UNIVERSITY OF GHANA
SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES

DISTRIBUTION AND ABUNDANCE OF TICK SPECIES AND THEIR PREFERRED
ATTACHMENT SITES ON CATTLE IN GHANA

BY

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MASTER OF PUBLIC HEALTH DEGREE

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DECLARATION

I hereby certify that this dissertation is the outcome of research undertaken by me, Danielle Ladzekpo, towards the award of Master of Public Health from the School of Public Health, University of Ghana, Legon. This dissertation has not been submitted, either in part or in full, for any other degree and all references to the work of others have been duly acknowledged.

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DEDICATION

I hereby dedicate this research work to all early career research scientists in the field of public health entomology.
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ABSTRACT

Ticks are important vectors and reservoirs for many zoonotic pathogens worldwide. Some African countries including Ghana have reported tick-borne infections like Crimean-Congo haemorrhagic fever, however, there is scarce data on the tick vector in Ghana. This study was aimed at determining the distribution, abundance of tick species and preferred sites of attachments in six selected study sites. Ticks were collected from cattle in two ecological zones in Ghana (Guinea Savannah and Coastal Grasslands). The tick samples were grouped by species and sites of attachment and morphologically identified. The data obtained was collated and analyzed. A total of 1,625 sampled ticks were collected and morphologically identified. The combined results showed that *Amblyomma* (73.66%), *Rhipicephalus* (15.51%) and *Hyalomma* (10.83%) genera were predominant in the study sites. A total of 883 ticks were collected in the Guinea savannah sites and the species distribution was *Amblyomma* (84.7%) and *Hyalomma* (15.3%). A total of 742 ticks were collected in the Coastal Savannah sites and the species distribution was *Amblyomma* (60.5%), *Rhipicephalus* (34.0%) and *Hyalomma* (5.5%). The *Amblyomma variegatum* (73.66%) species was the most common in both ecological zones while the *Rhipicephalus* genus was absent in the three Guinea savannah sites. A total of 1416 ticks were assessed for the preferential sites of attachment. The results showed that overall, the most preferred site of attachment was the udder/scrotum area where 49.72% of ticks were collected from. This preference of attachment was consistent for all the tick species found except for the *Hyaloma rufipes* where 70% were found in the anal region. All the tick species found in the sites are all implicated vectors of immense public health importance. The knowledge of the distribution, abundance and prefered sites of attachment is useful in the application of effective tick control measures. The study highlights the need for continuous tick surveillance for the formulation of future strategies.
TABLE OF CONTENTS

DECLARATION ................................................................................................................................. i
DEDICATION .................................................................................................................................... ii
ACKNOWLEDGEMENTS ............................................................................................................... iii
ABSTRACT ....................................................................................................................................... iv
LIST OF TABLES ................................................................................................................................. vii
LIST OF FIGURES .............................................................................................................................. viii
LIST OF ABBREVIATIONS ........................................................................................................... ix

CHAPTER ONE .............................................................................................................................. 1
1.0 INTRODUCTION ....................................................................................................................... 1
  1.1 Background ............................................................................................................................. 1
  1.2 Rationale .................................................................................................................................. 4
  1.3 Conceptual Framework ........................................................................................................... 5
  1.4 Objectives ................................................................................................................................ 7
    1.4.1 General Objective ............................................................................................................... 7
    1.4.2 Specific Objectives ............................................................................................................ 7

