

**DETECTION OF ZONOTIC *BABESIA* SPECIES IN GREATER ACCRA,  
GHANA**

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**(10284117)**

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## DECLARATION

I do hereby declare that this thesis is my own work produced from research under the joint supervision of Prof. Patrick Ferdinand Ayeh-Kumi and Dr. Patience Borkor Tetteh -Quarcoo, all of the Department of Medical Microbiology, under the College of Health Sciences, University of Ghana. This work has not been previously submitted partially or wholly for the award of a degree in any University. References to the works of other investigators have been duly acknowledged.

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**DEDICATION**

I dedicate this piece to all researchers and my lovely family.

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## ABSTRACT

*Babesia* species are intra-erythrocytic protozoa of the phylum apicomplexa. They are either round or pear shaped, often characterized by a tetrad configuration (Maltese cross). The merozoite stage of *Babesia spp* 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 5.0 $\mu$ m. This parasite is transmitted by hard ticks and can cause a zoonotic disease known as babesiosis. Human babesiosis is usually asymptomatic except in immuno-compromised people in whom symptoms present like malaria, yet treatment for these two diseases is different.

These similarities can increase the possibility of misdiagnosing a patient with malaria when he or she is really suffering from babesiosis or vice versa leading to an inappropriate treatment choice. Ghana is a malaria endemic country; thus, general malaise is usually treated as malaria. This study was conducted to detect *Babesia sp* in the blood of patients who had been diagnosed with malaria. There was screening for *Babesia* in cattle and dogs as well, in order to suggest a possible transmission of the parasite from these animals to humans since it is zoonotic.

Whole blood samples were taken from One hundred and fifty (150) malaria positive cases, thirty (30) sick cattle and thirty-three (33) sick dogs. Microscopy (Giemsa stained thin smears) and polymerase chain reaction (PCR) were techniques employed for the detection of *Babesia sp*. *Babesia* infection was confirmed in nine (30%) cattle samples, one (3%) dog sample but none in humans. *B. canis* was found in the dog but the cattle *Babesia sp* were unspecified. Although *Babesia* infection was not detected in humans, there is the possibility of having zoonotic *Babesia* species in Accra, as long as *Babesia* was detected in cattle and dogs with which humans live in close proximity.

## CHAPTER ONE

### 1. INTRODUCTION

#### 1.1 Background

*Babesia* species are intra-erythrocytic protozoa of the phylum apicomplexa, they were first described in herds of cattle by Babes in 1888 (Bock *et al.*, 2004). They are either round or pear shaped, often characterized by a tetrad configuration known as the Maltese cross. The merozoite stage of *Babesia sp* has diagnostic significance and is found as intracellular inclusions of infected red blood cells. The trophozoite stages appear as ring forms which measure about 1.0 to 5.0  $\mu\text{m}$ ; they are non-motile cytoplasmic rings with chromatin dots as nuclei and may resemble *Plasmodium falciparum*.

There are over one hundred known *Babesia species*; depending on the type of host, different species have been reported to infect specific hosts. Four of these species, *Babesia microti*, *B. duncani*, *B. divergens*, *B. bovis* and *B. venatorum* are the species often known to infect humans (Vannier & Krause, 2009) although other species have been implicated in a few human babesiosis cases. It is noteworthy that, the species that infect humans have their natural vertebrate animal hosts; babesiosis is a zoonosis. The natural hosts of these four species are the white footed mouse and the deer for *B. microti*; cattle for *B. bovis* and *B. divergens* and the roe deer for *B. venatorum*. However, with the advent of possible cross infections, this study will look out for the dog *Babesia sp.* (*B. canis*) in humans in addition to the species mentioned above known to infect humans except *B. venatorum*.

Symptoms of babesiosis in humans can range from subclinical to severe (Kim *et al.*, 2007). Symptoms, if any, usually develop within one (1) to nine (9) weeks after exposure

especially in immuno-compromised people (Leibly, 2011). Clinical manifestations of babesiosis are hemolytic anemia and nonspecific flu-like symptoms (fever, chills, body aches, weakness, fatigue), however, some patients risk having splenomegaly, hepatomegaly, jaundice, blood pressure instability and myocardial infarctions in severe cases. A combination of Atovaquone and Azithromycin or clindamycin and quinine is used in the treatment of babesiosis (Krause *et al.*, 2000).

## **1.2 Problem Statement**

The symptoms of *Babesia* infection are similar to that of malaria and the morphology of *Plasmodium falciparum* is similar to that of *Babesia*. Therefore, accurate diagnosis of babesiosis continues to be a challenge. There is not much information on the presence or absence of *Babesia* species that infect humans in Ghana; unlike the United States of America, Europe and some other parts of the world where infection in humans is established and documented (Hildebrandt *et al.*, 2007).

If *Babesia* infection is misdiagnosed as malaria, the patient is likely to suffer adversely because babesiosis cannot be treated with the current anti-malarial drugs (Centeno-Lima *et al.*, 2003). Furthermore, immuno-competent asymptomatic carriers of human *Babesia sp.* are likely to donate blood; immuno-compromised patients who receive such blood and blood products will be at a greater risk of being infected with *Babesia* which may lead to disease.

## **1.3 Justification**

This study, when conducted, will result in the detection of *Babesia species* in Accra, Ghana. This will give an idea of the state of babesiosis in Ghana since not much

information on *Babesia* infection is available and as a matter of fact, there is no published data on human *Babesia* infection in Ghana.

Ghana is a malaria endemic region and there is the possibility that babesiosis might be misdiagnosed as malaria since routine diagnosis of malaria employs the microscopic examination of Giemsa-stained thick blood films which does not characterise the parasites into species. Therefore, in the quest to detect *Babesia* infection in this study, thin blood films will be prepared to enable the speciation of the parasites. This speciation will reveal *Babesia* parasites (in cases of the classical Maltese cross or paired pear-shaped morphology) or even the kind of *Plasmodium species* infecting the patient. Screening of cattle and dogs for *Babesia* will be necessary to have an idea of the species infecting them. If species infecting these animals are known to infect humans, there can be a hypothesis that, cross infection from these animals to humans is possible since they inhabit the same habitat as humans.

Some cases of babesiosis found in other parts of the world were not confirmed until there was a review of slides, initially diagnosed as *Plasmodium falciparum* malaria in blood smears of patients (Kunimoto *et al.*, 1998; Marathe *et al.*, 2005 Ramharter *et al.*, 2010; Centeno-Lima *et al.*, 2003). This study is important because, knowledge of the state of *Babesia* infection in Ghana will inform our medical personnel to broaden their investigations to include other tests like *Babesia* tests in patients with malaria-like symptoms in order to reduce misdiagnosis of malaria.

This study will also inform health personnel on the need for further investigations into “drug-resistant malaria” for the administration of appropriate treatment regimens in order to reduce any adverse conditions that misdiagnosis may lead to.

Depending on the outcome, this study will also raise concerns about the possibility of including *Babesia sp.* screening as one of the criteria that declares a person fit to donate blood. This is because recipients of blood and blood products are mostly immunocompromised and will be at a higher risk of being infected if the blood they receive is infected with human *Babesia sp.*

#### **1.4 Aim**

To determine the occurrence of human *Babesia* species in Accra

#### **1.5 Specific Objectives:**

1. To detect *Babesia species* in blood samples of “malaria positive” patients.
2. To screen blood of sick cattle and dogs for *Babesia* infection.

#### **1.6 Hypotheses:**

1. There is a possibility that some humans in Accra are infected with *Babesia*.
2. Cattle and dogs are infected with *Babesia*, some of which may infect humans.

## CHAPTER TWO

### 2. LITERATURE REVIEW

Babesiosis is an emerging tick-transmitted zoonotic disease caused by *Babesia sp* and occasionally infects humans. In 1956, the first human case was described (Skrabalo and Deanovic, 1957) though the parasite, *Babesia* had been described earlier in 1888 in animals by Victor Babes (Vannier & Krause, 2012). Almost fifty years since babesiosis was first reported in humans, the epidemiology of the disease has changed from a few isolated cases to the establishment of endemic areas in southern New England, New York, New Jersey and the Northern Midwest over the past fifty years (Hunfeld *et al.*, 2008). However, isolated cases are reported over a wide geographic area in Europe, Asia, Africa and South America (Hildebrandt *et al.*, 2007).

Signs of *Babesia* infection range from asymptomatic to severe cases leading to death (Vannier *et al.*, 2008). The disease was initially thought to infect only asplenic individuals until other cases were recorded in people with intact spleens (Chiang & Haller, 2011).

#### 2.1 The Pathogen

*Babesia* was first discovered by and named after a Hungarian microbiologist and pathologist, Victor Babes (Bock *et al.*, 2004). He thought it was a bacterium causing haemoglobinuria in cattle in Romania and initially named it *Haematococcus bovis* but this changed afterwards to *Babesia bovis* (Angus, 1996). Later in 1893, Smith and Kilborne discovered the cause of “Texas fever” in cattle in the United States as *Pyrosoma bigeminum* (now *Babesia bigemina*). They also established the fact that *Babesia sp* was tick transmitted; this was the first time an arthropod was implicated in the transmission of a disease causing organism to mammals (Smith and Kilborne, 1893). The pathogen has

various characteristics similar to *Plasmodium species* especially *P. falciparum*. There are various species of *Babesia* most of which cause diseases in animals and a few reported to cause diseases in humans. The parasite is transmitted by ixodid ticks (hard ticks) and infection with this parasite may lead to a disease known as babesiosis.

### 2.1.1 Classification

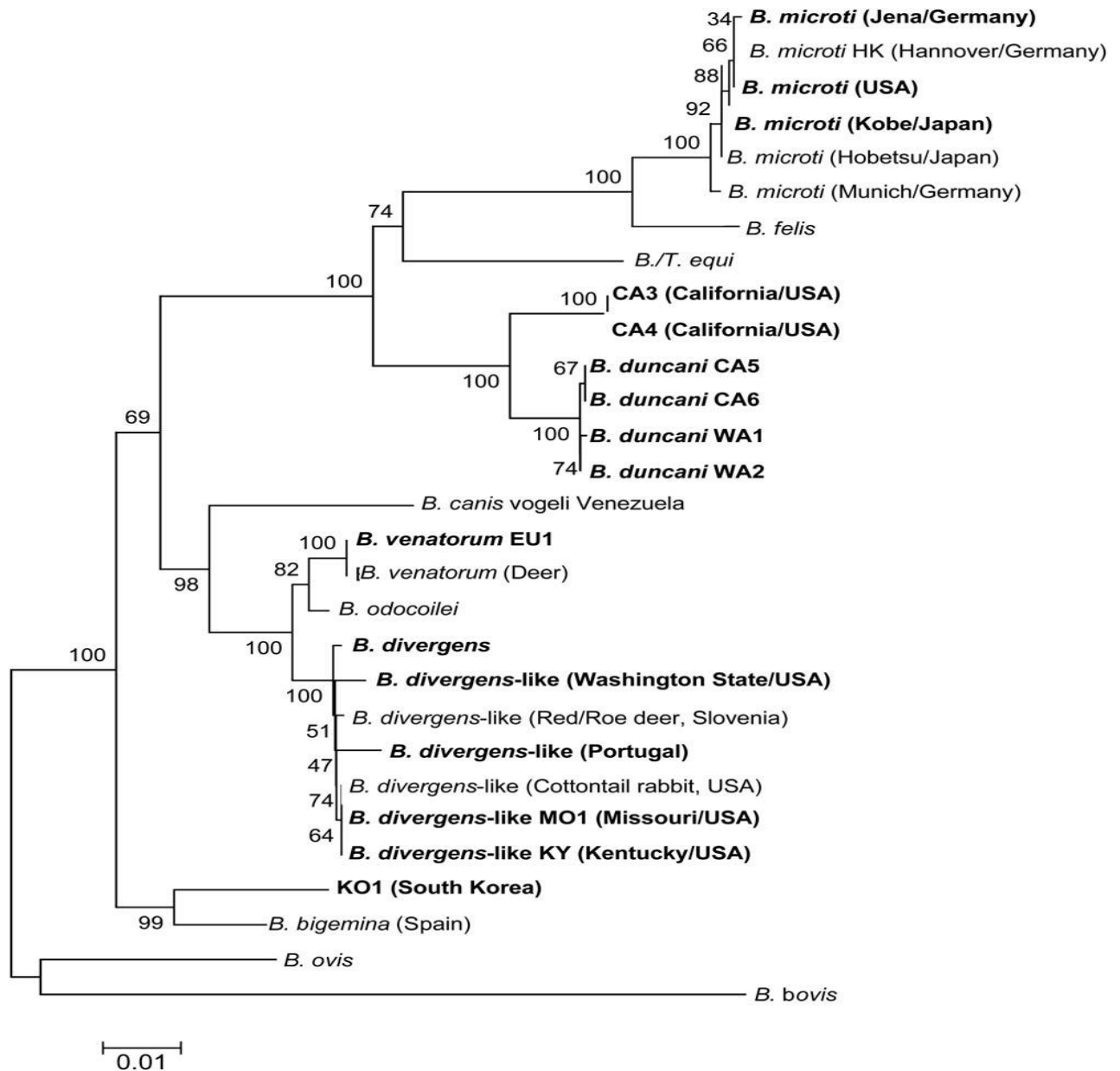
The genus *Babesia* belongs to the kingdom protista; phylum apicomplexa; class coccidiasida; order piroplasmida; family babesiidae (Levin, 1971) and there are over one hundred species described. The phylum, apicomplexa, also comprises of parasites like *Plasmodium species*, *Toxoplasma gondii* and *Cryptosporidium parvum* that cause diseases in humans.

*Babesia* species are traditionally divided into large *Babesia* such as *B. bigemina*, *B. canis*, *B. divergens* and others and are known as *Babesia sensu stricto* (s.s.) and small *Babesia* such as *B. microti* (Gray *et al.*, 2010). These two groups can be differentiated from each other based on their size and trans-ovarial transmission from female adult ticks to their offsprings which occurs in large *Babesia sp* unlike in small *Babesia sp* (Uilenberg, 2006). Also, phylogenetic tree (Figure 1) based on the 18S rRNA gene sequence differentiates large *Babesia sp* from small *Babesia sp* (Criado-Fornelio *et al.*, 2003). These phylogenetic relationships were determined by neighbour-joining analysis of 18S rRNA gene sequences. The absence of trans-ovarial transmission is among the features that small *Babesia sp* like *B. microti* share with *Theileria sp* (Criado-Fornelio *et al.*, 2003).

