013). The genetic diversity of merozoite surface antigen (MSA-1) among Babesia bovis detected from cattle populations in Thailand, Brazil and Ghana. J Vet Med Sci. **75**: 1463-1470.

Nakayima, J., Nakao, R., Alhassan, A., Mahama, C., Afakye, K., & Sugimoto, C. (2012). Molecular epidemiological studies on animal trypanosomiases in Ghana. Parasites & Vectors. **5**:217. https://doi.org/10.1186/1756-3305-5-217.

Nalubamba, K., S., Mudenda, N., B., Namwila, M., M., Mulega, C., S., Bwalya, E., C., M'kandawire, E., Saasa, N., Hankanga, C., Oparaocha, E., & Simuunza, M. (2015). A study of Naturally Acquired Canine Babesiosis Caused by Single and Mixed *Babesia* species in Zambia: *Clinicopathological Findings and Case Management*. **2015**: 1-9.

Nasiba, Y. (2017). Minister expresses fears Ghana might miss SDGs targets. https://www.myjoyonline.com/news/2017/July-11th/minister-….

New, D., L., Quinn, J., B., Qureshi, M., Z., & Sigler, S., J. (1997). Vertically transmitted babesiosis. *J Pediatr*. **131**(1 Pt 1): 163-164.

University of Ghana http://ugspace.ug.edu.gh

Nejash, A., & Bekele, T. (2016). Bovine Theileriosis and its Control: A Review. *Advances in Biological Research*. **10**(4): 200-212.

O'Meara, W., P., Mangeni, J., N., Steketee, R., & Greenwood, B. (2010). Changes in the burden of malaria in sub-Saharan Africa. *Lancet Infect Dis*. **10**(8):545–55.

Osorno, B., M., Vega, C., Ristic, M., Robbles, C., & Ibarra, S. (1976). Isolation of *Babesia sp*. from asymptomatic human beings. Veterinary Parasitology**.** **2**(1): 111-120.

Ota, N., Mizuno, D., Kuboki, N., Igarashi, I., Nakamura, Y., Yamashina, H., Hanzaike, T., Fujii, K., Onoe, S., Hata, H., Kondo, S., Matsui, S., Koga, M., Matsumoto, K., In-okuma, H., & Yokoyama, N. Epidemiological survey of *Theileria* orientalis infection in grazing cattle in the eastern part of Hokkaido. *Japan J Vet Med Sci*. **71**(7): 937-944.

Owusu, I., A. (2015). Detection of Zoonotic *Babesia* species in Greater Accra, Ghana. *UG Space*. ugspace.ug.edu.gh/handle/123456789/8595.

Paddock, C., D., Lane, R., S., Staples, J., E., & Labruna, M., B. (2016). Changing Paradigms for Tick-Borne Diseases in the Americas: Forum on Microbial Threats; Board on Global Health; Health and Medicine Division; National Academies of Sciences, Engineering, and Medicine. Washington (DC). National Academies Press (US). 21st September 2016.

Pain, A., & Mahabile, M. (1997). Profitability and household income contribution of small ruminants to small scale farmers in Botswana. Small Ruminant Resources. **25**(1): 9-15.

Penzhorn, B., L. (2011). Why is Southern African canine babesiosis so virulent? An evolutionary perspective. **4**:51.

Persing, D. H., Herwaldt, B. L., Glaser, C., Lane, R. S., Thomford, J. W., Mathiesen, D., Peter, B. A., Krause, J., Douglas F., Phillip, M. D., Patricia A., & Conrad, D.V.M. (1995). Infection with a *Babesia*-like organism in Northern California. *New England Journal of Medicine*. **332**: 298-303.

University of Ghana http://ugspace.ug.edu.gh

Pieszko, M. (2015). Molecular regulation of the macroschizont to merozoite Differentiation in *Theileria* annulata. PhD Thesis, University of Glasgow, UK. p. 308.

Pike, RM. (1979). Laboratory-associated infections: summary and analysis of 3921 cases. *Health Lab. Sci*. **13**: 105-114.

Pike, MR. (1979). Laboratory-associated infections: incidence, fatalities, causes, and prevention. *Ann Rev Microbiol*. **33**: 41-66.

Pike, RM., Sulkin, SF., & Sohiulze, MI. (1965). Continuing importance of laboratory-acquired infects. *Am J Pubic Nations Health*. **55**: 190-199.

Plumb, D., C. (2015). Plumb's Veterinary Drug Handbook. *Wiley-Blackwell*. Ames**. 8**: 1296.

Pohl, A., E. (2013). Epidemiology study of tick-borne diseases in cattle in Minas Gerais, Brazil (Doctoral dissertation, 1mu).

Preston, P., M. (2001). Theilerioses. In: Service, M. W., Ed., Encyclopedia of Arthropod-Transmitted Infections of Man and Domesticated Animals. *CABI Publishing, Wallingford*. 487-502.

Pulford, D., McFadden, A., Hamilton, J., S., & Donald, J. (2016). Investigation of the index case herd and identification of the genotypes of *Theileria* orientalis associated with outbreaks of bovine anaemia in New Zealand in 2012. *NZ Vet J*. **64**(1): 2-8.

Quick, R. E., Herwaldt, B. L., Thomford, J. W., Gorret, M. E., Eberhard, M. L., Wilson, M., Spach D. H., Dickson, J. W., Telford, S. R., Steingart, K. R., Pollock, R., Persing, D. H., Kobayashi, J. M., Juranek, D. D., & Conrad, P. A. (1993) Babesisosis in Washington State: A New Species of Babesia? *Annals of Internal Medicine*, 119(4), 283-290. doi:10.7326/003-4819-119-4-1993 08150-00006.

Ramharter, M., Walochnik, J., Lagler, H., Winkler, S., Wernsdorfer, W. H., Stoiser, B., & Graninger, W. (2010). Clinical and Molecular Characterization of a Near Fatal Case of Human Babesiosis in Austria. *Journal of Travel Medicine,* **17**(6), 416–419. http://doi.org/10.1111/j.1708-8305.2010.00446.x.