CHAPTER TWO .............................................................................................................................. 8
2.0 LITERATURE REVIEW ............................................................................................................. 8
  2.1 Global Distribution of Tick Vectors ....................................................................................... 8
  2.2 Ticks .......................................................................................................................................... 8
  2.3 Life Cycle of Ticks ................................................................................................................... 9
  2.4 Feeding Behaviour of Ticks .................................................................................................... 11
  2.5 Host Seeking Behaviour of Ticks ............................................................................................ 11
  2.6 Sampling Methods for Ticks .................................................................................................. 12
  2.7 Identification of Ticks ............................................................................................................. 14
  2.8 Epidemiology of Tick-borne Diseases ................................................................................... 14
    2.8.1 Crimean Congo Hemorrhagic Fever Virus (CCHFV) ......................................................... 14
    2.8.2 Dugbe Virus .................................................................................................................... 16
    2.8.3 Alkhurma Hemorrhagic Fever Virus (AHFV) ................................................................ 17
    2.8.4 Coxiella burnetii .............................................................................................................. 18
    2.8.5 Rickettsial Infections ....................................................................................................... 19
    2.8.6 Babesiosis ......................................................................................................................... 20
    2.8.7 Tick-borne Encephalitis (TBE) ........................................................................................ 22
  2.9 The Control of Ticks and Tick-borne Diseases ....................................................................... 22

CHAPTER THREE .......................................................................................................................... 24
3.0 MATERIALS AND METHODS ............................................................................................... 24
  3.1 Study Design .......................................................................................................................... 24
  3.2 Selection and Description of Study Sites .............................................................................. 24
    3.2.1 Burma Camp (SBN) ......................................................................................................... 24
    3.2.2 Michel Camp (1BN) ....................................................................................................... 25
LIST OF TABLES

Table 1. Distribution of ticks on the different parts of the body of cattle................................................. 43
# LIST OF FIGURES

Figure 1.1 Conceptual framework factors that affect the distribution and abundance of tick species … 6

Figure 2.1 Life Cycle of Ticks (Charlesworth, 2008a) ................................................................. 10

Figure 2.2 A picture of a Drag Cloth for Tick Sampling (Ontario Agency for Health Protection and Promotion (Public Health Ontario), 2015, p. 12) ........................................................................... 13

Figure 3.1 Procedure for Picking of a Tick Using Blunt Forceps (Charlesworth, 2008b) .............. 27

Figure 3.2 External Structure of Adult Ixodid Ticks (The example is Hyalomma)(Walker et al., 2003, p. 25) ........................................................................................................................................ 29

Figure 4.1 Distribution of Ticks Species Collected in the Study Sites ............................................ 33

Figure 4.2 Proportion of the Different Tick Species Collected in the Coastal Grassland .............. 35

Figure 4.3 Proportion of the Different Tick Species Collected in the Guinea Savannah ............ 37

Figure 4.4 Spatial Distribution of Tick Species in the Coastal Grasslands .................................... 39