*Babesia* species that infect humans have been put into four main groups (Gray *et al.*, 2010): The first group consists of *B. microti*, small parasites (<3 µm), apparently associated with rodents (Goethert & Telford III, 2003) and form a species complex in which nearly all human isolates belong (Fujisawa *et al.*, 2011). The second group includes

*B. duncani* and *B. duncani*-like organisms. These are small *Babesia sp* that are morphologically similar but phylogenetically distinct from *B. microti* and are related to *Babesia sp* associated with dogs and wildlife in the western United States (Kjemtrup & Conrad, 2000). *B. divergens*, *B. divergens*-like parasites and *B. venatorum* (EU1) belong to group three and also comprise the large *Babesia sp* group (Gray *et al.*, 2010). *B. divergens* is a parasite of cattle and *B. venatorum* infects roe deers (Herwaldt *et al.*, 2003). A single strain from Korea, the KOREA 1 (KO1) strain, closely related to ovine *Babesia sp* and also belonging to the large *Babesia sp* group yet separate from the *B. divergens* group of large *Babesia sp* (Kim *et al.*, 2007) makes up the fourth group of *Babesia sp* that has been reported to infect humans.

Morphologically, *B. microti* was known to be a single species exclusively found in microtine rodents but recently, it is rather considered a complex of closely related sub-species, a number of which are found in hosts other than rodents (Gray *et al.*, 2010).



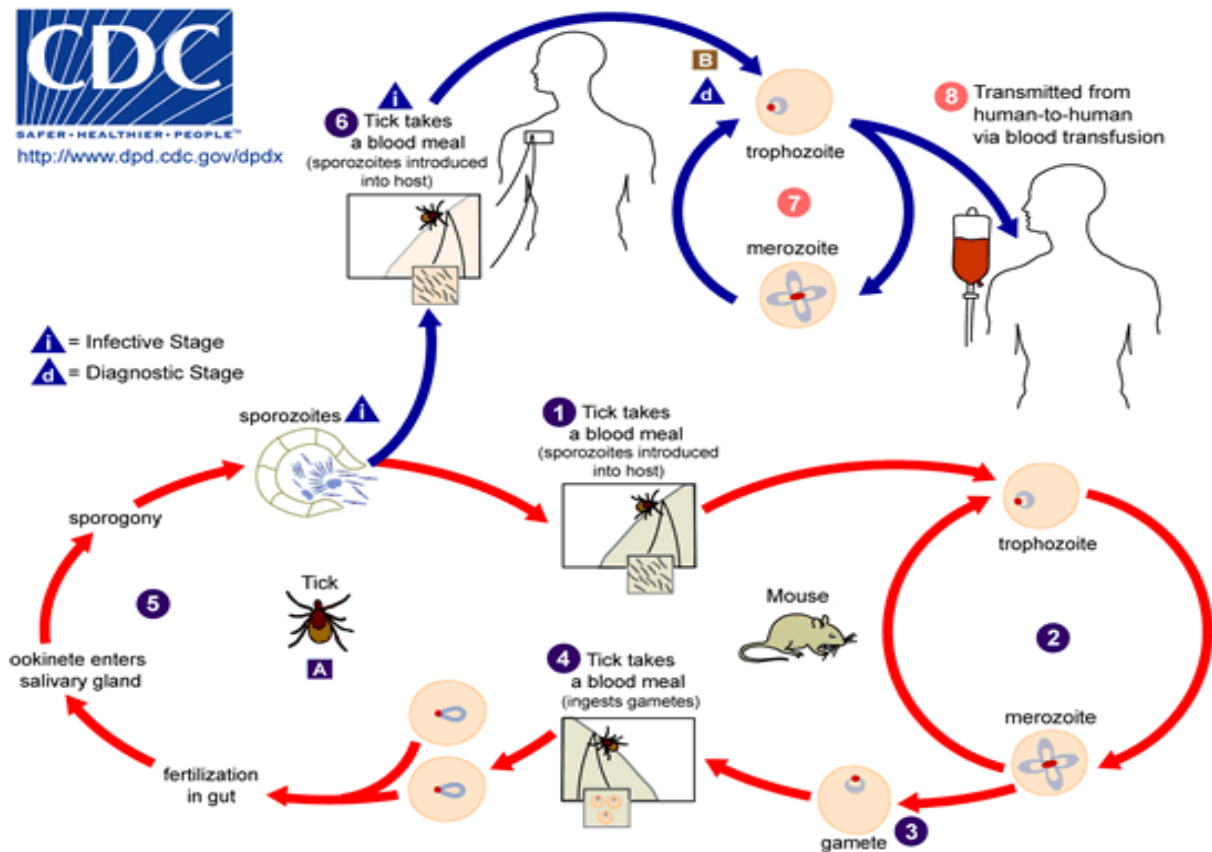
**Figure 1: Phylogenetic relationships of Babesia isolates (adapted from (Gray et al., 2010))**

### 2.1.2 Life cycle and transmission

Two hosts are involved in the development and transmission of *Babesia sp*; a vertebrate (intermediate host), and an Ixodid tick (definitive host). Depending on the *Babesia* species, there are different animal hosts, like rodents, cattle, dogs and other vertebrates. Also, different species of ticks in the ixodidae family transmit different *Babesia* species. The *Babesia*-infected tick introduces sporozoites into its host during a blood meal. The

sporozoites attach to and enter erythrocytes where they mature into trophozoites, each of which buds to form four merozoites (Vannier & Krause, 2012). These merozoites exit the erythrocytes by rupturing them and then infect other red blood cells.

In the blood, there is differentiation of some of the parasites into male and female gametocytes which when ingested by the vector (hard tick) accumulate in their guts (Vannier & Krause, 2012). The gametocytes differentiate into gametes in the guts of the ticks and then fuse to form zygotes that move across the epithelial walls of the guts into the haemolymph for maturation into ookinetes. The ookinetes then migrate to the salivary glands and other tissues where they develop into dormant sporoblasts (Rudzinska *et al.*, 1984) from which the sporozoites develop. In *Babesia sensu stricto* (s.s.) species (large *Babesia*), the ovaries of ticks are also invaded by the parasites leading to trans-ovarial transmission to their offspring (Gray *et al.*, 2010). Thousands of sporozoites are introduced into the host as infected nymphs feed (Vannier & Krause, 2012) and the cycle continues. Humans accidentally enter the cycle when bitten by infected ticks.



**Figure 2: Life Cycle and transmission of Babesia (Extracted from <http://www.dpd.cdc.gov/dpdx>)**

Trans-placental or vertical transmissions where pregnant mothers transmit the parasite to their babies have been reported in a few cases for *B. microti* (Fox *et al.*, 2006). The parasite has also been known to be transmitted through blood transfusion. In view of the fact that recipients of blood and blood products frequently are immuno-compromised or have underlying medical conditions, most cases of transfusion-transmitted babesiosis are often severe and approximately twenty percent (20%) of cases have been fatal (Herwaldt *et al.*, 2011). About 10% of cases occur in non-endemic areas because persons may become infected at endemic sites and subsequently donate blood in non-endemic areas or because units of contaminated blood are exported to these non-endemic areas (Herwaldt *et al.*, 2011).

The white-footed mouse (*Peromyscus leucopus*) is the primary reservoir host for *B. microti* (Spielman *et al.*, 1985). Although adult ticks may transmit *B. microti*, most cases result from exposure to nymphal ticks during the period from late spring through summer (Spielman *et al.*, 1985). In the United States, tick vectors for transmission of *B. duncani*, *B. duncani*-type, and *B. divergens*-like parasites are yet to be conclusively identified (Hunfeld *et al.*, 2008), but the *Ixodes scapularis* is known as the vector for transmission of *B. microti* (Herwaldt *et al.*, 2003). In Europe, the sheep tick *Ixodes ricinus* has been identified as the primary vector for transmission of *B. divergens* and *B. venatorum* (Gray *et al.*, 2010; Hunfeld *et al.*, 2008).

Although babesiosis is reported throughout the year, most infections occur from early summer through to late fall (Herwaldt *et al.*, 2011) in temperate regions. Adult female ticks lay eggs and the larvae hatch during summer (Spielman *et al.*, 1985); as the larvae take a blood meal from infected white-footed mice (*Peromyscus leucopus*), they also become infected with *B. microti* later in summer. There may be other small rodents that carry *B. microti* though the white-footed mice are the primary reservoir hosts. Moulting of larvae into nymphs occurs in spring and these infected nymphs feed on humans or mice thereby infecting them with the parasite. From late spring to summer, most babesiosis cases occur (Vannier & Krause, 2012) following the developmental cycle of the ticks. Nymphs moult into adults in fall which feed mostly on the white footed deer. These deer do not get infected with *B. microti*; they provide the adult ticks with blood for proliferation of the tick population. Thus, female ticks lay eggs that hatch into larvae free of *B. microti* (no trans-ovarian transmission) and the cycle continues (Vannier & Krause, 2012) as the larvae moult into nymphs and feed on other hosts. Unlike *B. microti* and other small *Babesia sp*, there is trans-ovarian transmission of large *Babesia sp* from infected adult female to its larvae (Uilenberg, 2006).

On the other hand, there are only two seasons in the tropics. Thus, weather conditions allow for proliferation of ticks throughout the year, hence, possible transmission is year-round. Given the widespread distribution of the tick vectors that transmit *Babesia* species in temperate latitudes, the prevalence of human babesiosis may be underestimated, although the endemic range of babesiosis may never reach the enzootic range of the tick species known to transmit *Babesia* to humans.

## **2.2 The Disease**

Infection of a susceptible host with *Babesia sp* may lead to a disease known as babesiosis. In humans, it is usually qualified as human babesiosis. In animals, it is generally referred to as tick fever; in cattle, it is sometimes called cattle fever or Texas fever.

### **2.2.1 Pathogenesis of *Babesia***

In heavy infections, the primary pathological pathway is haemolysis which leads to anaemia and in some cases jaundice (Gray *et al.*, 2010). Without intervention, decreased number of erythrocytes as a result of haemolysis decreases the efficiency of gas transport leading to anoxia and toxic effects and these may cause organ failure eventually leading to death. It has been observed that the degree of anaemia is not always directly related to the level of parasitaemia, thus, haemolysis does not only occur in infected cells but also in uninfected erythrocytes due to excessive production of pro-inflammatory cytokines causing symptoms like fever, myalgia, renal insufficiency, coagulopathy and hypotension (Clark & Jacobson, 1998; Krause *et al.*, 2007).

### **2.2.2 Disease characteristics**

The disease pattern in symptomatic *Babesia* infected individuals is usually unspecific; just as any fever-causing infection, unspecific flu-like symptoms like fever, chills, body aches,

weakness and fatigue are apparent in *Babesia* infection. Unlike malaria infection, babesiosis presents haemolytic anaemia. Symptoms of the disease become more apparent in humans when the parasites multiply within the blood (Becker *et al.*, 2009). There is usually persistent non-periodic high fever (40-41 degrees Celsius), chills, intense sweats, headache, myalgia and lumbar and abdominal pain. There may also be vomiting and diarrhea (Gray *et al.*, 2010). High levels of haemolysis may lead to jaundice, anoxia, as well as toxic effects, and the disease may cause failure in major organs like the heart, kidneys, liver and lungs (Hunfeld *et al.*, 2008).

### **2.2.3 Laboratory diagnosis**

*Babesia* is generally diagnosed by identifying the parasite on thin blood smears stained with Giemsa or Wright's stain (Vannier *et al.*, 2008). Trophozoites of *B. microti* and *B. duncani* are similar in structure. They are usually pleomorphic rings which could take various forms like oval, pear-shaped, round or amoeboid. In rare instances, tetrads of these species are arranged in a cross-like pattern known as the Maltese cross (Conrad *et al.*, 2006). Merozoites of *B. divergens* and *B. venatorum* typically appear as paired pear-shaped forms and rarely appear as tetrads in human erythrocytes (Hunfeld *et al.*, 2008). Ring forms of *Babesia sp* are similar to that of *Plasmodium falciparum* making it quite difficult to microscopically differentiate *P. falciparum* from *Babesia sp* especially in syndemic areas (Zhou *et al.*, 2014).

However, certain distinguishing features of *Babesia* are the pleomorphic nature of the rings, the presence of extracellular forms in thin blood smears, the absence or rare presence of gametocytes in blood smears as well as the absence of haemozoin in *Babesia* infected erythrocytes (Vannier & Krause, 2012). It has been noted that *Babesia sp* parasitaemia level is often low, ranging from one percent (1%) to ten percent (10%)

though it can be as high as eighty percent (80%) (Meldrum *et al.*, 1992). Due to the low level of parasitaemia, it is advisable to view at least three hundred microscopic fields before reporting (Vannier & Krause, 2012). When blood smears are negative for *Babesia*, yet there is a strong suspicion of babesiosis, a more sensitive and specific technique for detecting *Babesia* DNA in the blood is the polymerase chain reaction (PCR) (Krause *et al.*, 1996). This is a molecular technique used to amplify small, unidentifiable quantities of nucleic acid into identifiable quantities. From 1991 through to 2000, new *Babesia* and *Babesia*-like parasites have been identified and characterised using molecular techniques (Herwaldt *et al.*, 2003). Some of such new *Babesia* strains include the Washington 1 (WA1) (Quick *et al.*, 1993), California 1(CA1) (Persing *et al.*, 1995), Missouri 1 (MO1) (Herwaldt *et al.*, 1996 ) and European Union 1 (EU1) (Herwaldt *et al.*, 2003).

Serological techniques have also been used to diagnose babesiosis in certain cases. An antibody detection test, indirect immunofluorescence assay is the standard for detecting *Babesia* antibodies (Krause *et al.*, 1996). There is also an immunoblot assay for the detection of antibodies against *B. microti* (Ryan *et al.*, 2001).

To add to it, animal inoculation can be done when other laboratory tests are inconclusive. This is done by injecting a laboratory animal with a sample of blood from the patient. Within two to four weeks, species like *B. microti* become present in the blood of the inoculated animal (Krause *et al.*, 1996) if the patient was infected.

#### **2.2.4 Treatment**

Successful treatment of babesiosis with quinine sulphate and clindamycin hydrochloride was first described in 1982 for the treatment of a newborn that was infected through blood transfusion (Wittner *et al.*, 1982). Unfortunately, patients experience side effects like tinnitus, vertigo and gastrointestinal upset with this combination therapy and this has led

to the search for alternative therapy (Chiang & Haller, 2011). The current drug of choice for treatment of *Babesia* infection is a combination of atovaquone and azithromycin; a seven to ten day therapy has been shown to be effective with minimal side effects (Krause *et al.*, 2000). However, it is recommended to treat patients with severe babesiosis with intravenous clindamycin and oral quinine (Wormser *et al.*, 2006).