University of Ghana http://ugspace.ug.edu.gh

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B., M., Whitty, C., J. (2004). Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ. **329**(7476): 1212.

Ríos, L., Alvarez, G., & Blair, S. (2003). Serological and parasitological study and report of the first case of human babesiosis in Colombia. *Rev. Soc. Bras. Med. Trop*. **36**: 493-498.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubalek, Z., Foldvari, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalska, E., & Kazimirova, M. (2014). *Ixodes Ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Front. Public Health **2**:251. doi: 10.3389/fpubh.2014.00251.

Rollend, L., Bent, S. J., Krause, P. J., Usmani-brown, S., Steeves, T. K., States, S. A., Lepore, L., Ryan, R., Dias, F., Mamoun, C. B., & Diuk-wasser, M. A. (2013). Quantitative PCR for Detection of *Babesia microti* in *Ixodes scapularis* Ticks and in Human Blood. *Vector-borne and zoonotic diseases*, **13**(11), 1–7. http://doi.org/10.1089/vbz.2011.0935.

Rosa, C., T., Pazzi, P., Nagel, S., McClure, V., Christy, J., Troskie, M., & Dvir, E. (2014). 'Theileriosis in six dogs in South Africa and its potential clinical significance'. *J S Afr Vet Assoc*. **85**(1): 1114. http://dx.doi.org/10.4102/jsava.v85i1.1114.

Roshni, N., Aleksandra, M., D., John, C., N., & Delong, L. (2017). Autoimmune hemolytic anemia associated with babesiosis. *Biomark Res*. **5:**14.

Roncalli, A., R. (2001). The history of Italian parasitology. *Vet Parasitol*. **98**: 3-30.

Rudzinska, M., A., Trager, W., Lewengrub, S., J., & Gubert, E. (1976). An electron microscopic study of *Babesia microti* invading erythrocytes. Cell Tissue Res. **169**: 323-334.

Saad, F., Khan, K., Ali, S., & ul Akbar, N. (2015). Zoonotic significance and Prophylactic Measure against babesiosis. *Int. J. Curr. Microbiol. App. Sci*. **4**(7): 938-953.

Safar, E., H. (2017). Laboratory-acquired blood-borne parasites from accidental exposure. *J Arab Soc Med Res*. **12**(1): 1-5.

University of Ghana http://ugspace.ug.edu.gh

Sanjaya, N., Paparini, A., Latimer, M., Andriolo, K., Dasilva, A., J., Wilson, H., Xayavong, M., V., Peter, J. C. Philip, J., & Peter, J. I. (2012). First report of human babesiosis in Australia. *Med. J. Aust*. **196**(5): 350-352.

Salem, N., Y., & Farag, H., S. (2014). Clinical, Hematologic, and Molecular Findings in naturally Occurring *Babesia* canis vogeli in Egyptian Dogs. Vet Med intern. **2014**: 1-6. http://dx.doi.org/10.1155/2014/270345.

Sawczuk, M., Maciejewska, A., & Skotarczak, B. (2008). Identification and molecular characterization of *Theileria sp*. infecting red deer (*Cervus elaphus*) in northwestern Poland. *Eur J Wildl Res*. **54**: 225-230.

Scott, J., D. (2017). First record of locally acquired human babesiosis in Canada caused by *Babesia duncani*: a case report. *Sage Open Medical Case Reports*. **5**: 1-4. doi: 10.1177/2050313x17725645.

Schetters, T., P., Moubri, K., Précigout, E., Kleuskens, J., Scholtes, N., C., & Gorenflot, A. (1997). Different *Babesia canis* isolates, different diseases. *Parasitology*. **115**(5): 485-493.

Schnittger, L., Rodriguez, A., E., Florin-Christensen, M, Morrison, D., A. (2012). *Babesia*: a world emerging. *Infect Genet Evol*. **12**: 1788-1809.

Schnittger, L., Yin, H., Qi, B., Gubbels, M., J., Beyer, D., Niemann, S., Jongejan, F., & Ahmed, J., S. (2004). Simultaneous detection and differentiation of *Theileria* and *Babesia* parasites infecting small ruminants by reverse line blotting. *Parasitol Res*. **92**(3): 189-196.

Silva, M., G., Marques, P., X., & Oliva, A. (2010). Detection of *Babesia* and *Theileria species* infection in cattle from Portugal using a reverse line blotting method. *Veterinary Parasitology*. **174**: 199-205.

Sivakumar, T., Hayashida, K., Sugimoto, C., & Yokoyama, N. (2014). Evolution and genetic diversity of *Theileria*. *Infect. Genet. Evol*. **27**: 250-263.

University of Ghana http://ugspace.ug.edu.gh

Simões, P., B., Cardoso, L., Araujo, M., Yisaschar-Mekuzas, Y., & Baneth, G. (2011) 'Babesiosis due to the canine *Babesia* microti-like small piroplasm in dogs - first report from Portugal and possible and possible vertical transmission' *Parasites and Vectors***. 4**: 50. http://dx.doi.org/10.1186/1756-3305-4-50.

Skrabalo, Z., & Deanovic, Z. (1957). Piroplasmosis in man; report of a case. Doc *Med Geogr Trop*. **9**(1): 11-16.

Smeenk, I., Kelly, P. J., Wray, K., Musuka, G., Trees, J., & Jongejan, F. (2000). *Babesia bovis* and *B. bigemina* DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. *Journal of the South African Veterinary Association,* **71**(1), 21–24. http://doi.org/10.4102/jsava.v71i1.671.

Smith, T., & Kilborne, F., L. (1893). Investigation into the nature, causation, and prevention of southern cattle fever. *US Dept Agr Bur Anim Indust Bull*. **1**: 1-301.

Sobczyk, A. S., Kotomski, G., Gorski, P., & Chowicz, A. W. (2005). Usefulness of touchdown pcr assay for the diagnosis of atypical cases of *Babesia canis canis* infections in dogs. *Bulletin- Veterinary Institute in Pulawy*. **49**: 407–410.