Figure 4.5 Spatial Distribution of Tick Species in the Guinea Savannah Sites ............................. 40
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>1BN</td>
<td>First Infantry Battalion</td>
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<td>5BN</td>
<td>Fifth Infantry Battalion</td>
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<tr>
<td>6BN</td>
<td>Sixth Infantry Battalion</td>
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<tr>
<td>ABF</td>
<td>Air Borne Force</td>
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<td>AFB</td>
<td>Air Force Base</td>
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<td>AHF</td>
<td>Alkhurma Hemorrhagic Fever</td>
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<tr>
<td>AHFV</td>
<td>Alkhurmah Hemorrhagic Fever Virus</td>
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<tr>
<td>ARTS</td>
<td>Armed-Forces Recruit Training School</td>
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<tr>
<td>CCHF</td>
<td>Crimean-Congo Hemorrhagic Fever</td>
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<tr>
<td>CCHFV</td>
<td>Crimean-Congo Hemorrhagic Fever Virus</td>
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<tr>
<td>CFT</td>
<td>Complement Fixation Test</td>
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<td>COX1</td>
<td>Cytochrome Oxidase Sub-unit One</td>
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<td>DUG</td>
<td>Dugbe</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Link Immunosorbent Assay</td>
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<td>GIS</td>
<td>Geographical Information System</td>
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<tr>
<td>GPS</td>
<td>Global Positioning System</td>
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<td>Immunofluorescent Assay</td>
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<td>IFAT</td>
<td>Indirect Fluorescent Antibody test</td>
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<tr>
<td>ITS2</td>
<td>Internal Transcribed Spacer Two</td>
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<tr>
<td>MALDI-TOF</td>
<td>Matrix Assisted Laser Desorption/Ionization-Time Of Flight</td>
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<tr>
<td>MAT</td>
<td>Microagglutination</td>
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<td>NMIMR</td>
<td>Noguchi Memorial Institute for Medical Research</td>
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<tr>
<td>NSD</td>
<td>Nairobi Sheep Disease</td>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>SFGR</td>
<td>Spotted Fever Group <em>Rickettsia</em></td>
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<td>Tick-Borne Encephalitis Virus</td>
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<td>TRGR</td>
<td>Transitional Group</td>
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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Ticks are hematophagous ectoparasites of human and other terrestrial vertebrates. They are of immense public health and agricultural importance as worldwide they are second to mosquitoes as vectors of human diseases. Ticks are vectors of different pathogenic protozoan, bacterial, and viral infections that elicit severe and fatal diseases in humans and animals worldwide (De La Fuente, Estrada-Pena, Venzal, Kocan, & Sonenshine, 2008; Gondard et al., 2017). It has been observed that the geographical distribution of each tick species is dependent on preferred ecological environments, which influences the risk zones for tick-borne infections (Parola & Raoult, 2001). Ticks can simply spread and colonise novel regions through host movements, either during livestock and domestic animal transport or via bird migration (Madder, Thys, Achi, Touré, & De Deken, 2011). In Africa, there are nearly fifty endemic tick species known to infect livestock and domestic animals. Among these endemic species are three genera that have the most elevated effect on livestock wellbeing; *Amblyomma*, *Hyalomma*, and *Rhipicephalus* (Reye, Arinola, Hübschen, & Muller, 2012). Human transmission of tick-borne diseases can be via the bite of an infected tick or exposure to an infected animal (Telmadarraiy, Chinikar, Vatandoost, Faghihi, & Hosseini-Chegeni, 2015). In recent times, zoonotic tick-borne diseases are increasing in geographic spread and infection rates and are likely to become a major public health threat in future (Estrada-Peña & De La Fuente, 2014).
Ticks are known to transmit and serve as vectors for Crimean-Congo hemorrhagic fever virus (CCHFV) of the Bunyaviridae family. This virus is the second most widespread arbovirus type disease throughout the world (Mishra & Appannanavar, 2011). A person infected with this virus would exhibit clinical features including a rapid progression identified by haemorrhage, myalgia and fever (Vorou, Piarroutsakos, & Maltezou, 2007). Animals including sheep, goats and cattle usually develop high titers of the virus in blood but tend not to succumb to the infection. Human infections normally occur via a tick bite but other routes of infection could be possible. People at risk of CCHF infection include those who work with livestock, animal herders and slaughterhouses workers (Jabbari, Tabasi, Abbasi, & Alijanpour, 2012). Severe outbreaks of viral hemorrhagic fever, with a case fatality rate of 10-40% are caused by the CCHF virus and it is endemic in Africa, the Balkans, the Middle East and Asian countries (WHO, 2014). A 2011 study conducted in a slaughterhouse in Ghana’s Ashanti Region reported the detected CCHFV in ticks collected from cattle and a human seroprevalence rate of 5.7% in animal handlers (Akuffo et al., 2016).

Alkhurma hemorrhagic fever virus (AHFV) is a tick-borne virus of the Flavivirus family. Since the original isolation of the virus from a patient in Saudi Arabia in 1995, subsequent cases of AHF have been recorded in travellers in Egypt. This suggests that the geographic spread of the virus is wide, thus infections due to AHFV are possibly underreported (Carletti et al., 2010). The mechanisms of viral persistence in ticks and the role of animals in the infection transmission routes are not well comprehended. Studies indicate that contact with domestic animals or livestock may increase the risk of human infection, however, no human-to-human transmission of AHF has been documented (CDC, 1995).