In 1987, a different way of treating babesiosis was described, known as exchange transfusion where two to three blood volumes are immediately transfused to an infected person, followed by 600mg intravenous clindamycin four times daily and 600 mg oral quinine three times daily (Gorenflot, *et al.*, 1987). Later, a case was reported whereby parasitaemia was reduced from thirty-five percent (35%) to three percent (3%) within a few hours following exchange transfusion (Brasseur & Gorenflot, 1992). It is important to treat people with parasitaemia showing mild or no symptoms to clear the parasites in order to avoid instances of blood transfusion-transmitted *Babesia* infections (Wormser *et al.*, 2006).

### **2.3 Distribution of Human Babesiosis**

Many animals are infected by over 100 *Babesia* species but only a few species have been documented to infect humans (Spielman, 1976; Vannier *et al.*, 2008). *B. microti* causes most cases in the United States, such cases occur in the Northeast and upper Midwest, primarily from May through October (Vannier *et al.*, 2008).

The emergence of babesiosis in these regions was attributed to the increase in white tailed deer population as well as encroachment of local communities on wildlife habitats (Spielman *et al.*, 1985; Hayes & Piesman, 2003). Few other cases caused by *B. duncani* and *B. duncani*-type organisms were identified on the Pacific Coast from northern California to Washington State (Kjemtrup & Conrad, 2000). Random cases of infection

with *B. divergens*-like organisms were found in Kentucky, Missouri, and Washington State (Herwaldt *et al.*, 2004).

In Europe, babesiosis caught medical attention in 1957 and during the early nineties (1990s), nineteen cases had been reported (Brasseur & Gorenflot, 1992). Most of these cases were found in normo-splenic patients who resided in rural areas. Over fifty percent (50%) of the nineteen cases were in France and the British Isles and *B. divergens* transmitted by *Ixodes ricinus* was reported to account for fourteen of the cases. Most reported cases in Europe have been attributed to *B. divergens*, and a few have been caused by *B. venatorum* formerly known as EU1 and *B. microti* (Hunfeld *et al.*, 2008; Gray *et al.*, 2010). *B. microti*-like organisms have been shown to cause illness in Japan and Taiwan, whereas a new *Babesia* agent (KO1 strain) has been identified in South Korea (Kim *et al.*, 2007).

In Africa, Australia, and South America, infrequent cases have been reported (Hunfeld *et al.*, 2008). Cases caused by uncharacterised *Babesia* species were documented; three in Egypt, one in Mozambique, and two in South Africa (Kjemtrup and Conrad, 2000; El-Bahnasawy *et al.*, 2011). A case of *B. divergens*-like infection was reported on the Canary Islands, off the coast of West Africa (Gray *et al.*, 2010).

## **2.4 Review of Case Reports**

Majority of *Babesia* infections in humans have been acquired and or reported in the temperate region especially the United States and Europe (Marathe *et al.*, 2005). Sporadic cases of babesiosis have been reported in other parts of the world aside the United States and Europe. Places like China, Taiwan, Egypt, South Mexico and India have reported few cases of babesiosis.

In the United States, most cases of babesiosis reported have been caused by *B. microti*, then a few others by *B. duncani*, *B. divergens*-like parasites and some new strains. It was found in northeast upper Midwest that *B. microti* caused several hundreds of cases ranging from sub-clinical to fatal in both spleen-intact and asplenic individuals (Herwaldt *et al.*, 1995). In Washington state and California, five cases were recorded due to *B. duncani* in spleen intact patients and four severe-fatal cases in asplenic patients due to CA1-CA4 (California strains) (Gray *et al.*, 2010). Also, three different types of isolates of *B. divergens*-like parasites were reported to cause disease in three asplenic patients each from Washington State, Kentucky and Missouri (Quick *et al.*, 1993; Herwaldt *et al.*, 2003). A fatal transfusion related case of babesiosis due to *B. microti* was reported in a forty-three-year old splenectomised woman who lived in Delaware, a non-babesiosis endemic State. She had Diamond-Blackfan syndrome and had been transfusion dependent for almost her entire life, therefore an investigation of fourteen of her donors revealed that, one donor who resided in New Jersey had elevated antibodies for *B. microti*. Although she was being treated for babesiosis, the patient died three days after admission as a result of acute respiratory distress syndrome and multisystem organ failure related to babesiosis (Zhao *et al.*, 2009).

Away from North America, there was a study by Osorno *et al.* (1976) to determine the presence of *Babesia* infection in Mexico. One hundred and one (101) asymptomatic individuals were screened for *B. canis* using indirect hemagglutination assay (IHA). Thirty-eight (38) individuals reacted and the blood of three of these individuals inoculated in splenectomised hamsters showed *Babesia* in peripheral blood of the hamsters. This was the first documented findings of a latent form of human babesiosis (Osorno *et al.*, 1976). Then in Canada, the first case of human babesiosis was reported as an imported case from Connecticut; a 66-year-old male from Connecticut developed general malaise symptoms

and gastrointestinal distress while passing through Canada after returning from a tour in Alaska. Initial diagnosis after detecting intra-erythrocytic parasites was *P. falciparum* infection but the patient's clinical and travel history made malaria an unlikely diagnosis. After a request to review the patient's microscopic slides, the hematopathologist diagnosed *Babesia* infection. A titre of 1/64 for *B. microti* was obtained with patient's acute serum, confirming his infection with *B. microti* (Kunimoto *et al.*, 1998)

In Europe, cases have been recorded in Austria, Italy, Germany and Portugal. About fifteen years ago, more than thirty (30) cases of human babesiosis reported in Europe had been caused by *B. divergens*, a species that infects cattle (Zintl *et al.*, 2003). In Austria, a case of babesiosis, initially suspected to be malaria was diagnosed in a patient. Microscopy of this patient revealed that erythrocytes were infected with more than one parasite with up to six intracellular ring forms. An antibody test for *Plasmodium* was negative, since the case happened in Europe; clinicians suspected *B. divergens* infection but an antigen test for *B. divergens* was also negative. The patient later mentioned his stay in Massachusetts which prompted physicians to investigate for *B. microti*; PCR amplicons revealed the presence of *B. microti* (Ramharter *et al.*, 2010). The first case of babesiosis in Portugal was reported in a sixty-six year old splenectomised male in 1998. Microscopic examination of the patient's blood showed parasitaemia of about thirty percent (30%) and PCR confirmed infection with *B. divergens* (Centeno-Lima *et al.*, 2003).

In Asia, China recorded two cases but the causative *Babesia* species were not characterised (Zhou *et al.*, 2014); cases of babesiosis caused by *B. microti*-like parasites have been reported in Japan (Saito-Ito *et al.*, 2000) and in an asymptomatic woman in Taiwan (Shih *et al.*, 1997). Korea has reported a severe case due to an ovine *Babesia*-like strain named KO1 (for KOREA 1) in an asplenic patient (Kim *et al.*, 2007). A case of an

intra-erythrocytic parasite suspected to be *Babesia sp* was diagnosed in a fifty-five year old male with a normal spleen in India (Marathe *et al.*, 2005). In this case, extracellular merozoites were seen in the stained thin film of the patient's blood which raised suspicion for *Babesia*, however, tests were done to detect malaria but all turned negative. Although no other tests were done to confirm the presence of *Babesia sp*, the patient was treated for babesiosis and was afebrile after two days of treatment and after seven days, no parasites were seen in his peripheral blood.

## CHAPTER THREE

### 3. MATERIALS AND METHODS

#### 3.1 Study Design

This study was a cross-sectional one. Samples were collected at a point in time from a population without following up on the study subjects after sample collection.

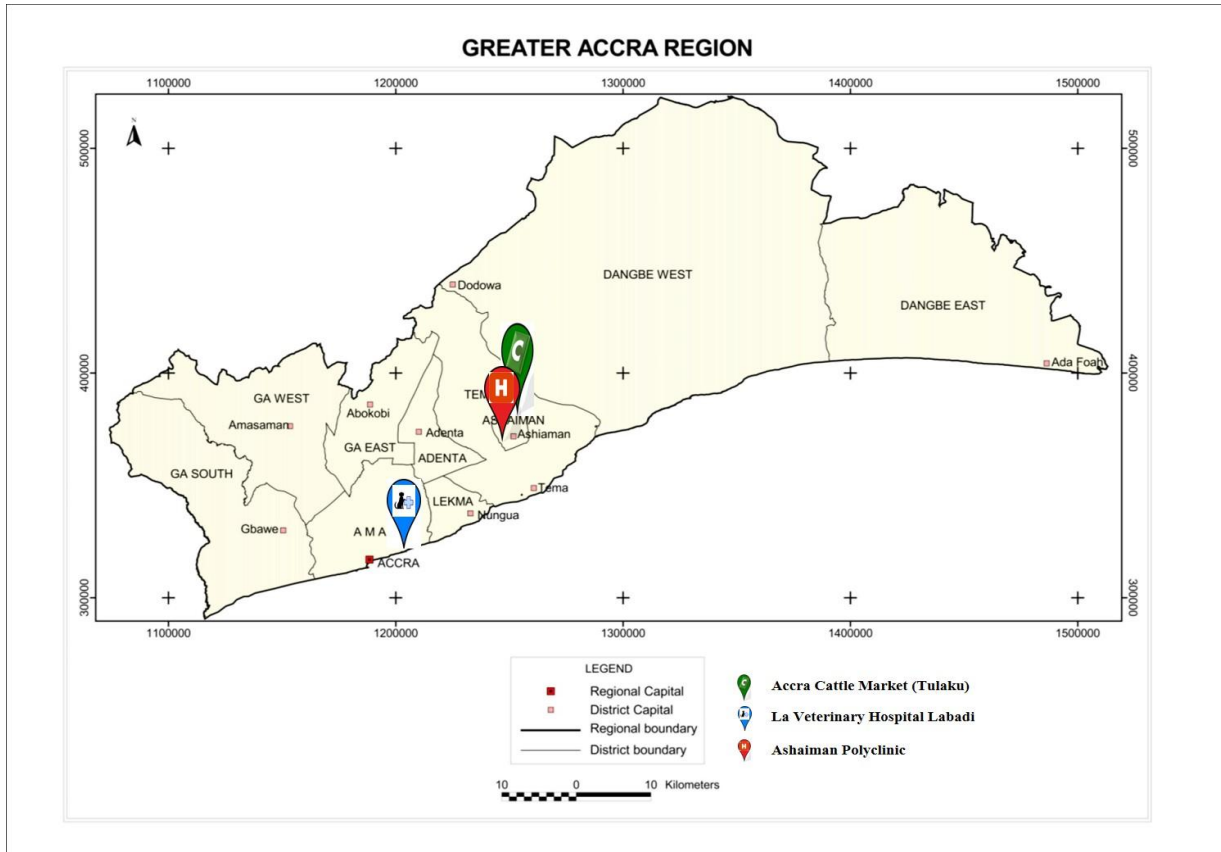
#### 3.2 Study Sites

The whole study was conducted in some parts of the Greater Accra region of Ghana. This is the smallest region in terms of area yet the most densely populated of the ten regions of Ghana. It occupies 1.4 percent (3,245 square kilometers) of Ghana's land surface and has a population of 4,010,045 as at 2010. The capital city of the Greater Accra region is Accra which is also the nation's capital. For political administration, Greater Accra is divided into ten sections; Accra Metropolitan Area, Tema Metropolitan Area, Ashaiman Municipality, Ga East District, Ga West District, Ga South, Dangme East District, Dangme West District, Adenta, and Lekma. The region is at the southern-most part of Ghana and lies on the coast of the Gulf of Guinea with a coastline of about 225 kilometers. The vegetation is mainly coastal savannah shrubs interspersed with thickets. Temperature in Greater Accra ranges between 20 and 30 degrees Celsius with an annual rainfall ranging from 635 millimeters (at areas along the coast) to 1,140 millimeters (at areas relatively distant from the coast). Two rainfall seasons are experienced; April to July and September to October with peaks in June and October respectively (<http://www.ghana.gov.gh/index.php/about-ghana/regions/greater-accra>).

Human subjects for the study were recruited at the laboratory of the Ashaiman Polyclinic which in the Ashaiman Municipality at Latitude: 5.673954, longitude: -0.023797. The laboratory has eight (8) staff members on call who rotate; five work during the day shift and two for the evening shift. It offers a wide range of laboratory diagnosis and serves an average of seventy (70) clients daily.

Cattle blood samples were taken from the Accra Cattle Market at Tulaku also in the Ashaiman Municipality at Latitude: 5.680211, longitude: -0.024091. The market covers an area of about nine hundred square meters (900m<sup>2</sup>). It is the main sales point for most cattle that are brought from places outside Accra especially Northern Ghana, Burkina Faso, Mali and Niger. The main function of the market is to house cattle that are brought in until they are all sold out but a few cattle owners take the opportunity to keep their cattle there for longer periods, thus rearing them. While awaiting sale, the cattle are sent out to graze in the morning around 7am and return around 5pm. The cattle are kept in an enclosed concrete wall which they share with sheep and goat at one side. Within the wall is an open kraal in which some owners keep their cattle. There is a pool constructed to serve as a water source for the cattle as well as an emergency slaughter area for slaughtering animals that become weak after travelling long distances (refer to Figures 11-15 in results). On the market premises are offices for Agriculture Extension Service Department (veterinary service), Environmental Sanitation and Association of Cattle Owners.

The dog blood samples were taken from the La Veterinary Hospital located in Labadi, within the Accra Metropolis at Latitude: 5.603717, longitude: -0.186964. It has five veterinary officers on rotation and provides general clinic and surgery services to a wide range of animals mostly pets and livestock.



**Figure 3: Map of Accra showing approximate locations of the three study sites (Adapted and modified from 2010 Population and Housing Census-GSS, June 2013)**

**Legend:** The **green balloon with the letter “c”** within points to an approximate location of the Accra cattle market, Tulaku; the **blue balloon with a black dog** within points to an approximate location of the Veterinary Hospital, Labadi; the **red balloon with the letter ”H”** within points to the approximate location of the Ashaiman Polyclinic.

### 3.3 Sample Sizes

One hundred and fifty (150) human blood samples, thirty (30) cattle blood samples and thirty-three (33) dog blood samples were collected and used for the study. These sample

sizes were calculated using the formula, 
$$\mathbf{n} = \frac{z^2 \cdot P \cdot (1-P)}{m^2}$$

Where:

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

### **3.3.1 Human subjects**

At a 95% confidence level and a corresponding  $Z$ -value of 1.96; Prevalence ( $P$ ) of 11.5% (0.115) obtained from a study by Hunfeld *et al.* (2002); Margin of error ( $m$ ) of 0.05,

Human sample size =  $(1.96^2 \times 0.115 \times 0.885) / 0.05^2 = 156.39(2dp) \sim 157$  humans.