Solano-Gallego, L., & Baneth, G. (2011). Babesiosis in dogs and cats – expanding parasitological and clinical spectra. *Vet Parasitol*. **181**: 48-60.

Solano-Gallego, L., Sainz, A., Roura, X., Estrada-Pena, A., & Miro, G., A. (2016). Review of canine babesiosis: The European perspectives. *Parasit Vectors*. **9**: 336. doi 10.1186/s137-016-1596-0.

Spielman A, Wilson M., L., Levine, J., F., & Piesman, J. (1985). Ecology of Ixodes dammini-borne human babesiosis and Lyme disease. Annu Rev Entomol. **30**:439-60.

University of Ghana http://ugspace.ug.edu.gh

Stjernberg, L., & Berglund, J. (2005). Detecting ticks on light versus dark clothing. Randomized controlled trial. Scand J Infect Dis. **37**(5): 361-364.

Stoler, J., & Awandare, G., A. (2016). Febrile illness diagnostics and the malaria – industrial complex: a socio-environmental perspective. *BMC Infectious Diseases*. **16:** 683. doi 10.1186/s12879-016-2025-x.

Swilks, E., Fell, S., A., Hammer, J., F., Sales, N., Krebs, G., L., & Jenkins, Cheryl. (2017). Transplacental transmission of *Theileria* orientalis occurs at a low rate in field-affected cattle: infection in utero does not appear to be a major cause of abortion. *Parasites & Vectors*. **10**: 227. doi 10.1186/s1307-017-2166-9.

Taboada, J., & Merchant, S., R. (1991). Babesiosis of companion animals and man. *Vet. Clin. North Am. Small Anim. Pract*. **21**(1): 103-123.

Tanyel, E., Guler, N., Hokelek, M., Ulger, F., & Sunbul, M. (2015). A case of severe babesiosis treated successfully with exchange transfusion. International Journal of Infectious Diseases (IJID). **38**: 83-85. doi: 10.1016/j.ijid.2015.07.019.

Tarimo, M., A. (2013). Studies on the prevalence of east coast fever among cattle in Kilosa district. Master Thesis. *Sokoine, University of Agriculture*. Morogoro, Tanzania. 63.

The Center for Food Security and Public Health (CFSPH). Last Updated, 2008. Bovine Babesiosis. Iowa state university, Ames, Iowa. www.cfsph.iastate.edu/Factsheets/pdfs/bovine_babesiosis.pdf.

Theiler, A. (1904). The transmission of East Coast fever by ticks. *Transvaal Agricultural Journal*. **3**: 71-86.

Tivura, M., Asante, I., van Wyk, A., Gyaase, S., Malik, N., Mahama, E., Hostetler, D., M., Fernandez, F., M., Asante, K., P., Kaur, H., & Owusu-Agyei, S. (2016). Quality of Artemisinin-based Combination Therapy for malaria found in Ghanaian markets and public health implications of their use. *BMC Pharmacol Toxicol*. **17**: 48. doi: [10.1186/s40360-016-0089-2].

University of Ghana http://ugspace.ug.edu.gh

Tolkacz, K., Bednarska, M., Alsarraf, M., Dwużnik, D., Grzybek, M., Welc-Falęciak, R., Behnke, J., M., & Bajer, A. (2017). Prevalence, genetic identity and vertical transmission of Babesia microti in three naturally infected species of vole, Micotus spp. (Cricetidae). *Parasites & Vectors*. **10**:66.

Uilenberg, G. (2001). Babesiosis. In: Service, M. W., Ed., Encyclopedia of Arthropod-Transmitted Infections of Man and Domesticated Animals. CABI Publishing, Wallingford. **46**: 53-60.

Uilenberg, G. (2006). *Babesia* – A historical overview. *Vet Parasitol*. **138**: 3-10.

Uilenberg, G. (2006). *Theilerial species* of domestic livestock. In: Irvin AD, Cunningham MP, Young AS, eds. Advances in the Control of Theileriosis. *The Hague, Netherlands: Martinus Nijhoff Publishers*. **1981**: 4-37.

Uilenberg, G. Franssen, F., F., Perié, N., M., & Spanjer, A., A. (1989). "Three groups of *Babesia* canis distinguished and a proposal for nomenclature". *Vet Quarterly*. **11**(1): 33-40.

Uilenberg, G., Schreuder, B., E., C. (1976). Studies on *Theileriidae* (Sporozoa) in Tanzania. I. Tick transmission of *Haematoxenus veliferus*. *Tropenmed Parasit*. **27**:106–111.

United Nations, UN. (2015). Sustainable Development Goals. Last updated, 20th September, 2016.

United States Food and Drug Administration (2014). Fatalities Reported to FDA Following Blood Collection and Transfusion. Silver Spring, MD: Food and Drug Administration.

Vannier, E., Borggraefe, I., Telford, S., R., Menon, S., Brauns, T., Spielman, A., Gelfand, J., A., Wortis, H., H. (2004). Age-Associated decline in resistance to *Babesia microti* is genetically determined. *J Infect Dis*. **189**(9): 1721-1728. https://doi.org/10.1086/382965.

Vannier, EC., Diuk-Wasser, MA., Ben, M. C., & Krause, PJ. (2015). *Babesiosis. Infect Dis Clin North Am*. **29**(2): 357-370.

University of Ghana http://ugspace.ug.edu.gh

Vannier, E., Gewurz, B., E., & Krause, P., J. (2008). Human babesiosis. *Infect. Dis. Clin. North Am*. **22**(3): 469-488. Available in PMC, 2014 Apr 24. doi: 10.1016/j.idc.2008.03.010.

Vannier, E., & Krause, P., J. (2009). Update on babesiosis. *Interd Persp on Infect Dis*. **2009**: 1-9.

Vannier, E., & Krause, P., J. (2012). Human babesiosis. *New Engl J Med* **366**: 2397-2467.