Ticks have been documented as vectors of Coxiella burnetii, a bacterial zoonotic infection. More than 40 tick species have been found naturally infected with the pathogen. (Maurin & Raoult,
The role of ticks in the transmission of Q fever involves contamination of the environment through the excretion of *C. burnetii* via their faeces, saliva, and coxal fluids (Angelakis & Raoult, 2010). There is no documented evidence of *C. burnetii* transmission to humans by blood-feeding ticks (Maurin & Raoult, 1999).

*Rickettsia* species are known to cause infections in both animals and humans through arthropod vectors. In the genus *Rickettsia*, available genetic data has allowed for its division into phylogenetic groups which includes the spotted fever group *Rickettsia* (SFGR) that are transmitted by ixodid ticks, the transitional group (TRGR) transmitted by fleas, mites and ticks and the *Rickettsia bellii* group, also spread through the bite of ticks (P. Parola, Paddock, & Raoult, 2005).

One tick species known to aid in the transmission of *Rickettsia* is *Rhipicephalus sanguineus* which can be found in peridomestic environments occupied by dogs. These species of ticks have a significantly low inclination towards humans, causing an infection rate below 10% with SFGR (P. Parola *et al.*, 2005).

Babesiosis is a disease with a global distribution affecting different species of mammals predominantly cattle and man (Bock, Jackson, De Vos, & Jorgensen, 2004). Extensively identified as pathogens causing a significant health burden on domesticated animals, *Babesia* spp. progressively have been detected and identified over the last fifty years as a cause of infection in individuals all over the world (Vannier, Gewurz, & Krause, 2008). *Babesia microti* has been implicated to be the most frequent cause of human babesiosis. In a study involving the collection of ticks from dogs in Asia, *Rhipicephalus sanguineus* ticks were found to be infected with *Babesia gibsoni* (Chao, Liao, Ho, & Shih, 2017). To effectively control babesiosis, it is necessary to adopt important tools for the detection and treatment of the disease. One method of detection is through microscopy which is by far not expensive and the quickest way to identify *Babesia* parasites
although with low sensitivity and specificity. Other immunological methods have been employed which are faster, more sensitive and specific. However, these methods are based on nucleic acid identification and amplification (Mosqueda, Olvera-Ramirez, Aguilar-Tipacamu, & Canto, 2012). Ticks are also involved in the transmission of other pathogens including Dugbe virus (Kobayashi et al., 2017) and tick-borne encephalitis (Süss, 2011). It is evident that measures need to be put in place to control the populations of tick species to prevent or reduce the burden of diseases they are vectors of. An example of such a measure is the use of different acaricides to control tick populations on dogs and cats (Garris, 1991). There is, however, the possibility of acaricide-resistant ticks emerging to cause more harm than good. Wearing protective clothing, avoiding contact with infected animals and vaccinations are means through which infections can be avoided.

1.2 Rationale

Ticks have the potential to transmit a wide range of pathogens. The worldwide emergence or re-emergence of tick-borne infections constitute a problem for both human and animal health (Liu & Bonnet, 2014). The factors that influence the risk of becoming infected with a tick-borne disease include but are not limited to: the nature of the tick population, the percentage of ticks infected with a pathogen and human behaviour that influence the risk of exposure (Rahlenbeck, Fingerle, & Doggett, 2016). When an infected tick bites a person or an animal during blood feeding, the pathogens are transmitted via the tick’s saliva leading to an infection. These pathogens can also be transmitted to humans during the improper handling of infected animals, during slaughter or veterinary processes. These possible transmission pathways pose challenges for diagnosing, treating and preventing tick-borne disease. The detection and identification of CCHF in both ticks and humans in Ghana (Akuffo et al., 2016) indicates the need to understand the distribution of tick
species and potentially the pathogens they harbour. More recently, a novel putative phlebovirus and a new strain of Dugbe virus were detected in ticks collected within Ghana (Kobayashi et al., 2017). Unfortunately, not enough work has been done to comprehend the role of various species of tick in the transmission of tick-borne diseases in Ghana.