### **3.3.2 Sample size of sick dogs**

At a 90% confidence level,  $Z = 1.65$ ; Prevalence ( $P$ ) of 14.8% (0.148) from a study by Matjila *et al.* (2004); Margin of error,  $m = 0.1$ .

Sample size = 34.33 ~ 35 dogs.

### **3.3.3 Sample size of sick cattle**

At 90% confidence, estimated prevalence of 50% (0.5);  $Z = 1.65$  and  $m = 0.1$

Sample size = 68 cattle.

However, due to time constraints and elimination of certain samples, sample sizes used for the study were lesser than proposed as indicated earlier but this did not significantly affect the outcome of the study.

### **3.4 Sample Collection**

#### **3.4.1 Review samples**

One hundred (100) human blood samples that had been screened by thick film routine microscopy and reported to be positive for malaria were collected from private medical laboratories in Accra. These samples were re-screened for the presence of *Babesia* in case of possible misdiagnosis or co-infection of *Plasmodium sp* and *Babesia sp*.

#### **3.4.2 Community samples**

Fifty (50) human Blood samples were collected from consenting members of a cattle rearing community in Ashaiman who were diagnosed with malaria after laboratory examination of Giemsa-stained thick blood films at the Ashaiman Polyclinic.

#### **3.4.3 Animal samples**

Whole blood samples were taken from the jugular vein of sick cattle at the Cattle Market in “Tulaku”. The ages of the cattle were determined using the type of dentition they had. Cattle with a pair or more of permanent lower incisors were considered adults and those with milk teeth were grouped as juveniles. Using the presence or absence of the udder as well as inspecting their genitals, the gender of the cattle was determined. Adult females had udders unlike the male, in the juveniles, the presence of the scrotal sac in between the hind legs indicate a male while those with four small teats are female. In cases where checking the genitals becomes difficult, the manner of urination is used; those that urinate from the navel region where their genital organ is located are males whereas those that urinate from under the tail are females.

Blood samples from the cardiac vein of sick dogs visiting the La Veterinary Hospital were also collected. Ticks found on each animal were collected into well labeled, screw-cap, plastic containers.

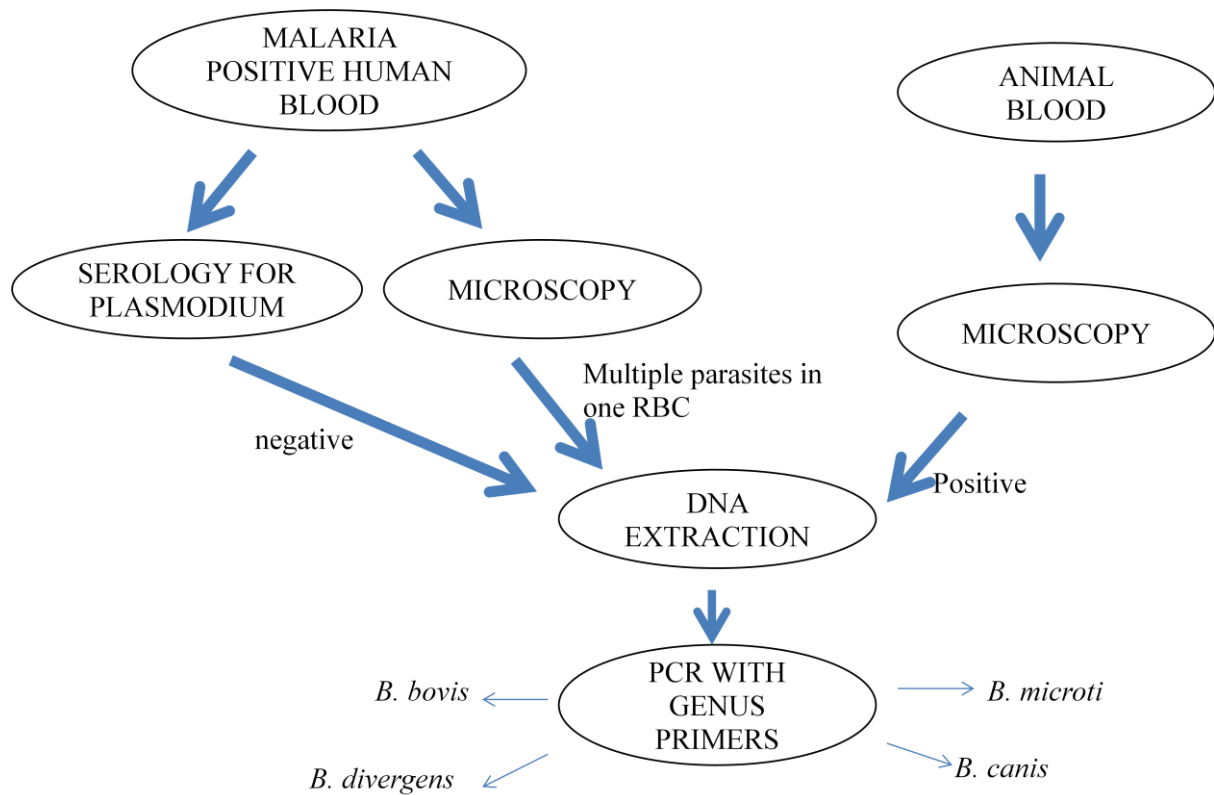
All blood samples were collected into well labeled EDTA tubes and kept in cold boxes for transport to the parasitology laboratory of the Medical Microbiology Department, University of Ghana. About 2 ml venous blood sample was taken from each of the selected subjects for screening. Microscopy and polymerase chain reaction (PCR) were the techniques employed for laboratory detection of *Babesia*.

### **3.5 Laboratory Procedures**

As summarized in Figure 4, blood samples collected were screened for *Babesia* infection by microscopically examining Giemsa stained thin blood smears. Deoxyribonucleic acid (DNA) was extracted from blood samples that were suspected to be infected with *Babesia*.

Blood samples from animals whose thin films showed intra-erythrocytic parasites were selected for DNA extraction; from humans, slides that showed intra-erythrocytic parasites with Babesia-like features such as Maltese cross, multiple parasites and others were selected for DNA extraction. Also, microscopic slides with haemo-parasites that did not react with a rapid diagnostic test kit (Clinogen Diagnostics, Japan) for the four human *Plasmodium sp* were selected for DNA extraction. The kit was designed to detect antigens in whole blood; it contained a membrane strip pre-coated with two monoclonal antibodies as two separate lines across the test strip. One line had antibodies specific for *P. falciparum* histidine rich protein-2 (Pf HRP-2) and another line had antibodies that were pan specific to the lactate dehydrogenase of *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*). Extracted DNA was amplified by polymerase chain reaction (PCR), targeting *Babesia* with a genus specific primer. Positive PCR products were again

amplified using species specific primers for four *Babesia* species; *B. microti*, *B. divergens*, *B. bovis* and *B. canis*. All laboratory safety precautions were followed.



**Figure 4: Flow chart summarising laboratory procedures**

**Small arrows** indicating PCR with species specific primers for *B. bovis*, *B. divergens*, *B. microti* and *B. canis* after PCR with genus primers for speciation

### 3.5.1 Microscopy

Thin and thick blood smears were made on clean labeled glass slides for each blood sample. The smears were air dried after which thin smears were fixed in absolute methanol. Dried smears were then flooded with a one in five (20%) dilution of Giemsa stain and held for 10 minutes. The stain was gently washed off with clean water and the slides air dried. The stained smears were viewed under a light microscope using the oil immersion objective.

### 3.5.2 DNA extraction

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

### 3.5.3 Molecular detection and identification

Polymerase chain reaction (PCR) was used for the detection of any *Babesia* DNA from microscopically positive blood samples by first using genus specific sets of primers to run a nested PCR. Bab5 (5'-AATTACCCAATCCTGACACAGG-3') and Bab8 (5'-TTTGGCAGTAGT TCGTCTTTAACA-3') were used for the first round of amplification and primers Bab6 (5'-GACACAGGGAGGTAGTGACAAGA-3') and Bab7 (5'-CCCAACTGCTCCTATTAACCATTAC-3') for the second round (Wei *et al.*, 2001). PCR products positive for this primer were amplified using species specific primers (Table 1).

**Table 1: Primer sequences**

Species	Primer sequence	Reference
<i>B. divergens</i>	GTTTCTGMCCCATCAGCTTGAC CAATATTAACACCACGCAAAAATTC	(Hilpertshauser <i>et al.</i> , 2006)
<i>B. microti</i>	Bm18Sf-AACAGGCATTCGCCTTGAAT Bm18Sr- CCAACTGCTCCTATTAACCATTACTCT	(Rollend <i>et al.</i> , 2013)
<i>B. canis</i>	BcW-A : CATCTAAGGAAGGCAGCAGG BcW-B: TTAATGGAAACGTCCTTGGC	(Sobczyk & Kotomski, 2005)
<i>B. bovis</i>	5'CACGAGGAAGGAACTACCGATGGTTGA-3' 5'-CCAAGGAGCTTCAACGTACGAGGTCA-3'	(Smeenk <i>et al.</i> , 2000)

The reaction solution for PCR contained 12.5 µl of One Taq 2X master mix with GC buffer (New England Biolabs Inc), 0.5 µl of 10 µM of each primer, 3.5 µl of One Taq high GC enhancer and 8 µl of the DNA in a final volume of 25 µl.

DNA amplification was done using a MiniCycler MJ Research thermal cycler. Nested PCR was carried out for genus identification; species specific primers were used to re-run samples that were positive for the nested PCR. Conditions for the species specific primers are summarized in Table 2 below.

A total of 30 cycles was carried out for nest one; consisting of denaturing at 94°C for 30seconds, 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.

The amplified DNA (5 µl) was then subjected to electrophoresis in 2% agarose gel and detected by ethidium bromide staining and UV trans-illumination. The expected target size was 400-bp and the band size was measured using a 100bp DNA ladder (New England Biolabs Inc.).

**Table 2: Thermo-cycling conditions for species specific primers**

<b>Primer</b>	<b>Denaturation</b>	<b>Annealing</b>	<b>Extention</b>	<b>Cycles</b>	<b>Fragment size</b>
<i>B. divergens</i>	94°C (30seconds)	61°C (30seconds)	72°C (45seconds)	35	353bp
<i>B. microti</i>	95°C (15seconds)	59°C (1minute)	68°C (1minute)	35	104bp
<i>B canis</i>	94°C (2minutes)	60°C (30seconds)	72°C (30seconds)	35	509bp
<i>B. bovis</i>	94°C (30seconds)	63°C (30seconds)	72°C (1minute)	35	350bp

### 3.5.4 Gel electrophoresis

Agarose gel with wells was placed in the electrophoresis gel tank such that the wells were at the negative terminal of the tank. The tank was filled with 1X TAE buffer until the gel was completely covered. A two microlitre volume of blue loading buffer was mixed with ten microlitres of PCR products on a paraffin film. The mixture was carefully loaded into the gel wells submerged in TAE buffer using a micropipette. The 100 base pair ladder was loaded into the first well and PCR products were loaded each into subsequent wells after mixing with the loading buffer. The set-up was run at a voltage of 100V until the dye line was approximately eighty percent of the way down the gel. After a run, the gel was viewed on a ultra-violet light screen and a pictorial view of the gel was captured with a camera.

### 3.6 Statistical Analysis

Descriptive statistics was the main tool employed to analyse the data collected. Tables and graphical displays were used where necessary and appropriate to summarise data. Results on the frequencies of the different *Babesia species* identified were used to determine the most prevalent species. Also the frequencies of samples positive for *Babesia* were used to

determine which vertebrate species (humans, cattle and dogs) carried the highest parasite load.

### **3.7 Research Clearance**

This work received ethical clearance from the Ethics and Protocol Review Committee of the School of Medicine and Dentistry, under the College of Health Sciences of the University of Ghana. Samples were collected with the consent of patients and owners of animals (cattle and dogs) whose blood samples were taken for the study.

## CHAPTER FOUR

### 4. RESULTS

A total of one hundred and fifty (150) human blood samples were collected from patients who had been diagnosed as being infected with *Plasmodium* after examining Giemsa stained thick smear slides of their blood. Giemsa stained thin smears of all such cases were positive for intra-erythrocytic parasites. As indicated in Table 3 below, six (6) of these samples were suspected to contain *Babesia*. This was based on *Babesia*-like features such as multiple parasites infecting a single erythrocyte seen on the slides and/or non-reactivity of infected samples with rapid diagnostic test for *Plasmodium* using a kit that detects the histidine-rich region of *Plasmodium*. These 6 human blood samples were selected for DNA extraction for molecular detection of *Babesia*. PCR using *Babesia* genus primers did not confirm any of these human samples to be infected with *Babesia*.

Ten (10) out of thirty (30) blood samples collected from sick cattle were suspected to be infected with *Babesia* based on microscopy, nine out of these ten samples subjected to PCR were positive with the genus primer. This indicates that, 33% and 30% of the total cattle samples were positive using microscopy and PCR respectively.

Out of 33 blood samples taken from sick dogs, 3 (9%) showed positive for piroplasms with microscopy and only one of the three were positive with PCR.