Vermeil C, Menut J, Miegeville M, Cruziat J, Julienne F, Morin O, Roger AP, Marjolet M, Bouillard C (1983). Babesiasis, pediatric malaria: does confusion exist in Africa? Bull Soc Pathol Exot Filiales. 1983 Dec; 76(5 Pt 2):797-804.

van Zyl, M. (1995). Prediction of survival in hospitalized cases of canine babesiosis: a retro-spective investigation employing serum biochemical parameters and signalment data [M.S. thesis] Pretoria, South Africa: University of Pretoria.

Vinodkumar, K., Shyma, V., Justin, D., K., Ashok, S., Anu, J., P., Mini, K., Muhammedkutty, V., Sasidharan, S., & Chullipparambil, S. (2016). Fatal *Theileria* orientalis N2 genotype infection among Asian water buffaloes (Bubalus bubalis) in a commercial dairy farm in Kerala, India. *Parasitology*. **143**(1): 69-74. doi: 10.1017/S0031182015001468.

Wagner, G., G., Holman, P., & Waghela, S. (2002). Babesiosis and heartwater: threats without boundaries. *Vet Clin. Food Anim*. **18:** 417-430.

Wei, Q., Tsuji, M., Zamoto, A., Kohsaki, M., Matsui, T., Shiota, T., Telford III, S. R., & Ishi-hara, C. (2001). Human babesiosis in Japan: isolation of *Babesia microti*-like parasites from an asymptomatic transfusion donor and from a rodent from an area where babesiosis is endemic. *Journal of Clinical Microbiology*, **39**, 2178-2183.

Whegang Youdom, S., Tahar, R., & Basco, L., K. (2017). Comparison of anti-malarial drugs efficacy in the treatment of uncomplicated malaria in African children and adults using network meta-analysis. Malaria Journal. **16**(1): 311. doi: 10.1186/s12936-017-1963-0.

University of Ghana http://ugspace.ug.edu.gh

Wormser, G., P., Lombardo, G., Silverblatt, F., El Khoury, M., Y., Prasad, A., Yelon, J., A., Sanda, A., Karim, S., Coku, L., & Savino, J., A. (2011). Babesiosis as a cause of fever in patients undergoing a splenectomy. Am Surg. **77**(3): 345-347.

Wikipedia. (2018). Life cycle Theileria species, T. microti. Date visited 25/07/2018. Retrieved form https://en.wikipedia.org/wiki/Theileria_microti#/media/File:Babesia_life_cycle_human_en.svg)

Wittner, M., Lederman, J., Tanowitz, H. B. Rosenbaum, G. S., and Weiss, L. M. (1996). Atovaquone in the treatment of *Babesia microti* infections in hamsters. *Am. J. Trop. Med. Hyg.* **55:** 219-222.

Yabsley, M., J., & Shock, B., C. (2013). Natural history of Zoonotic *Babesia*: role of wildlife reservoirs. *Int. J Parasitol*. **2**:18-31.

Yadhav, C., P., Chandana, Sai lalith kumar, M., V., Sujitha, Y., N., Lavanya, M., & Madhavilatha, Ch. (2015). An overview of Babesiosis. *Int. J. Res. Pharm, L. Sci*. **3**(1): 287-295.

Zahler, M., Schein, E., Rinder, H., & Gothe, R. (1998). Characteristic genotypes discriminate between *Babesia* canis isolates of differing vector specificity and pathogenicity to dogs. *Parasitol Res***. 84**: 544-548.

Zahler, M., Rinder, H., Schein, E., & Gothe, R. (2000b). Detection of a new pathogenic *Babesia microti*-like *species* in dogs. *Vet Parasitol*. **89**: 241-248.

Zanet, S., Trisciuoglio, A., Bottero, E., Fernández de Mera, I., G., Gortazar, C., Carpignano, M., G., & Ferroglio, E. (2014). Piroplasmosis in wildlife: Babesia and Theileria affecting free-ranging ungulates and carnivores in the Italian Alps. Parasites & Vectors. **7**:70. doi: 10.1186/1756-3305-7-70.

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APPENDICES

APPENDIX I
A. Ethical Clearance from EPRC, SBAHS, CHS, UG

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.:... 13th March, 2017.

Benjamin PulleNiriwa
Department of Medical Microbiology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu, Accra

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.6 – P 4.3/2016-2017**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 9th of March, 2017 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Occurrence of zoonotic babesia among cattle, dogs and their ticks and humans in the middle belt in Ghana"**

PRINCIPAL INVESTIGATOR: **Benjamin PulleNiriwa**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

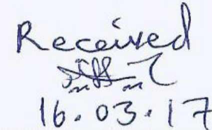
This ethical clearance is valid till 28th February, 2018.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed:

PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department



• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
• Email: administration@chs.edu.gh / provost@chs.edu.gh • Website: www.chs.ug.edu.gh

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B. Clearance from Holy Family Hospital, Techiman

Holy Family Hospital,

Post Office Box 36,

Techiman, Brong Ahafo Region.

14th July, 2017.

The Management Team,

Holy Family Hospital,

Post Office Box 36,

Techiman, Brong Ahafo Region.

Dear Sir/Madam,

PROJECT WORK

I am very grateful to the entire Management Team for releasing me to further my education. I am happy to inform management that, by the wonderful grace of God I have successfully defended my project proposal; "Occurrence of zoonotic Babesia among cattle, dogs and their ticks and humans in the middle belt of Ghana". I have been ethically cleared by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana to do my project.

I will start the project this month with sample collection, and every week or two weeks, I will send the samples to the Department of Medical Microbiology – School of Biomedical and Allied Health Sciences of the University of Ghana, Korle-Bu for molecular testing.