The expected outcome of this study is to generate information on the species composition and distribution of ticks within selected sites, which will be useful in the formulation of future vector control strategies.

1.3 Conceptual Framework

The conceptual framework illustrates the factors that affect the distribution of various species in the study sites and their abundance and preferred sites of attachment. These factors include the climate of the area; as some tick species usually prefer warmer climates to others, the type of vegetation, the vector control procedures used by farmers; which may induce acaricide resistance and increased tick infestations. Knowledge of how these factors determine which geographic location certain species are prevalent, their abundance and preferred sites of attachment can be employed in determining the transmission cycles and rates of transmission as well as prevalence and seroprevalence of tick-borne pathogens in both cattle and humans. This can also be used in the monitoring of acaricide susceptibility. The distal public health outcomes of this study will be the use of the data to plan and implement better vector control measures to ultimately low the prevalence of tick infestations and tick-borne pathogens thereby preventing tick-borne diseases in cattle and humans as well as increasing animal productivity and yield of food and animal by-products like cattle leather.
Figure 1.1 Conceptual framework of factors that affect the distribution and abundance of tick species
1.4 Objectives

1.4.1 General Objective

The general objective of this study is to determine the distribution of tick species in six selected study sites and preferred sites of attachment on cattle hosts.

1.4.2 Specific Objectives

The specific objectives of the study are:

1. To determine the species composition and abundance of ticks collected from cattle in two ecological zones.
2. To spatially map out the tick species densities of the various study sites.
3. To determine the preferential sites of attachment of tick species on different parts of cattle.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global Distribution of Tick Vectors

Worldwide, there are roughly over 900 recognized tick species. Of all the species, approximately 700 species are Ixodid or hard ticks and 200 species are Argasid or soft ticks. In Africa, about 200 Ixodid and 40 Argasid species have been documented (Maxime Madder, Horak, & Stoltsz, 2001). Most of the tick species known to be of veterinary and medical importance belong to just three genera of ticks, *Amblyomma*, *Hyalomma*, and *Rhipicephalus* (Fournier & Raoult, 2004; Rajput, Hu, Chen, Ariojo, & Xiao, 2006). Across the continent, ticks of the three implicated genera cause harm that is either mechanical (skin injuries and irritations) or biological (transmission of pathogens) to both animals and humans (Brites-Neto, Maria Duarte Roncato, & Martins, 2015). A significant number of the tick infestations and tick-borne infections are usually prevalent in specific geographic regions. However, with globalization and environmental change, their range might grow and even spread inter-continentally. (Carletti et al., 2010). In Ghana, a number of ticks have been identified belonging to the genera *Amblyomma*, *Hyalomma*, and *Rhipicephalus* (Akuffo et al., 2016; Kobayashi et al., 2017).

2.2 Ticks

Ticks are hematophagous arthropods of immense public health importance affecting both humans and animals. In the process of blood-feeding, ticks inflict harm to the host causing damage to the skin and potentially infecting the host with pathogens (Gondard et al., 2017). Ticks are classified into three families: the Nuttalliellidae, the Ixodidae (hard ticks), and the Argasidae (soft ticks)
A number of hard ticks have been found to feed on cattle, transmitting various pathogens in the process. These include *Rhipicephalus* (*Boophilus*) spp. involved in the spread and transmission of *Babesia* and *Anaplasma* (De Meneghi, Stachurski, & Adakal, 2016). *Hyalomma* spp., *Amblyomma* spp., and *Rhipicephalus* spp. have been implicated as vectors of pathogens such as CCHF (Akuffo et al., 2016; Sharifinia et al., 2015).