**Table 3: Summary of Microscopy and polymerase chain reaction (PCR) results from various hosts**

Host	Number of samples taken	Number positive- microscopy (%)	Number positive – PCR (%)
Humans	150	6 (4%)	0
Cattle	30	10 (33%)	9 (30%)
Dogs	33	3 (9%)	1 (3%)

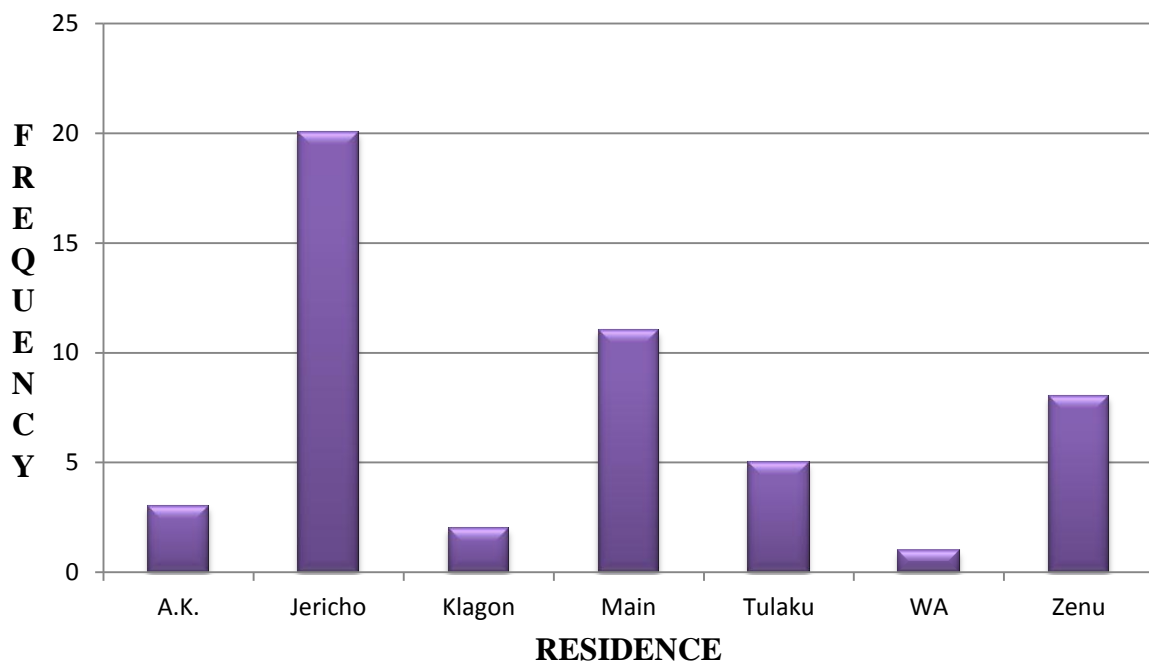
#### 4.1 Human Samples

Human blood samples collected were grouped into two categories; the first category, the “review samples”, were blood samples diagnosed to be *Plasmodium* positive with microscopy and collected from a community that had no cattle rearing activities. One hundred of such samples were taken out of which 96 were reactive to rapid test kit for *Plasmodium* antigen. The four that were non-reactive to the kit were neither positive when subjected to PCR using *Babesia* genus primers. The other category of samples, the “at-risk individuals”, were samples taken from the Ashaiman Polyclinic, an area where inhabitants are considered at risk because they live in a cattle rearing community. Fifty blood samples were collected from fifty patients from the Polyclinic.

Microscopic examination of Giemsa stained thin smears of the samples showed intra-erythrocytic parasites suspected to be *Plasmodium*, but two of the slides showed most of the infected RBCs having more than one intra-erythrocytic parasite with up to six parasites per RBC. There were extra-erythrocytic forms of the parasite and no hemozoin dots were seen on the smear. These features raised the suspicion of *Babesia sp* infection but no amplicons were detected when genus primer sets were used to amplify DNA extracted from the six blood samples suspected to be infected with *Babesia sp*.

#### 4.1.1 Sample size and demographics of humans subjects from the Ashaiman Polyclinic

A total of fifty (50) patients from the Ashaiman community who had tested positive for malaria at the Ashaiman Polyclinic were recruited for the study. Most of them were females making up 68.4% and the remaining 31.6% were males. The ages ranged from two (2) years to fifty-nine (59) years with a mean age of  $22.41 \pm 1.85$ . The age that occurred most (mode) was 25 years with a frequency of five (5). The median age was also 25 years. Information on residential suburbs showed that most of the patients resided in “Jericho” (Figure 5).



**Figure 5: Bar graph showing the frequency residence distribution of human subjects from the Ashaiman Polyclinic**

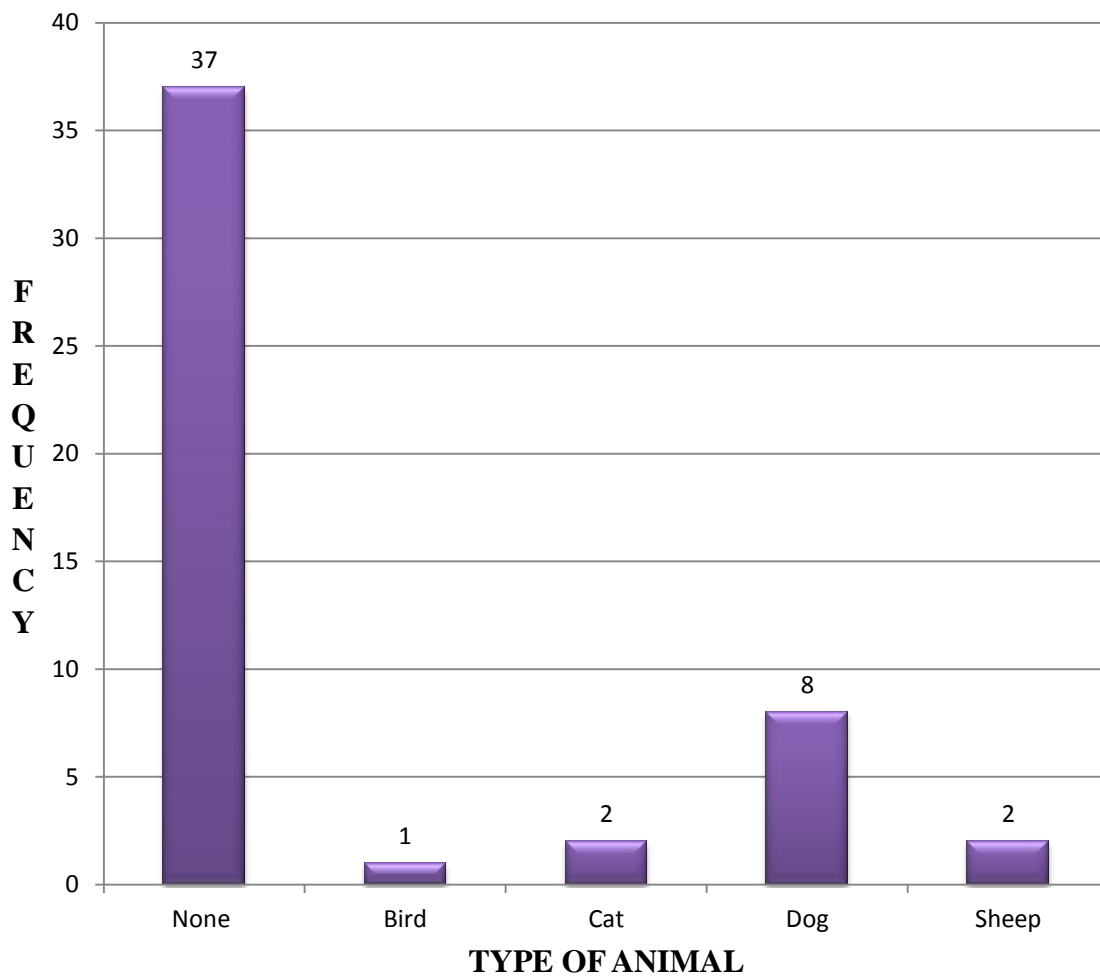
#### 4.1.2 Pre-disposing factors to *Babesia* infection

By way of oral interview, information on certain factors that could predispose an individual to *Babesia* infection was gathered from patients. Information such as, whether

the patient was splenectomised, had been transfused with blood, owned a domestic animal, or had ever been bitten by ticks was obtained from consenting patients. None of the patients had been splenectomised or recalled being bitted by ticks. Three (6%) of the patients admitted to had ever been blood transfused. Most of the patients (74%) did not keep domestic animals in their homes (Table 4). However, those that kept animals had majority being dogs.

**Table 4: Risk factors**

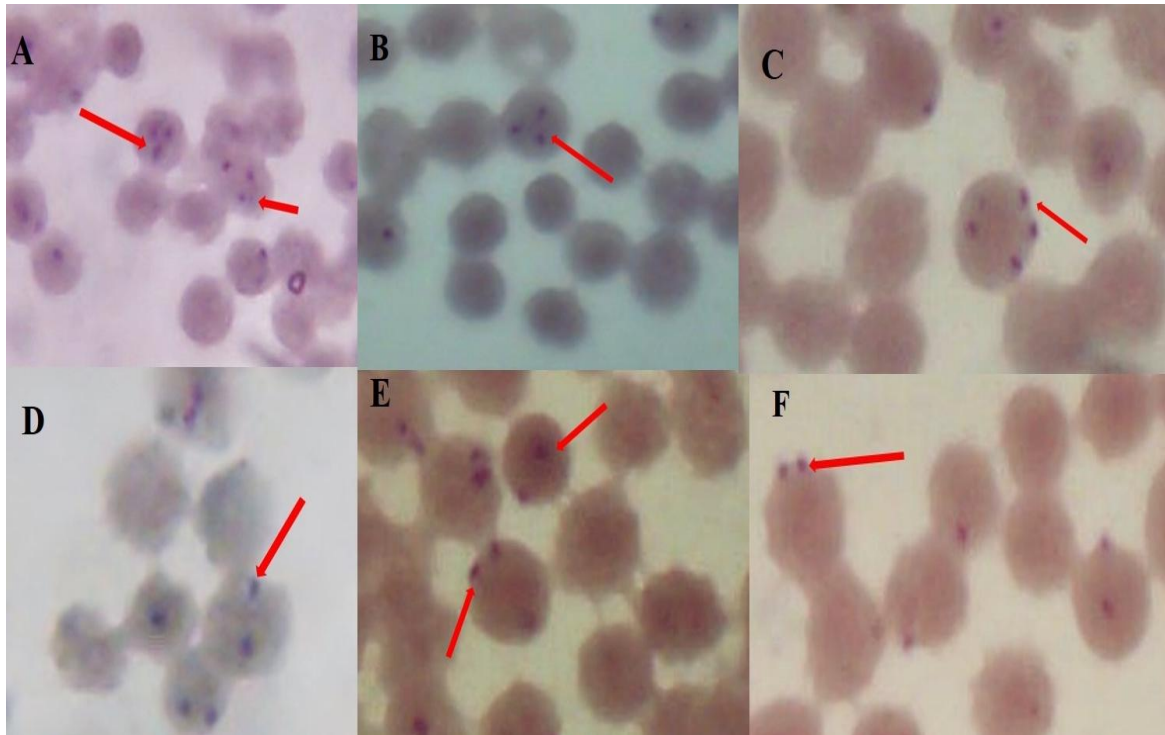
<b>PARAMETER</b>	<b>FREQUENCY</b>	<b>%</b>
<b>BLOOD TRANSFUSION</b>		
YES	3	6
NO	47	94
<b>SPLENECTOMY</b>		
YES	0	0
NO	50	100
<b>TICK BITE</b>		
YES	0	0
NO	50	100
<b>POSSESION OF DOMESTIC ANIMAL</b>		
YES	13	26
NO	37	74



**Figure 6: Frequency distribution of the type of domestic animals owned by human subjects from the Ashaiman Polyclinic**

#### **4.1.3 Microscopic detection of *Babesia* in human samples**

Pictures of multiple infections in red blood cells of the patients are shown in Figure 7. Various parasite configurations were observed; tetrad configuration (Figure 7C) in which three parasites aligned at one side in an arc-like array with the fourth parasite directly above the middle parasite in the arc; the triad configuration (Figure 7A&B), seen as three parasites arranged as if to form the three vertices of a triangle; paired parasites were also observed (Figure 7 D, E & F). Some of the parasites were positioned at the peripheries of the red blood cells (Figure 7 C, E&F).

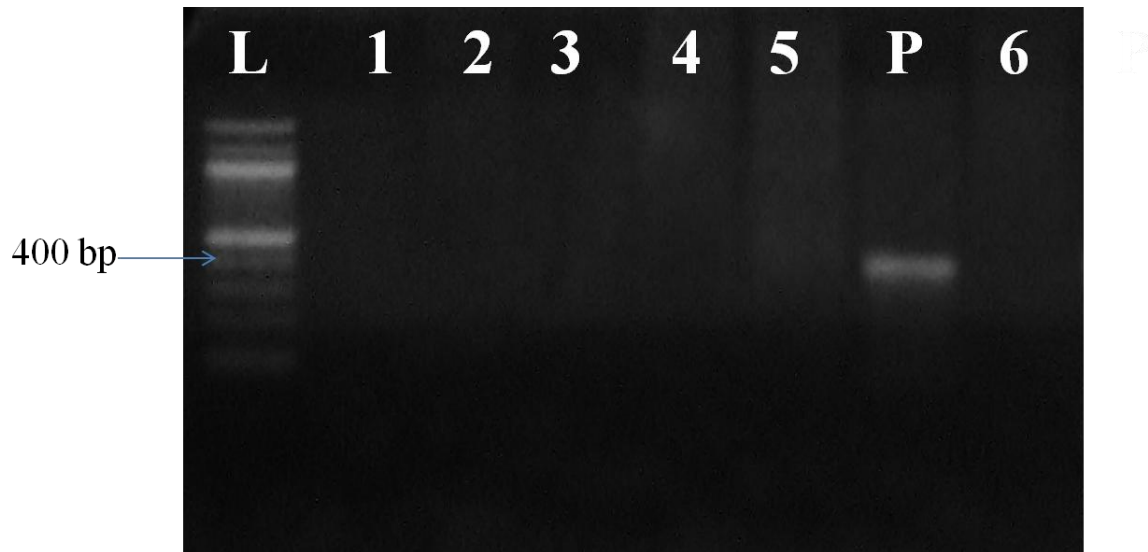


**Figure 7: Blood stages of intra-erythrocytic parasites as seen in thin blood films of human subjects**

**LEGEND:** C: tetrad parasite configuration infecting an erythrocyte; A&B: “triad” parasite configuration found infecting red blood cells; D, E&F: paired parasites infecting erythrocytes.

#### **4.1.4 Molecular detection of *Babesia* in humans**

DNA was extracted from the six (6) human blood samples suspected to be infected with *Babesia*. Extracted DNA was subjected to molecular detection using PCR with genus primer sets for *Babesia sp.* *Babesia* genes were amplified in none of the samples (Figure 8). Thus, *Babesia* infection was suspected in six of the samples but none of them was confirmed by PCR to be positive.



**Figure 8: Agarose gel showing PCR results with genus primers for *Babesia* on DNA extracted from human blood**

**LEGEND:** Lane L- 100bp DNA ladder; Lanes 1-6: human samples after PCR showing no bands  
Lane P: positive control

## 4.2 Cattle Samples

### 4.2.1 Demographic data of cattle

There were more adults (83.3%) than juveniles (16.7%) and only one of the infected cattle was a juvenile, the rest (8) were adults. Most of the cattle sampled were males (90%) while the remaining three (10%) were females.