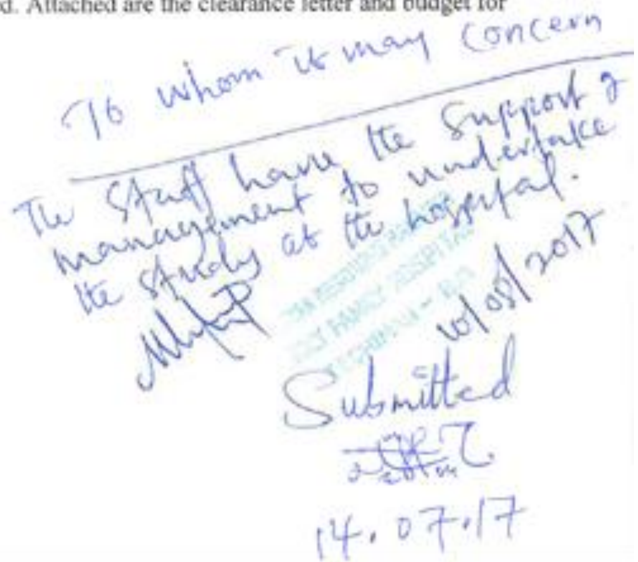
I would be grateful if management could support me either materially or financially to do the project. Any support given would be appreciated. Attached are the clearance letter and budget for the project. Thank you.

Yours faithfully

BENJAMIN PULLE NIRIWA (Lab.).

Tel: 0242015959761

CC,
Lab. IC.



To whom it may concern

The Staff have the Support of management to undertake the study at the hospital.

10/08/2017

Submitted

14.07.17

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C. Clearance from Kintampo Municipal's Hospital

Holy Family Hospital

Post Office Box 36

Techiman, Brong Ahafo Region.

14th July, 2017.

The Administration

Kintampo Municipal Hospital

Post Office Box 192

Kintampo, Brong Ahafo Region.

Dear Sir/Madam,

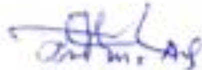
PERMISSION TO COLLECT SAMPLES FOR MY RESEARCH WORK

I am pursuing MPhil Medical Microbiology at the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences at the College of Health Sciences, University of Ghana – Korle-Bu Campus.

I am conducting a research project entitled "Babesiosis". I would be grateful if management could permit me to use samples collected from patients who are tested as malaria positives, for my project. I have been ethically cleared by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana, and have attached a copy of the clearance report to this application.

Your kind consideration would be greatly appreciated. Thank you.

Yours faithfully,



BENJAMIN PULLE NIRIWA

Tel. 0242015959

University of Ghana http://ugspace.ug.edu.gh

D. Clearance from Techiman Veterinary Office

UNIVERSITY OF GHANA
DEPARTMENT OF MEDICAL MICROBIOLOGY
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

Ref. No.:..

29th June, 2017

THE DIRECTOR
VETERINARY HOSPITAL
TECHIMAN
BRONG-AHAFO REGION

Dear Sir,

LETTER OF INTRODUCTION – MR. P BENJAMIN NIRIWA PULLE

Mr. Benjamin Niriwa Pulle is an MPhil student in the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, Korle Bu.

He is working on a project entitled "Babesiosis".

The project is under the supervision of Dr. Patience Tetteh-Quarcoo and Prof. Patrick F. Ayeh-Kumi (Department of Medical Microbiology, SBAHS, Korle Bu).

I write to seek your permission to allow Mr. Benjamin Niriwa Pulle to use your laboratory Department to enable him undertake his project.

Your kind assistance is highly solicited.

Thank you.

Yours faithfully,

[signature]

Dr. Patience Tetteh-Quarcoo
For: Head of Department

This is to certify that Benjamin Pulle Niriwa was allowed to Collect his project samples (blood) from Cattle and dogs under my Supervision. From 3/7/17.

[signature]
DEPUTY DIRECTOR
VETERINARY SERVICE
TECHIMAN - B/A

COLLEGE OF HEALTH SCIENCES

University of Ghana http://ugspace.ug.edu.gh

E. **Clearance from Kintampo Veterinary Office**

SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
DEPARTMENT OF MEDICAL MICROBIOLOGY

Phone: +233-302-665404/665383
Fax: +233-302-668286
E-mail: ugmsmicrob@chs.ug.edu.gh
My Ref. No.
Your Ref. No.

P O Box 4236
Accra.
Ghana

12th July, 2017

The Laboratory Manager
Veterinary Hospital
Kintampo
Brong-Ahafo Region.

Dear Sir,

LETTER OF INTRODUCTION – MR. BENJAMIN PULLE NIRIWA

Mr. Benjamin Pulle Niriwa is an Mphil student in the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, UG, Korle – Bu.

He is working on a project entitled "Babesiosis"

The project is under the supervision of Dr. Patience B. Tetteh-Quarcoo and Prof. Patrick F. Ayeh – Kumi (all of Department of Medical Microbiology, SBAHS, Korle-Bu)

I write to seek your permission to allow Mr. Benjamin Pulle Niriwa to use your laboratory to enable him undertake his project.

Your king assistance is highly solicited.

Thank you.

Yours faithfully,

Dr. Patience B. Tetteh-Quarcoo
For. Head of Department.

the above student was in Kintampo for sample collection ~~August~~ 2017 July
Bakpa David

MUNICIPAL VETERINARY OFFICER
BOX 43 VSD MoFA
KINTAMPO NORTH MUNICIPAL

University of Ghana http://ugspace.ug.edu.gh

APPENDIX II

INFORMED CONSENT FORM

University of Ghana

School of Biomedical and Allied Health Sciences

Department of Medical Microbiology

P.O.BOX 4236

Korle-Bu, Accra.

Study Title: Occurrence of *Babesia/Theileria* in the Middle Belt of Ghana

Dear Participant,

INFORMED CONSENT FORM

Your permission is being sought to participate in a study which is described below. Before you decide whether or not to participate, you can talk to anyone you feel comfortable with. If certain aspects are not clear to you, you are at liberty to seek further clarification and I will take time to explain better. If there are other questions or issues bothering your mind, do not hesitate to ask me for answers. Your participation in this study is entirely voluntary. The information you will provide and the outcome of the analysis of your samples provided will not be used in any way that would go against your interest. Your participation and test results will be coded, instead of your name and therefore will remain confidential. Therefore, if you decide not to consent or you consent and later decide to withdraw, there shall be no consequences attached to it and your decision shall be accepted.