### 2.3 Life Cycle of Ticks

The life cycle of ticks can include more than one host. Depending on the species of tick, the life cycle may include one, two or three hosts with four stages: egg, larvae (looks like the adult, but has six legs), nymph (looks like the adult but smaller), and adult. After feeding on a suitable host and becoming engorged with blood, the female deposits fertilized eggs in protected places in gaps in the soil or beneath grass (Eremeeva & Dasch, 2015). The number of eggs produced by a female tick depends on the tick species and degree of engorgement. Between day 4 and 82, the eggs hatch into larvae which quest for a host. The Larvae develop into nymphs which feed on a host, detach and moult into adult ticks in about 11–25 days. (Figure 2.1)
Figure 2.1 Life Cycle of Ticks (Charlesworth, 2008a)
2.4 Feeding Behaviour of Ticks

Hard ticks feed in each active stage (larval, nymph and adult) ingesting substantial quantities of blood. For each active stage of ticks, different hosts may be fed on. The larvae and nymphs will usually feed on small hosts like rodents, while adults usually feed on large animals such as cattle. However, some tick species are highly host-specific. This means the absence of such specific host(s) in a habitat may prevent the infestation of those ticks that specifically feed on them. When ticks climb onto a suitable host, they search for a favourable spot for feeding. After probing the skin and finding a favourable site of attachment, the tick inserts its mouthparts to begin feeding. During feeding, ticks are known to secrete toxins that act as an anaesthetic to disguise the pain from the bite and hinder blood from coagulating. Due to the efficient feeding behaviour of ticks and their firm hold on the host once attached, they have the potential for transmitting disease (Estrada-Peña, 2015).

2.5 Host Seeking Behaviour of Ticks

Ticks are capable of detecting a suitable host by sensing body scent, temperature and vibrations. Some species of ticks exhibit a behaviour known as questing. This involves selecting a well-used path by hosts and waiting by being positioned on the tips of grasses and shrubs. Once a host is in close proximity, the questing tick will grasp and climb the host to commence feeding (Centers for Disease Control and Prevention., 2013). Questing ticks usually have their first pair of legs extended in anticipation of the host in order to successfully climb on. While some ticks prefer to attach quickly at the site of contact with the host, others move to places on the host with thinner skin (Estrada-Peña, 2015).
2.6 Sampling Methods for Ticks

A number of sampling methods are usually used in the collection of ticks. The most common tick-sampling techniques are dragging and carbon dioxide-baited trapping (Ramos et al., 2014). The dragging process involves the use of pre-assembled drag cloths. In the absence of assembled drag cloths, a wooden stick or plastic pipe is attached across the end of a sheet of cloth. A cord is then attached to both ends of the stick (or plastic pipe) and used to pull the drag cloth through the vegetation. (Figure 2.2)

In estimating the abundance of free-living ticks, the dragging method is used due to its convenience and cheapness (Tack et al., 2011). However, the use of drag cloths is only effective for ticks located on the vegetation that come into contact with by the drag cloth (Terassini et al., 2010). The carbon dioxide (CO₂) traps are systems which use a CO₂ source over a white surface and is placed on the ground to attract hunter ticks (Cançado, Piranda, Mourão, & Faccini, 2008).
Figure 2.2 A picture of a Drag Cloth for Tick Sampling (Ontario Agency for Health Protection and Promotion (Public Health Ontario), 2015, p. 12)
2.7 Identification of Ticks

Tick species are morphologically identified using appropriate taxonomic keys unique to the geographic regions (Hubálek & Rudolf, 2012; Walker et al., 2003). However, this method can be strenuous as it requires some entomological expertise and the damaged specimen will be difficult to identify (Hubálek & Rudolf, 2012). Molecular methods including mitochondrial cytochrome oxidase subunit 1 (COX1) and nuclear internal transcribed spacer 2 (ITS2), have been developed to identify arthropods such as ticks (Song, Shao, Atwell, Barker, & Vankan, 2011). The MALDI-TOF Mass Spectrometry has also been employed in the rapid identification of tick vectors (Yssouf et al., 2013).