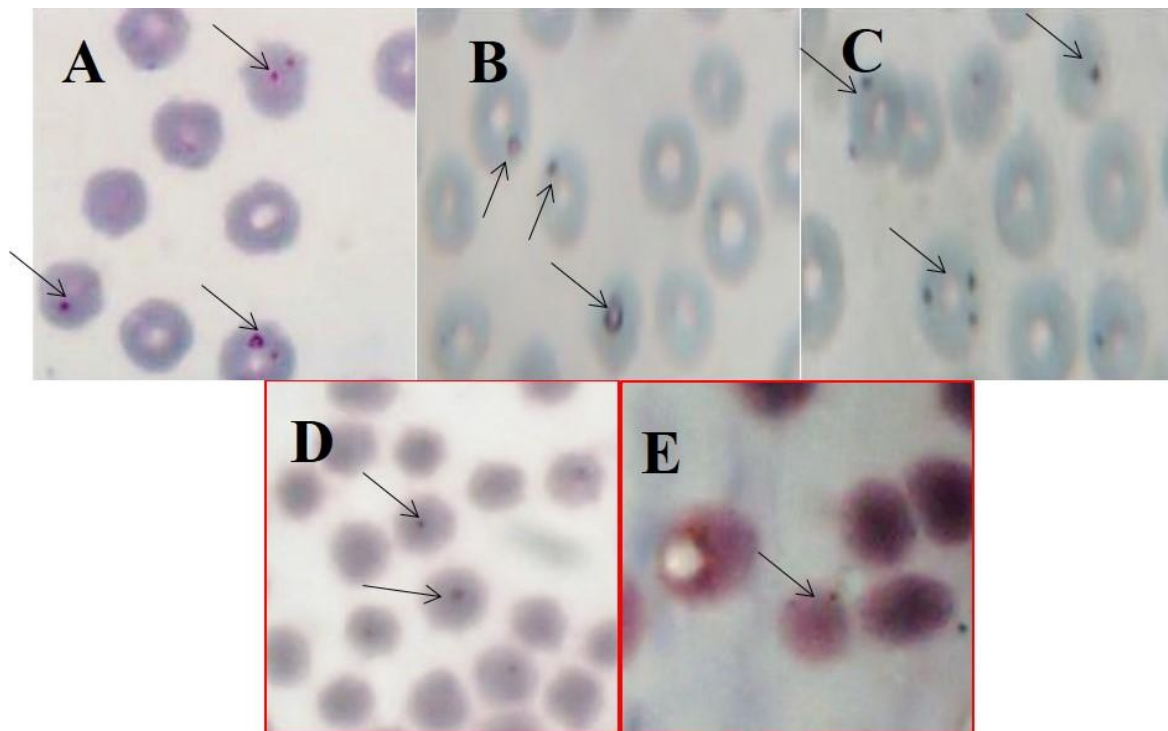
Half of the cattle had weak joints which was the most prevalent symptom followed by dermatitis (23.3%). The least frequent symptom was weight loss which was recorded in 10% of the cattle sampled. Each of the listed symptoms recorded at least one case of *Babesia* infection and the highest number of infections (4/8) was recorded in cattle with weakness (Table 5).

**Table 5: Demographic information on cattle**

PARAMETER	FREQUENCY	%	NO. INFECTED
<b>AGE</b>			
ADULT	25	83.3	8
JUVENILE	5	16.7	1
<b>GENDER</b>			
MALE	27	90	7
FEMALE	3	10	2
<b>SYMPTOMS</b>			
WEAKNESS	15	50	4
DERMATITIS	7	23.3	1
WEIGHT LOSS	3	10	1
ANOREXIA	5	16.7	3

#### 4.2.2 Microscopic detection of *Babesia* in blood samples of cattle

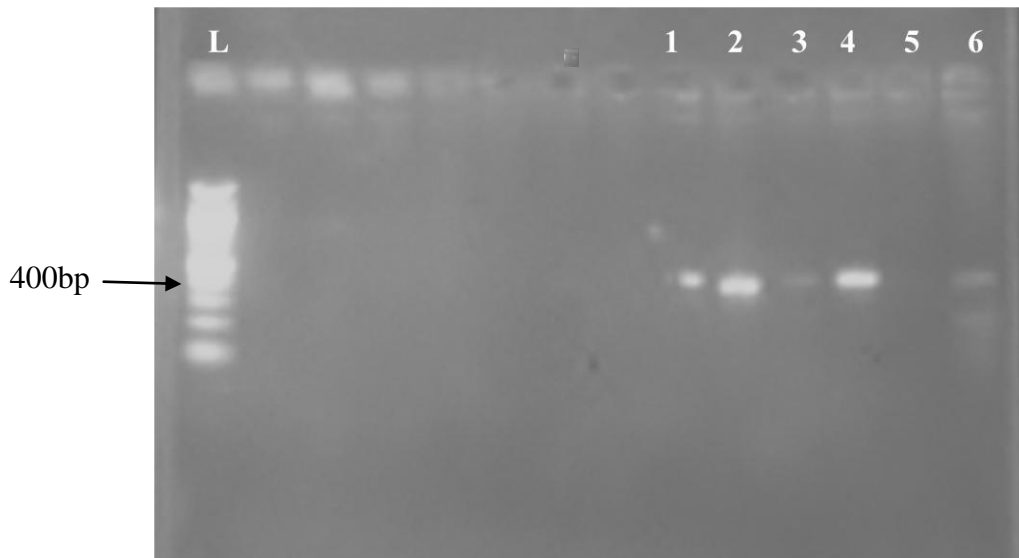
Intra-erythrocytic parasites were observed in ten (10) of the thirty cattle samples. No tetrad configuration was observed, there were few red blood cells infected with parasites in a triad configuration (Figure 9 C). Most of the infected erythrocytes had one or two parasites infecting them. Slides 8 A, B and C are slides from some of the nine samples that were confirmed to be infected with *Babesia sp.* Slides 8 D and E (red bordered) are from the single cattle sample that was not confirmed positive by PCR though intra-erythrocytic parasites were observed under the microscope.



**Figure 9: Blood stages of intra-erythrocytic parasites as seen in thin blood films of cattle blood samples. Arrows showing intra-erythrocytic parasites**

#### **4.2.3 Molecular detection of *Babesia* in cattle**

The ten samples that showed infected erythrocytes were subjected to PCR using genus primers after DNA extraction. Gel electrophoresis of the PCR products revealed 400 base pair bands in nine of the samples. Figure 10 shows a picture of one of the gels used.



**Figure 10: Agarose gel showing PCR results with genus primers for Babesia on some DNA samples extracted from cattle blood.**

**LEGEND:** Lane L - 100 base pair DNA ladder; Lanes 1-6 - PCR products showing band sizes of 400 base pairs.

#### 4.2.4 Cattle and housing conditions at the Accra Cattle Market

Below are pictures of cattle and their housing conditions at the Accra Cattle Market.



**Figure 11: A herd of cattle returning from grazing**



**Figure 12: Different cattle belonging to different owners housed in the same kraal. This makes it easier for ticks to transmit pathogens from cattle to cattle**



**Figure 13: Close contact of cattle owners, buyers and herdsmen with cattle at the Accra Cattle Market. This makes tick bites and subsequent transmission of *Babesia* possible**



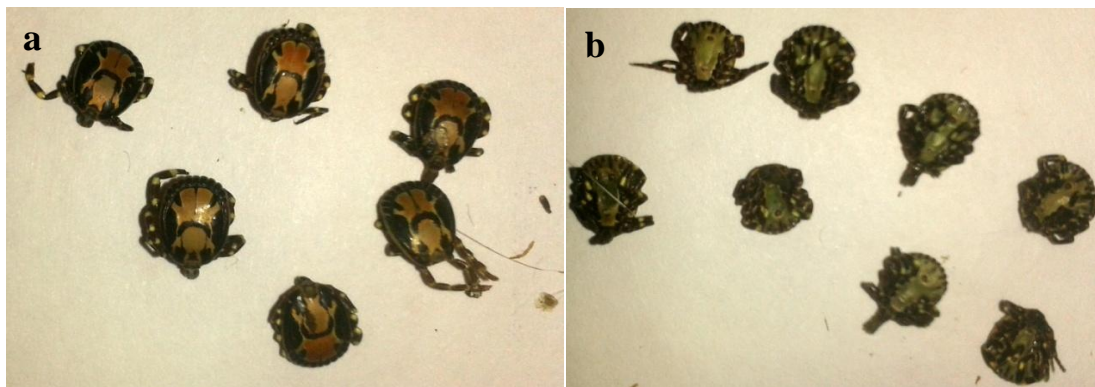
**Figure 14: Cattle with weak joints, thus cannot join the entire herd to graze**



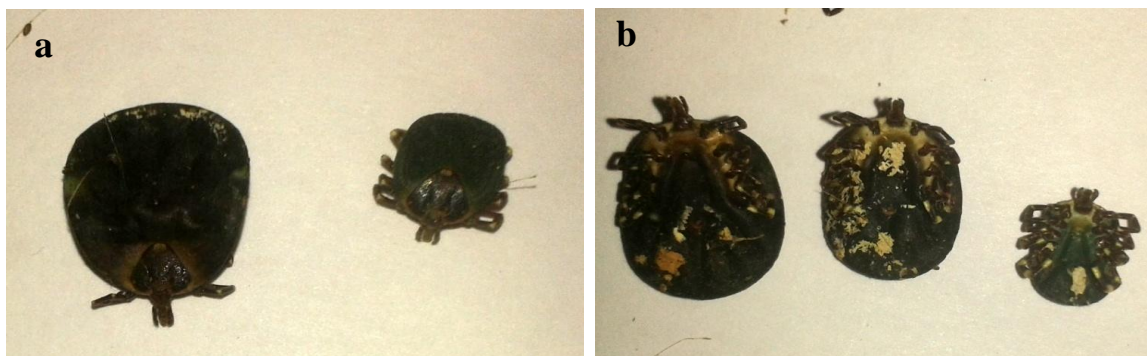
**Figure 15: An un-kept water supply from which cattle drink. Seen in the picture is a herdsman fetching some of the water for incapacitated cattle that cannot move to the pool to drink**

#### 4.2.5 Ticks on cattle

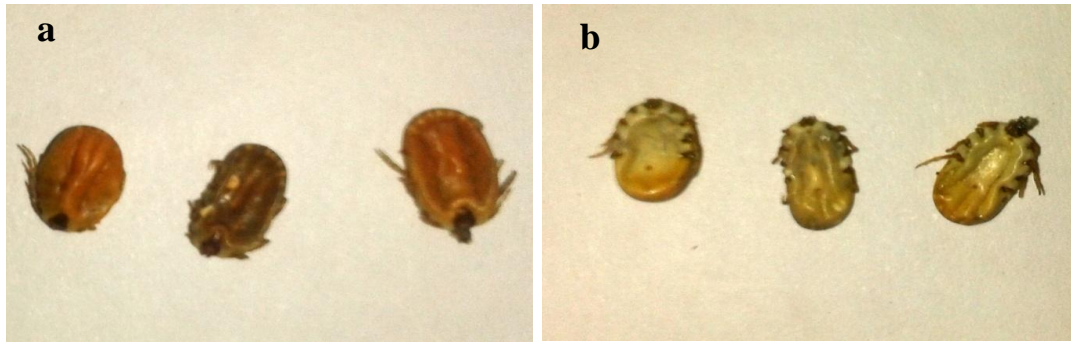
Three genera of hard ticks were collected from cattle at the Accra cattle market to have an idea of the ticks that these cattle were hosting. Five (5) *Boophilus* ticks were collected and they had an average length of 20 millimeters (Figure 19). Thirty-two (32) male *Amblyoma variegatum* with average length of 5.5 millimeters (Figure 16) and six (6) females of the same species with average length of 8 millimeters were collected (Figure 17). Three (3) *Rhipicephalus praetextatus* with average length of 5.6 millimeters (Figure 18) were collected.



**Figure 16: Dorsal (a) and ventral (b) views of male *Amblyoma variegatum* (tropical bont tick)**



**Figure 17: Dorsal (a) and ventral (b) views of female *Amblyoma variegatum* (tropical bont tick)**



**Figure 18: Dorsal (a) and ventral (b) views of *Rhipicephalus praetextatus* (cattle leg tick)**



**Figure 19: Dorsal (a) and ventral (b) views of *Boophilus* sp**

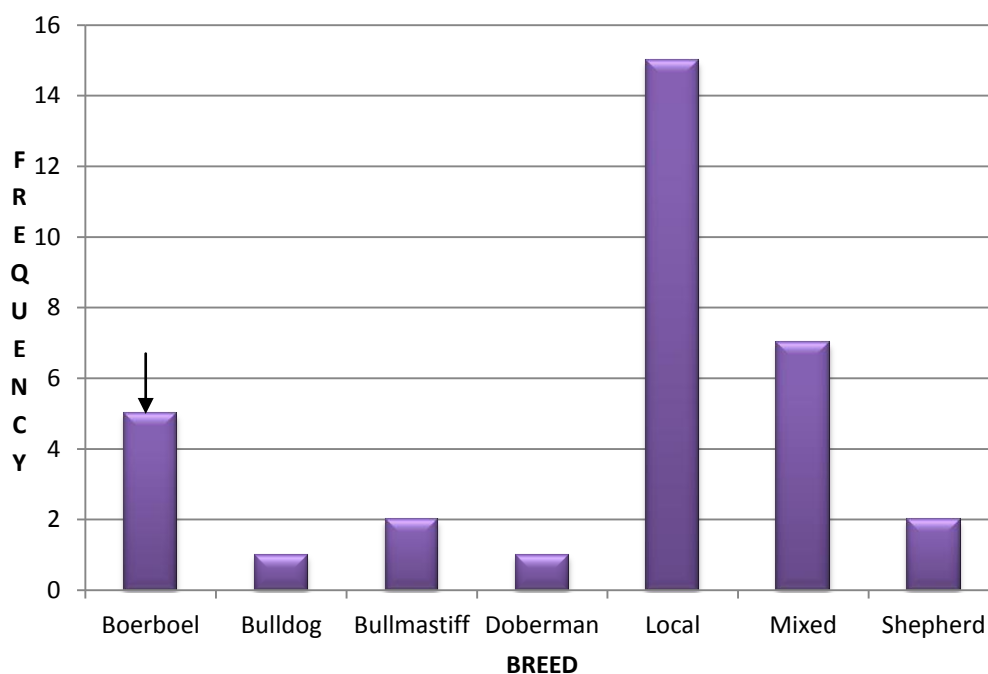
### 4.3 Dog Samples

#### 4.3.1 Demographic data of dogs

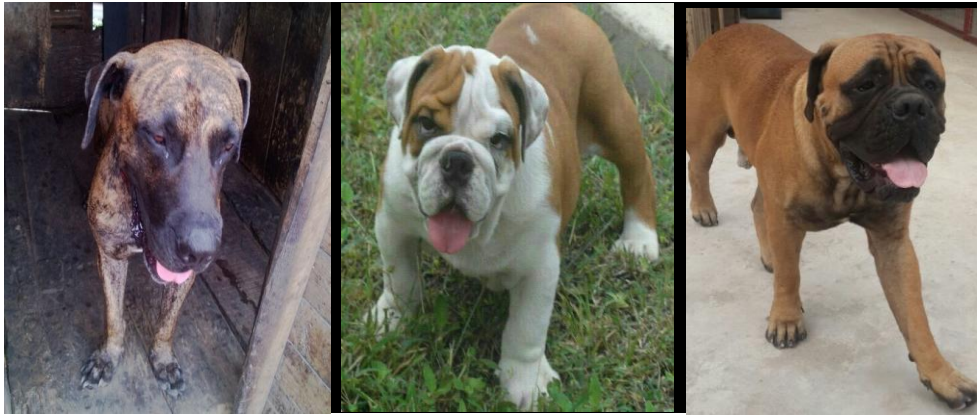
There were slightly more male (54.55%) than there were female (44.45%) dogs. Ages of dogs ranged from 2 months to 108 months with a mean age of 25.88 months  $\pm$ 4.45. The median age was 19 months and the modal age was 24 months. The dog in which *Babesia canis* was detected was an 8-month old male (Table 6). Most of the dogs sampled were local breeds (Figure 20) and most of them lived in Labadi (Figure 22). Pictures of some of the breeds of dogs visiting the La Veterinary Hospital are presented in Figure 21.