THE STUDY IN FEW WORDS

Piroplasms (order Piroplasmida) are eukaryotic parasites of the phylum Apicomplexa. Two main genera (*Babesia* and *Theileria*) of the piroplasmorida are responsible for diseases in both humans and animals. *Babesia species* are tick-transmitted parasites that cause diseases in domestic animals like dogs and cattle are usually infected with the disease but the species that infect the cattle have

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been known to also infect humans. Theileriosis is caused by parasites of the genus Theileria. Babesiosis and theileriosis can be transmitted from animals to humans through the bite of infected ticks; human to human transmission can be through blood transfusion or from mother to child during pregnancy. Symptoms of babesiosis in humans are similar to those of malaria but different drugs are used for treatment. Also, in terms of diagnosis, some *Babesia* and *Theileria species* can microscopically be mistaken as *Plasmodium species*. I am therefore conducting this study to determine the occurrence of *Babesia/Theileria* in the Middle Belt of Ghana.

Procedure

Your venous blood sample will be taken using a sterile needle and syringe. The blood will then be processed for the detection of *Babesia*/*Theileria* spp.

Risks

Pain may be felt during the venous blood sample taking but this pain is usually mild and may last for a few minutes. Otherwise, this study will not pose any fatal risks to your health.

Benefit

There may be no immediate personal benefit to you other than test results being communicated to your clinician and copies kept in your folder. However, this study will go a long way to inform some medical decisions of the Ghana health service to enable better health care services to the nation.

Confidentiality

Any information you give us will remain confidential and your blood sample will be number coded instead of using your name.

Contact

Any questions concerning this study may be addressed to Benjamin Pulle Niriwa - 0242015959 (or Dr. Patience B. Tetteh-Quarcoo - 0244 633251) of the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana, Korle-Bu.

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Participant: I understand all the above and hereby agree to participate or allow my ward to participate in this study.

______________________	______________________	________________
Name of participant	Signature/Thumbprint	Date
______________________	______________________	________________
Name of witness	Signature/Thumbprint	Date
______________________	______________________	________________
Name of investigator	Signature/Thumbprint	Date

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APPENDIX III

ADMINISTRATION OF QUESTIONNAIRES FOR DEMOGRAPHIC DATA

UNIVERSITY OF GHANA, LEGON

SCHOOL OF BIOMEDICL AND ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL MICROBIOLOGY, KORLE-BU

INTERVIEWER ADMINISTRATION OF QUESTIONNAIRES FOR (CATTLE REARERS) HERDSMEN AND THEIR FAMILIES ON:

OCCURRENCE OF ZOONOTIC *BABESIA* AMONGST CATTLE, DOGS; THEIR TICKS AND HUMANS IN THE MIDDLE BELT OF GHANA

Dear interviewee,

Good day, I am an MPhil Medical Microbiology final year student of University of Ghana-Legon. I am doing my project on the, "Occurrence of Zoonotic *Babesia* amongst Cattle, Dogs; Their Ticks and Humans in the Middle Belt of Ghana". I would be grateful if you could answer some few questions about your demography before we take your sample. The purpose of the study is to determine zoonotic *Babesia* species in humans, cattle, dogs, and ticks in the Middle Belt of Ghana.

The study's results will help us to know whether there is *Babesia* infection in the middle belt of Ghana or not. This will help to facilitate better health care and to formulate appropriate policies. The study is taking place in two selected municipalities within the Middle Belt of Ghana. Questioning and answering would take less than five (5) minutes and would be done in a place that is conveniently appropriate to you.

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Whatever information I would receive from you would be strictly confidential; your name/identity would not be disclosed to any other person. All your answers would be anonymous and cannot be traced back to you in any way. Please you are not going to be forced to provide answers to any question you do not want to answer, and you can stop the interview at any point of time.

It is entirely your choice to decide whether you participate or not. Thank you.

Specific Research Objectives

1. To screen for possible *Babesia/Theileria* in humans, sick cattle, and dogs blood.
2. To use molecular techniques to determine the specific *Babesia/Theileria species*.

SECTION A

DEMOGRAPHIC DATA OF CATTLE REARERS AND THEIR FAMILIES

Please tick where appropriate (√)

1. What is your name please?

   Answer …………………………….

2. What is your sex/gender?

   a. Male ( ) b. Female ( ) c. Other (Specify) …………………..

3. How many years are you?

   a. 1-10years ( ) b. 11-20years ( ) c. 21-30years ( ) d. 31-40years ( )

   e. 41-50years ( ) f. 51-60years ( ) g. 61-70years ( ) h. 71years and above (…….)

4. Where is your present resident? Answer ……………………………………………….

SECTION B

QUESTIONNAIRE TO CATTLE'S OWNERS FOR CATTLE'S SAMPLES

Please tick where appropriate (√)

1. What is the name of your community?

   Answer ……………………………………………

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2. What is the color description of the cow?

   a. White ( ) b. Black ( ) c. Brown ( ) d. Red ( ) e. Mixed colors ( )

   f. Others (Specify) ……………………..

3. What is the age of your cow?

   a. 1-6months ( ) b. 6-11months ( ) c. 1-3years ( ) d. 4-6years ( )

   e. 6-8years ( ) f. 8-10years ( ) g. 11years and above, (state age ………..).

4. What is the sex of the cow?

   a. Male ( ) b. Female ( ) c. Other, specify …………….

SECTION C

QUESTIONNAIRE TO DOG'S OWNERS FOR DOGS' SAMPLES

1. What is the name of your dog?

   Answer ……………………………….