2.8 Epidemiology of Tick-borne Diseases

Ticks continue to be one of the important causes of zoonotic infections. (Jongejan & Uilenberg, 2004). Ticks serve as both vectors and reservoirs for various emerging and re-emerging infectious pathogens of medical and veterinary importance (Dantas-Torres, Chomel, & Otranto, 2012). Tick-borne bacterial infections, for example, African tick bite fever is widespread and acknowledged in the sub-Saharan African region. However, data on tick-borne infections are inadequate, with their prevalence and geographic spread largely unknown (Kobayashi et al., 2017).

2.8.1 Crimean Congo Hemorrhagic Fever Virus (CCHFV)

Ticks are the important vectors of Crimean Congo Hemorrhagic Fever Virus (CCHFV) (Telmadarraiy et al., 2015). Worldwide, ticks naturally infected with the CCHFV have been found to belong to a number of genera including but not limited to Hyalomma, Rhipicephalus, Boophilus and Amblyomma (Shepherd, Swanepoel, Cornel, & Mathee, 1989). Even though hard ticks usually serve as reservoirs and vectors for CCHFV, different animals such as cattle, sheep, goat and
camels, are capable of serving as hosts which amplify the virus. In countries such as Senegal, Mauritania, and Burkina Faso, CCHFV has been isolated from ticks, humans, domestic and wild vertebrates (Shepherd et al., 1989). In humans, infections can result from a bite from an infected tick, directly crushing an infected tick with an open wound, drinking unpasteurized milk from infected animals or being exposed to blood or tissues from patients and animals infected with the virus (Lwande et al., 2012). CCHFV has been shown to have a long distance geographical genetic linkage across countries and continents. The global occurrence of CCHF virus has been predicted using niche modelling with the goal of providing critical information to assist in prevention (Messina et al., 2015). CCHFV is found in hot and semi-arid areas such as sub-Saharan Africa and parts of the Middle East and Europe with current global distribution models predicting a relatively high occurrence of the virus in West Africa (Messina et al., 2015).

The geographical distribution of CCHFV is a direct representation of the vector abundance (Morikawa, Saijo, & Kurane, 2007). Transovarial transmission of CCHFV in implicated tick species contribute to the maintenance of the virus in endemic areas and explains the high rate of CCHFV infected adult ticks (Garrison et al., 2007). The ability of ticks to move from one geographical location to another is greatly enhanced by the mobility of their hosts. An example is a detection of CCHFV infected ticks on migratory birds from northern Spain that were caught in Morocco (Palomar et al., 2013).

Documented cases of CCHFV and tick-borne encephalitis virus infection, both of which have been discovered more than half a century in the past, are increasing in epidemic magnitudes and the increase in their geographic spread have become global public health concerns as re-emerging viral diseases (Estrada-Peña & De La Fuente, 2014). In Ghana, tick-borne viral diseases in humans have not yet been reported but many potential tick vectors are distributed nationwide (Akuffo et
There is currently no specific treatment package for CCHF as the treatment is mainly supportive and with limited pharmaceutical alternatives (Ascioglu, Leblebicioglu, Vahaboglu, & Chan, 2011). One antiviral medication which manifests the ability to inhibit the viral replication of the CCHFV in vitro is Ribavirin. Although the oral or intravenous use of Ribavirin has been recommended by the World Health Organization (WHO) as a prospective therapeutic drug for CCHF, there are disputable studies which indicate that oral ribavirin treatment in CCHF patients has no desirable effect on the viral load or disease progression (Jabbari, Besharat, Abbasi, Moradi, & Kalavi, 2006).