**Table 6: Age and gender of dogs from La Veterinary Hospital**

PARAMETER	FREQUENCY	%	NO. INFECTED
<b>AGE (months)</b>			
0-12	12	36.36	1
13-24	10	30.30	0
25-36	5	15.15	0
37-48	0	0	0
49-60	3	9.09	0
61 and above	3	9.09	0
<b>GENDER</b>			
MALE	18	54.55	1
FEMALE	15	45.45	0



**Figure 20: Distribution of the different breeds of dogs visiting the La Veterinary Hospital.** Black arrow is indicating the breed of the infected dog



A brindle boerboel

A two-month old bulldog

A bullmastiff

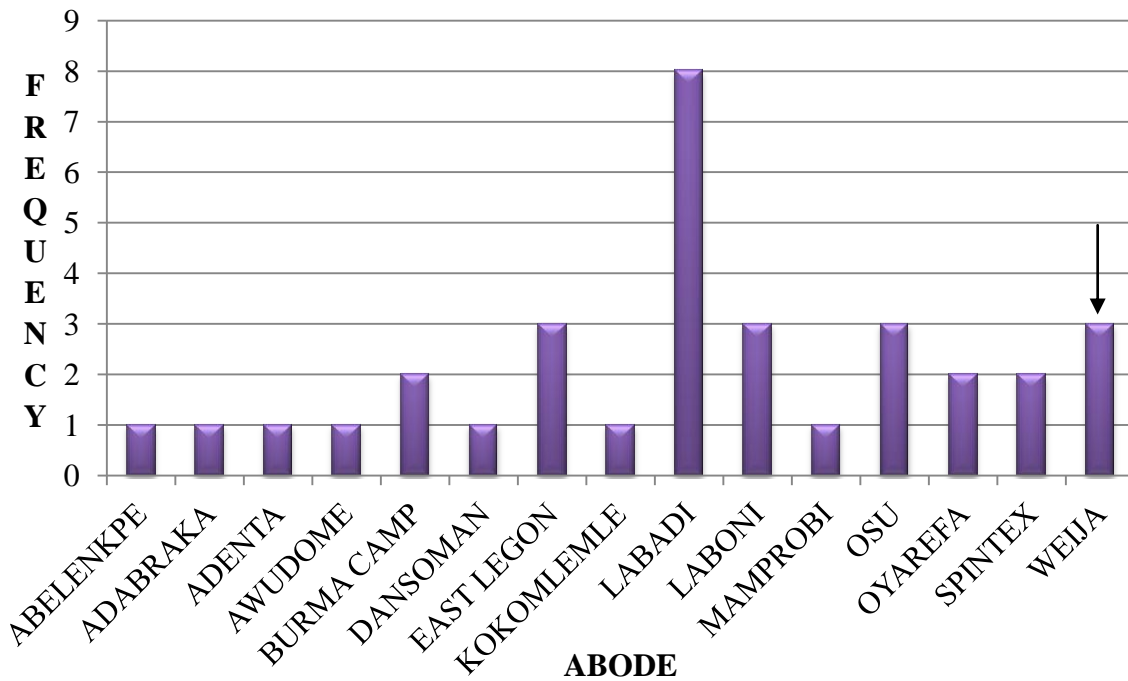


A Doberman

A local breed

A hybrid/mixed breed (local/Doberman)

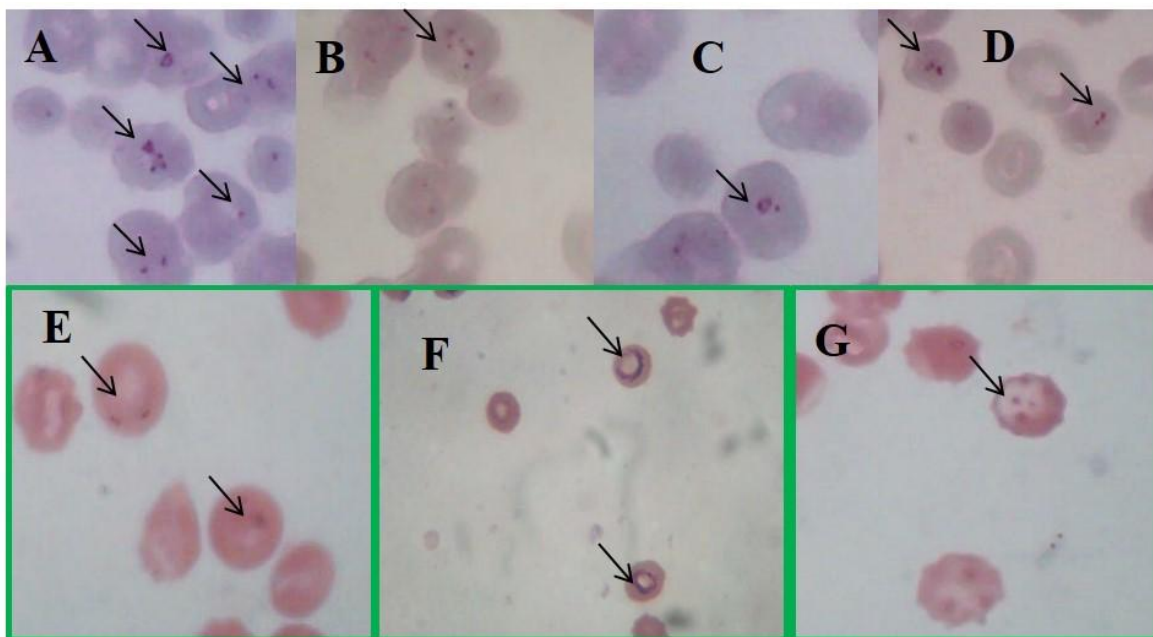
**Figure 21: Pictures of some dog breeds visiting the La Veterinary Hospital**



**Figure 22: Frequency distribution of where dogs visiting the La Veterinary Hospital live. Black arrow is indicating where the infected dog lived**

#### 4.3.2 Microscopic detection of *Babesia* in dogs

Intra-erythrocytic parasites were microscopically detected in three (3) of the thirty-three (33) dog blood samples. Some red blood cells were infected with more than three parasites (Figure 23: slides A and B), there were few red blood cells infected with parasites in a triad configuration (Figure 23G). Most of the infected erythrocytes had one or two parasites infecting them. A complete intra-erythrocytic ring was observed in one of the samples (Figure 23F). Slides E to G of figure 23 (green bordered) show slides from the single dog sample that was confirmed, infected with *Babesia canis*. Slides A to D are from the two dog blood samples that were not confirmed positive for *Babesia* infection by PCR although intra-erythrocytic parasites were observed in them.

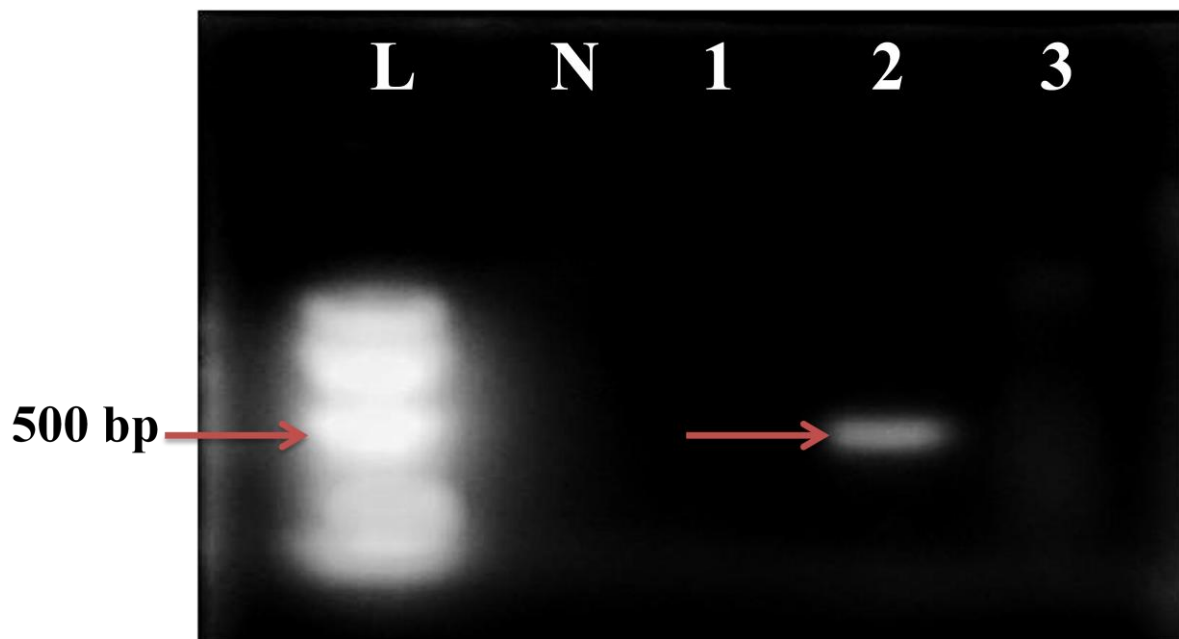


**Figure 23: Giemsa-stained thin blood smears from dogs with arrows showing intra-erythrocytic parasites**

**LEGEND:** A-D: portions of slides from two samples that were not confirmed by Pcr to be infected with *Babesia*; A & C: portions of the same slide; B & D: portions of the same slide; E-G: portions of a slide from the sample that was confirmed by PCR to be infected with *Babesia sp.*

#### 4.3.3 Molecular detection of *Babesia* in dogs.

One sample was confirmed to be infected with *Babesia sp* out of the 33 blood samples obtained from dogs. This single blood sample was subjected to a second run of PCR with three different species primers for Babesia. Amplification of DNA was observed in the PCR run with *B. canis* primers (Figure 24).

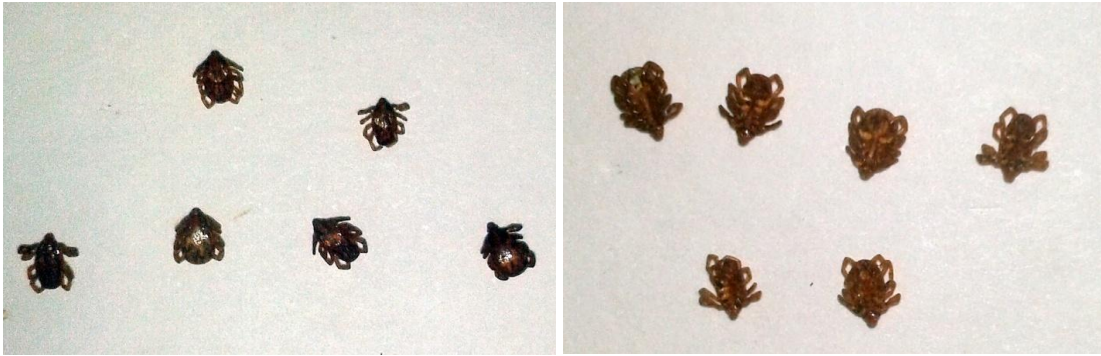


**Figure 24: Results of PCR amplification for speciation of Babesia from a dog**

**LEGEND:** Lane L- 100 base pair DNA ladder with arrow showing the 500 base pair band along which amplified products were expected to align. Lane N- negative control; Lane 1- PCR with *B. microti* primers showing no band; Lane 2- PCR with *B. canis* primers; arrow showing a band size around 500bp; Lane 3- PCR with *B. divergens* primers

#### 4.3.4 Ticks found on and around dogs at the La Veterinary Hospital

Ticks were collected from the inside of the ears and between the digits of dogs. Most of the hard ticks found on the dogs were the brown dog ticks (*Rhipicephalus sanguineus*) and argacid ticks (Figures 25-27).



**Figure 25: Dorsal and ventral views of questing *Rhipicephalus sanguineus* collected at the La Veterinary Hospital**



**Figure 26: Dorsal and ventral views of *Rhipicephalus sanguineus* picked from dogs visiting the La Veterinary Hospital**



**Figure 27: Dorsal and ventral views of argasid ticks picked from dogs visiting the La Veterinary Hospital**

## CHAPTER FIVE

### 5. DISCUSSION

#### 5.1 *Babesia* in Cattle

In this study, *Babesia sp* was detected mostly in cattle as compared to the dogs with percentage infections of 33% and 30% for microscopy and PCR respectively as against 9% and 3% in dogs. This may be due to the housing systems of these two animal groups; several cattle share one kraal but each dog whose sample was taken came from a separate home. This implies that, it is more efficient for an infected tick to move from one cattle to the other in the case where these cattle share the same kraal than for an infected tick to move from one dog to the other in a different home. It is also quite difficult to control ticks in this kind of cattle housing system where various farmers/cattle owners share the same kraal in which they keep their cattle as was observed at the Accra Cattle Market in Tulaku (Figure 12). Thus, if one farmer puts measures in place to control ticks on his farm, the practice will not be effective if other farmers do not check ticks on their cattle as well.

Microscopy revealed intra-erythrocytic parasites in ten (10) of the cattle samples out of which PCR confirmed nine to be positive when run against a genus primer set for *Babesia*. A comparison of intra-erythrocytic parasites seen in the blood sample that was negative for *Babesia sp* infection after PCR to those that were Positive for *Babesia* infection with PCR showed no significant morphological difference (Figure 9). The single cattle sample that was negative for *Babesia* with PCR though showed intra-erythrocytic parasites might have been infected with pathogens other than *Babesia sp*. *Theileria mutans*, *Theileria*

*velifera* and *Anaplasma sp* are among haemoparasites that occur as cytoplasmic inclusions in red blood cells.