2. What is the age of your dog?

   a. 1-6months ( ) b. 6-11months ( ) c. 1-3years ( ) d. 4-6years ( )

   e. 7years and above (state age …………)

3. What is the sex of your dog?

   a. Male ( ) b. Female ( ) c. Other, specify ………………………..

4. What is the resident of your dog?

   Answer………………………………

5. Temperature checked, ……°C

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APPENDIX IV

PREPARATION OF REAGENTS

Preparation of 10% Giemsa stain from the Stock

Daily preparation of a 1 in 10 dilutions (10%) of Giemsa stain is done by filtering the stock solution of Giemsa. After that, depending on the number of slides that I get per day, 1ml or 2ml of the stock Giemsa is take into a clean container and 9ml or 18ml of buffered water is added respectively.

Dilution of Primers with Nuclease Free Water

A 1 in 10 suspensions of the primers used for PCR were prepared by reconstituting 10µl of the desired primers with 90µl of nuclease free water to get a 100µl prepared primers with a final concentration of 10µM, ready for use. The table below shows examples of how some of the primers were reconstituted.

Reconstitution of Primers with Nuclease Free Water

Reconstitution of Primers with Nuclease Free Water

Primers	Quantity of Substance (in nmol)	Volume of nuclease free water, in µL, added
BcW-A	22.9	229
BcW-B	32.7	327
Bab 5	21.8	218
Bab 8	29.6	296
Bab 6	22.5	225
Bab7	37.2	372

How 2% Agarose Gel Was Prepared

The powder used for the preparation was commercially prepared. Two (2g) grams of the agarose gel powder were weighed with weighing balance. This was added into a clean two hundred (200ml) milliliters conical flask that was used to measure hundred milliliters (100ml) of TAE buffer. The mixture is heated to uniformly dissolve the powder. After that it left to cool to body temperature and twenty microliters (20ul) of Ethidium bromide is added into it. The mixture is then poured into a gel preparation tank containing combs with sizes that can contain up to 25ul of amplicons.

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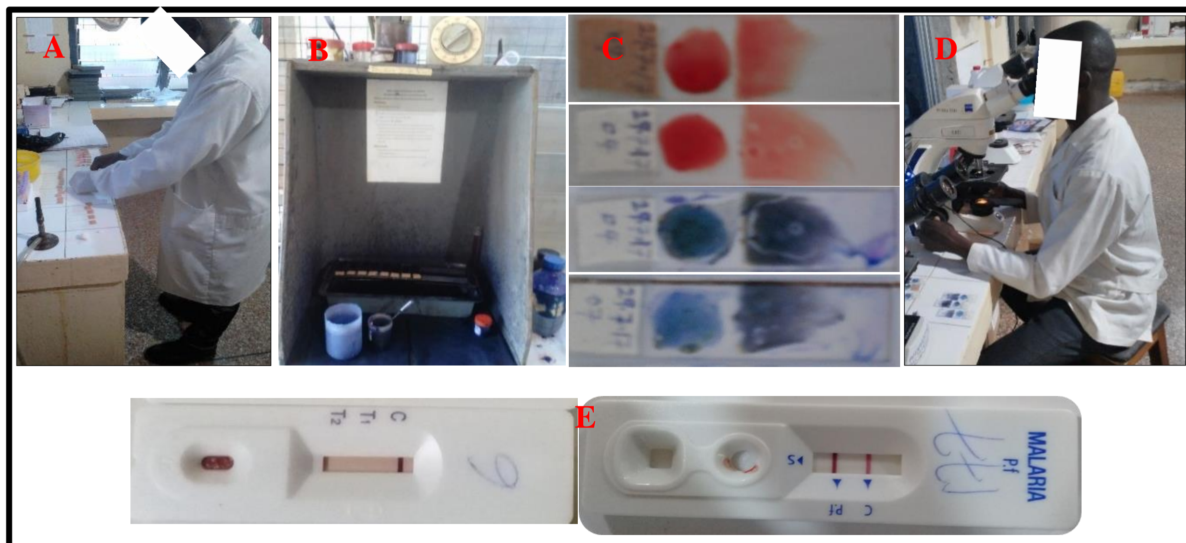
Dilution of 1X TAE Buffer with Distilled Water from 50X Stock Solution

To get a one litre (1L) volume of 1X TAE buffer for the Agarose gel preparation and gel electrophoresis; twenty milliters (20mL) of 50X concentrated TAE buffer was measured and diluted with nine hundred and eighty milliters (980mL) of distilled water as the working buffer.

Collection of Samples from the Various Study Groups



Preparation and Staining of Thick and Thin Smears with Giemsa for Microscopy and RDT for Plasmodium species

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Summary of Demographic Data of Cattle at the selected communities of the Middle Belt of Ghana

SUMMARY OF TECHIMAN CATTLE'S SAMPLES

Samples		J. T & S. H		Sansama		Tanoso – G.O		Tanoso - Buom		Tanoso - Mosque		Total	%
		No.	%	No.	%	No.	%	No.	%	No.	%		
Age (Years)	Adults	3	12	5	33.33	21	70	15	48.39	18	50	62	45.26
	Juveniles	22	88	10	66.67	9	30	16	51.61	18	50	75	54.74
	Total	25	100	15	100	30	100	31	100	36	100	137	100
Sex	Males	18	72.0	12	80.0	29	96.7	12	38.7	22	61.1	93	68
	Females	7	28.0	3	20.0	1	3.3	19	61.3	14	38.9	44	32
	Total	25	100	15	100	30	100	31	100	36	100	137	100
Cattle's Descrip-tion	White	17	68.0	5	33.3	19	63.3	14	45.2	25	69.4	80	58.4
	Black	0	0.0	1	6.7	1	3.3	6	19.4	3	8.3	11	8.0
	Brown	1	4.0	3	20.0	1	3.3	4	12.9	3	8.3	12	8.8
	Ash	0	0.0	0	0.0	2	6.7	0	0.0	0	0.0	2	1.5
	B & W	3	12.0	3	20.0	3	10.0	4	12.9	4	11.1	17	12.4
	Others	4	16.0	3	20.0	4	13.3	3	9.7	1	2.8	15	10.9
	Total	25	100	15	100	30	100*	31	100	36	100*	137	100