These parasites have been reported to infect cattle in Ghana with point prevalences of 92%, 44% and 12% respectively (Bell-Sakyi *et al.*, 2004). Also, some ticks collected from some cattle in this study (Figures 16, 17 &19) are known important vectors for some of these haemoparasites as will be discussed later in this section.

None of the nine samples that were confirmed positive for *Babesia* infection showed amplicons after subjecting them to PCR with four species specific primers for *Babesia*. Thus, none of these samples were confirmed to be infected with *B. bovis*, *B. divergens*, *B. microti* or *B. canis* using the respective primer sets for amplification with PCR.

Inability to detect the *Babesia* species infecting the cattle could be due to the fact that, none of the strains amplifiable by these primers were present in those nine samples from the cattle. *B. microti* and *B. canis* are not species known to infect cattle; therefore inability to detect amplicons after PCR with primers for *B. microti* and *B. canis* was quite expected. On the other hand, neither of the two species known to infect cattle, *B. bovis* and *B. divergens* were detected in any of the nine cattle samples after PCR with the respective primers; this may have been due to the fact that the *Babesia* species infecting these cattle are species other than the ones mentioned above. Other species like *B. bigemina* have been known to infect cattle in Ghana with a prevalence of 6% (Bell-Sakyi *et al.*, 2004); this species might have been the infecting parasite instead of *B. bovis* and *B. divergens*. To resolve this shortfall, genome sequencing can be done to determine the strains infecting these cattle, as was done in Korea, Washington and California to detect strains KO1, WA1 and CA1 respectively (Kim *et al.*, 2007; Quick *et al.*, 1993; Persing *et al.*, 1995).

Nevertheless, *Babesia sp* was detected in cattle, indicating that these livestock were infected with the parasite and to infer, there is the possibility of parasite transmission from the cattle to the people who are in close proximity with them as seen in Figure 13.

Thus, the possibility of tick bites and subsequent infection with *Babesia sp* in humans is higher in individuals who associate closely with the cattle. These people may have over the years built immunity to, or coexisted with the parasite and so remain asymptomatic to the infection. The danger however, lies with asymptomatic cases donating blood to already immuno-compromised patients, thus transmitting the infection which may cause disease in these patients. This is evident in a case in the United States where a patient who lived in a babesiosis non-endemic region was diagnosed with babesiosis; it was later confirmed that she contracted the infection earlier from her blood donor who lived in an endemic area (Zhao *et al.*, 2009).

Though the species infecting the cattle in this study were not known, literature has it that, *B. divergens* which naturally infects cattle has been reported to cause most of the severe cases of human babesiosis in Europe (Brasseur & Gorenflot, 1992). Also, another *Babesia* of cattle, *B. bovis* was the species that caused illness in the first case of human babesiosis in 1956, reported by Skrabalo and Deanovic (1957). Therefore, there is a possibility that any of the strains of *Babesia* infecting these nine cattle could be zoonotic. To add to it, a case reported in Korea showed that the strain detected in the patient was closely related to *Babesia sp* in sheep (ovine) than the species known to infect humans (Kim *et al.*, 2007). This is evident that the number of zoonotic *Babesia* species has been underestimated and thus any species should be considered potentially zoonotic.

Ticks found on some of the cattle were *Amblyoma variegatum* (tropical bont tick) (Figures 16 & 17) which is an important vector of some *Ehrlichia* species which cause bovine

ehrlichiosis and heartwater in cattle, goats and sheep. They also transmit *Theileria sp* that cause benign bovine theileriosis.

*Rhipicephalus praetextatus* was also found (Figure 18); these ticks feed mostly at the limb joints of cattle but have been known not to transmit any pathogens. However, they can cause toxicosis leading to paralysis in their hosts. Also, *Boophilus sp* (Figure 19), an important vector of *B. bovis*, *B. bigemina* and *B. divergens* was also collected from some cattle. Some *Boophilus sp* transmit *Anaplasma marginale*, *Anaplasma centrale* (causative organisms of anaplasmosis) and *Borrelia theileri*.

## **5.2 Babesia in Dogs**

Dogs have been known to be infected with *B. canis* complex none of which have been reported to cause disease in humans so far, although a sero-prevalence study in Mexico recorded about 37.6% of the study population having antibodies against *B. canis* (Osorno *et al.*, 1976). This was evidence of infection of the humans with *B. canis* but none of them had had the disease after infection. There has also been a case of babesiosis in an Egyptian boy suspected to have been contracted from his pet dog (El-Bahnasawy *et al.*, 2011). These reports informed the inclusion of dogs in this study and since dogs are closest to humans as compared to cattle, it was necessary to investigate if there was the slightest chance that dogs could also be infected with *Babesia* species that infect humans.

Microscopy revealed that blood samples from three (3) dogs contained intra- erythrocytic parasites which were assumed to be *Babesia* species but PCR confirmed *Babesia* infection in only one sample when the genus primer sets were used. One could argue that what were seen on the microscopic slides were mere artifacts but they were as much similar to what were seen on the confirmed sample's slide (refer to Figure 23). Far from being artifacts, the two groups of uncharacterised intra-erythrocytic parasites could be other organisms

other than *Babesia*, thus, explains why they were not detected with PCR using *Babesia* primers. Also, the ticks found on the dogs and those that were questing, were mostly the brown dog ticks (Figures 25 & 26) which are important vectors of *Babesia canis* and *Ehrlichia canis* causing disease in dogs. They are also vectors of *Rickettsia conorii* which causes Mediterranean spotted fever in humans and may also transmit *Coxiella burnetii* causing Q-fever.

The single sample that was confirmed by PCR to be positive for *Babesia species* was subjected to another round of PCR, this time using a set of primers that amplify *B. canis*. As suspected, the dog parasite, *B. canis* was detected in this sample. It is pertinent however, to know that these dogs are infected with *Babesia* even if none of the species detected have been known to cause disease in humans. Nevertheless, dog owners and dog keepers should ensure that ticks are controlled on their dogs, even if not for their own health, for the health of their dogs. They should as well minimise direct contact with the dogs to reduce chances of being bitten by ticks, because, brown ticks as noted earlier are vectors of human pathogens that cause spotted fever and Q-fever and the fact that human *Babesia sp* has not been detected in dogs yet does not mean that such an event is not possible. A study in Mexico revealed that thirty-eight out of one hundred and one (101) asymptomatic individuals were found to carry antibodies against *Babesia canis* (Osorno *et al.*, 1976) indicating that, they had one time or the other been infected with the parasite although disease resulting from *B. canis* infection has not been reported. The disease had not manifested in these individuals probably because they were immuno-competent, however, we might not be able to say same about immuno-compromised individuals.

### **5.3 Babesia in Humans**

This study did not confirm *Babesia* infection in the blood samples of malaria positive patients, although six (6) were suspected to be infected with intra-erythrocytic parasites other than *Plasmodium sp.*

However, the presence of the infection in cattle and dogs suggest possible infection in humans due to the fact that these are animals that humans in Accra and Ghana associate closely with. The major limitation of this study which played a role in the outcome of the study was the lack of cooperation of cattle herdsman making them not consent to partake in the study. From Figure 13, these people who work with the cattle associate more closely with the animals than the mere inhabitants of the community. Therefore the chances of detecting zoonotic *Babesia* species in humans will be higher in those who are in close association with them than other population.

## CHAPTER SIX

### 6. CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

From this study, the prevalence of *Babesia* infecting dogs visiting the La Veterinary Hospital in April was 3% after using microscopy for initial detection and PCR to confirm. Prevalence of *Babesia* infection in cattle at the Accra Cattle Market was 30%. Thus, per the second specific objective, sick cattle and dogs were screened and *Babesia* infection was detected in 3% of sick dogs and 30% of sick cattle. This supports the hypothesis that cattle and dogs in Accra are infected with *Babesia sp* but as to whether these species infect humans was not determined. *Babesia* infection was not confirmed in any of the one hundred and fifty human blood samples used in this study as was set out to achieve in the first objective. However, the detection of *Babesia sp* in the animals indicates the possibility of a zoonotic transmission from these animals to humans who live in close proximity to them. There should therefore be no haste to dismiss the possibility of zoonotic *Babesia* in our setting.

#### 6.2 Recommendations

The study of *Babesia* infection is very limited in Ghana and this study can be considered as one of the baseline assessments of *Babesia* infection in various hosts in Greater Accra Ghana. In order to consolidate the knowledge in this area to inform policy, the following recommendations are being made:

1. Phylogenetic analysis of the unspecified cattle *Babesia* found in this study should be done in order to know how close these parasites are to the ones known to infect humans.

2. Future works in this area should include all persons presenting with malaria-like symptoms as well as a larger sample size. Such a study should investigate all samples with Microscopy, Serology and PCR in order to ascertain the specificity and sensitivity of these methods to the detection of *Babesia sp* in blood samples.
3. A study to investigate *Babesia sp* infection in specific groups like seemingly resistant malaria cases, blood donors, hemolytic anaemia or jaundiced cases as well as splenectomised and other immune-compromised individuals should be conducted in order to determine which groups of people with certain clinical conditions are most at risk of babesiosis.
4. There should also be community based studies including asymptomatic people for the detection of *Babesia* infection.
5. Finally, a One-Health approach towards the study on *Babesia* nation-wide should be carried out, particularly the involvement of Entomologists who will take care of the vector and transmission aspect; Veterinarians and Animal Biologists who will take care of the Natural and/ or Reservoir hosts; Health care professionals who will handle the human aspect; as well as professionals in other fields where necessary.

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## APPENDICES

### Appendix I

#### Preparation of 20% Giemsa from Stock

Giemsa stock solution was filtered and diluted with distilled water in 1:5 ratios to obtain a 20% solution. To ensure a smooth and uniform mixture, the solution was filtered after dilution. Fresh 20% Giemsa solution was prepared as and when needed.

### Appendix II

#### Preparation of Protease

1.2 millilitres of protease solvent was dispensed into a vial containing lyophilized Qiagen protease according to manufacturer's instructions and stored at a temperature of 2-8 degrees Celsius.

### Appendix III

#### Activation of Wash Buffers 1 and 2 (AW1 and AW2)

Twenty-five milliliters (25 ml) of absolute ethanol was added to a nineteen milliliter (19 ml) wash buffer 1 concentrate to obtain 44 ml Buffer AW1.

Thirty milliliters (30 ml) of absolute ethanol was added to a thirteen milliliter (13 ml) wash buffer 2 concentrate to obtain 43 ml Buffer AW2.

Both buffers were stored at room temperature.

**Appendix IV****Suspension of Primers**

Lyophilized primers were reconstituted into solution by adding appropriate volumes of nuclease free water depending on the number of moles of primers and depending on the desired primer concentration. The table below shows the amount of substance (number of moles) of the lyophilized primers in nanomoles (nmol) and the volume of nuclease-free water added to obtain a concentration of one hundred micro-molars (100  $\mu$ M)

<b>Primers</b>	<b>Amount of substance(nmol)</b>	<b>Volume of nuclease free water added (<math>\mu</math>L)</b>
PBTQBdivF	34.3	343
PBTQBdivR	35.7	357
Bm18Sf	29.7	297
Bm18Sr	26.3	263
BcW-A	22.9	229
BcW-B	32.7	327
PBTQBbovF	28.2	282
PBTQBbovF	28.5	285
Bab 5	21.8	218
Bab 8	29.6	296
Bab 6	22.5	225
Bab 7	37.2	372

In order to obtain a 10  $\mu$ M primer concentration for PCR solution, 10  $\mu$ L of the 100  $\mu$ M primer solution was dispensed into a sterile, well labeled eppendorf tube and diluted with

90  $\mu\text{L}$  of nuclease-free water. Thus, a 100  $\mu\text{L}$  primer solution with a final concentration of 10  $\mu\text{M}$  was obtained.

## **Appendix V**

### **Preparation of 2% Agarose Gel**

Agarose powder was suspended in 1X TAE buffer in a ratio 1g (agarose): 50 ml (TAE). The suspension, contained in a microwavable flask was heated in a microwave oven for about three minutes to bring to the boil until the agarose was completely dissolved. After every thirty seconds, the microwave was stopped in order to swirl the gel solution to ensure uniform mixing in the course of dissolution. The prepared agarose was allowed to cool to a temperature of 50 degrees Celsius before adding ethidium bromide stain. Two and a half microlitres (2.5  $\mu\text{L}$ ) of the stain was added per 50 mL of agarose and swirled to obtain a uniform mixture. The mixture was then poured into a gel tray with well comb(s) in place. The gel was poured to submerge about one-fourth to half the length of the combs. The set up was allowed to cool at room temperature for about twenty minutes before carefully removing the combs.

## **Appendix VI**

### **Preparation of 1X TAE Buffer from 50X Stock Solution**

Twenty milliliters (20 mL) of 50X TAE was diluted with 980 mL of distilled water in order to obtain a one litre (1L) volume of 1X TAE buffer.

## **Appendix VII**

### **Informed Consent**

Participant ID Number:

Participant Age:

Gender:

### **PROJECT TITLE: DETECTION OF HUMAN BABESIA SPECIES IN GREATER ACCRA, GHANA**

Dear Participant,

#### **CONSENT TO PARTICIPATE IN A RESEARCH PROJECT**

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 and therefore will remain confidential instead of your name. 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 in good faith.

#### **The study in a few words**

Babesiosis is a tick-transmitted zoonotic disease caused by a group of parasites of the genus *Babesia*. Domestic animals like dogs and cattle are usually infected with the disease but the species that infect the cattle have been known to also infect humans. Babesiosis

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. I am conducting this study to determine the occurrence of human babesiosis in Accra.

### **Procedure**

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

### **Risks**

Pain may be felt at the site where the needle will be inserted into your vein to draw blood but this pain is usually mild and may last for a few hours. 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 Miss Irene Amoakoh Owusu (0245423473) of the Department of Microbiology, University of Ghana School of Biomedical and Applied Sciences, Korle-Bu.

**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

## Appendix VIII

### Ethical Clearance



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

---

MS-AA/C.2/Vol.18<sup>A</sup>

30<sup>th</sup> June, 2015

My Ref. No.....

**Ms. Irene Amoakoh Owusu**  
Dept. Of Medical Microbiology  
SBAHS, Korle-bu

#### ETHICAL CLEARANCE

Protocol Identification Number: **MS-Et/M.9 – P 3.3/2014-2015**

The Ethical and Protocol Review Committee of the College of Health Sciences on 26<sup>th</sup> June, 2015 unanimously approved your research proposal.

**TITLE OF PROTOCOL: "Occurrence and Speciation of Human Babesia in Greater Accra, Ghana"**

**PRINCIPAL INVESTIGATOR: Ms. Irene Amoakoh Owusu**

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 November, 2015.**

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

Signed: .....

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

cc: Provost, CHS  
Head of Department  
Research Office

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