SUMMARY OF KINTAMPO CATTLE'S SAMPLES

Samples		Komadai		Techira #2		Mossi-Akura		Suronuase		Total (T)	%
		No.	%	No.	%	No.	%	No.	%		
Age (Years)	Adults	4	50	10	58.82	16	69.57	13	59.09	43	61.43
	Juveniles	4	50	7	41.18	7	30.43	9	40.91	27	38.57
	Total	8	100.00	17	100.00	23	100.00	22	100.00	70	100.00
Sex	Males	2	25.00	9	52.94	10	43.48	9	40.91	30	42.86
	Females	6	75.00	8	47.06	13	56.52	13	59.09	40	57.14
	Total	8	100.00	17	100.00	23	100.00	22	100.00	70	100.00
Cattle's Descrip-tion	White	3	37.50	7	41.18	9	39.13	12	54.55	31	44.29
	Black	2	25.00	2	11.76	1	4.35	4	18.18	9	12.86
	Brown	2	25.00	3	17.65	3	13.04	2	9.09	10	14.29
	Ash	1	12.50	0	0.00	1	4.35	0	0.00	2	2.86
	B & W	0	0.00	3	17.65	3	13.04	3	13.64	9	12.86
	Others	0	0.00	2	11.76	6	26.09	1	4.55	9	12.86
	Total	8	100.00	17	100.00	23	100.00	22	100.00	70	100

No. = Number, T = Total, and % = Percentages, B & W = Black and White, G. O = Godfred Oduro, J. T & S. H = James Town and Slaughter House. 100* is 99.9 which is approximately 100% as shown on the table 2 above. Sampling is still in progress with kraal yet to be sampled at Kintampo and the human's samples. In Techiman, the cattle sample is higher than that of Kintampo because one of my kraals at Tanoso (Godfred Oduro's kraal) was used as an index case for research into the occurrence of Trypanosoma species amongst cattle in the Techiman Municipality.

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Demographic Data of Cattle Sampled for *Babesia/Theileria species* at the Middle Belt of Ghana; Techiman,

Parameter	Freq.	%	*Babesia/Theileria species*				*Trypanosoma species*			
			Pos.	%	Neg.	%	Pos.	%	Neg.	%
Age										
Adults	62	45.26	8	47.06	54	45	13	59.09	49	42.61
Juveniles	75	54.74	9	52.94	66	55	9	40.91	66	57.39
Total	137	100	17	100	120	100	22	100	115	100
Gender										
Males	93	67.88	13	76.47	80	66.67	15	68.18	78	67.83
Females	44	32.12	4	23.53	40	33.33	7	31.82	37	32.17
Total	137	100	17	100	120	100	22	100	115	100
Description										
White	80	58.39	9	52.94	71	59.17	11	50	69	60
Black	11	8.03	0	0.00	11	9.17	2	9.09	9	7.83
Brown	12	8.76	1	5.88	11	9.17	2	9.09	10	8.7
Ash	3	2.19	1	5.88	2	1.67	2	9.09	1	0.87
Black/White	17	12.41	2	11.76	15	12.5	3	13.64	14	12.17
Others	14	10.21	4	23.53	10	8.33	2	9.09	12	10.43
Total	137	100	17	100	120	100	22	100	115	100
Symptoms										
Weight Loss	67	48.91	5	29.41	62	51.67	10	45.45	57	49.57
Weakness	43	31.39	7	41.18	36	30	3	13.63	40	34.78
Anorexia	12	8.76	3	17.65	9	7.5	3	13.63	9	7.83
Dermatitis	10	7.30	1	5.88	9	7.5	5	22.73	5	4.35
Nil	5	3.65	1	5.88	4	3.33	1	4.55	4	3.48
Total	137	100	17	100	120	100	22	100	115	100

Terms: Freq., - Frequency, Pos., - Positive, Neg., - Negative, and % - Percentage. Trypanosoma species were seen, so I recorded it.

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Demographic Data of Cattle's Samples at the Middle Belt of Ghana; Kintampo, 2017-2018

Parameter	**Freq.**	**%**	***Babesia species***				***Trypanosoma species***			
			Pos.	%	Neg.	%	Pos.	%	Neg.	%
Age										
Adults	43	61.43	7	43.75	36	66.67	1	100	42	60.87
Juveniles	27	38.57	9	56.25	18	33.33	0	0.00	27	39.13
Total	70	100	16	100	54	100	1	100	69	100
Gender										
Males	30	42.86	8	50.00	22	40.74	0	0.00	30	43.48
Females	40	57.14	8	50.00	32	59.26	1	100	39	56.52
Total	70	100	16	100	54	100	1	100	69	100
Description										
White	31	44.29	10	62.50	21	38.89	0	0.00	31	44.93
Black	8	11.43	2	12.50	6	11.11	0	0.00	8	11.59
Brown	11	15.71	2	12.50	9	16.67	0	0.00	11	15.94
Ash	2	2.86	0	0.00	2	3.70	0	0.00	2	2.90
Black/White	9	12.86	0	0.00	9	16.67	1	100	8	11.59
Other	9	12.86	2	12.50	7	12.96	0	0.00	9	13.04
Total	70	100	16	100	54	100	1	100	69	100
Symptoms										
Weight Loss	21	30.00	3	18.75	18	33.33	0	0.00	21	30.43
Weakness	32	45.71	8	50.00	24	44.44	0	0.00	32	46.38
Anorexia	10	14.29	3	18.75	7	12.96	0	0.00	10	14.49
Dermatitis	6	8.57	2	12.50	4	7.41	1	100	5	7.25
Nil	1	1.43	0	0.00	1	1.85	0	0.00	1	1.45
Total	70	100	16	100	54	100	1	100	69	100

Terms: Freq., - Frequency, Pos., - Positive, Neg., - Negative, and % - Percentage.