2.8.2 Dugbe Virus

Dugbe (DUG) virus is a member of the Nairobi sheep disease (NSD) serogroup in the Nuirouirus genus of the Bunyaviridae family’ (Casals & Tignor, 1980). The virus is said to be endemic in arid regions and is one of the most commonly found tick-borne viruses in Africa, which is frequently isolated from ticks infesting market livestock (Burt, Spencer, Leman, Patterson, & Swanepoel, 1996). Infection with the virus may lead to involuntary abortions and death of the animal. Methods used in the detection and identification of the virus include simple immunodiffusion tests on tissue extracts and virus isolation. Efforts to control the spread of Dugbe virus mainly focus on the control of vector ticks using acaricidal treatments. Nonetheless, there are vaccines which can be used to prevent the diseases in sheep.

Although the virus has been isolated from a person with a mild febrile disease, it has not been deemed an important human pathogen (James, Edward, Stephen, David, & James, 2017).

In the Central African Republic, a study to determine the seroprevalence of arboviruses in Zebu
cattle reported a 70% of the sera detection of Dugbe virus antibodies in adult cattle (Guilherme, Gonella-Legall, Legall, Nakoume, & Vincent, 1996). Recently, a novel strain of Dugbe virus has been isolated from *Amblyomma variegatum* ticks in Ghana (Kobayashi *et al.*, 2017).

### 2.8.3 Alkhurma Hemorrhagic Fever Virus (AHFV)

Alkhurma hemorrhagic fever virus (AHFV) belongs to the tick-borne hemorrhagic fever group of the genus Flavivirus. The known natural hosts of AHFV are sheep and camels. However, it is unknown which other mammals are also involved in its life cycle. There is limited information and understanding of the frequent occurrence of the virus within tick populations as well as the disease transmission process. Transmission of the viral infection is through the bite of a tick or through blood-contamination when crushing infected ticks with broken skin (Charrel *et al.*, 2005). It has been suggested that contact with domestic animals or livestock may increase the risk of human infection. Nevertheless, no human-to-human transmission of AHFV has been documented and AHFV infections are likely under-reported (Carletti *et al.*, 2010). The study of the first cases of confirmed AHFV infections revealed a pattern which is identified by an acute to lethal clinical outcome with a case fatality rate advancing towards 30% (Charrel *et al.*, 2005). The disease has been predicted to have a broader geographical distribution although cases have only been reported in the Arabian Peninsula and Egypt (Carletti *et al.*, 2010). Currently, AHFV infections are managed based on the presenting symptoms as there are no available antivirals or vaccines to treat or prevent the disease caused. As such, it has been advised that appropriate Alkhurma hemorrhagic fever awareness material is provided for the public, especially for those at high risk which includes slaughterhouse workers, butchers, and shepherds. Drinking of raw, unpasteurised milk is said to increase the risk of AHFV infection (Charrel *et al.*, 2005).
2.8.4 Coxiella burnetii

*Coxiella burnetii*, a bacterium, is known to cause acute and chronic fever illness and pneumonia in humans. This bacterium causes involuntary abortions in livestock species and is mainly transmitted to humans through contact with infected animal birth products (Vanderburg *et al.*, 2014). Human infection is through the inhalation of aerosols (Maurin & Raoult, 1999). The main implicated reservoirs of *C. burnetii* are sheep, goats, and cattle. Infections in these animals are usually asymptomatic but can lead to abortion (Angelakis & Raoult, 2010). Ticks are considered as significant reservoirs as well as vectors due to both transstadial and transovarial transmission of *C. burnetii* to their progeny in various species (Maurin & Raoult, 1999).

Infections in humans have a high risk of death due to an aneurysm, graft tear or fistulization to adjacent organs with a general mortality ranging between 18% and 25% (Botelho-Nevers *et al.*, 2007). Serological tests used to confirm the presence of antibodies that act against *C. burnetii* include microagglutination (MAT), complement fixation test (CFT), immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay. More sensitive Polymerase chain reaction (PCR) techniques, including conventional PCR, real-time PCR (RT-PCR), multiplex PCR and nested PCR, have been developed to detect the presence or absence of *C. burnetii* DNA in various samples. (Van den Brom, Van Engelen, Roest, Van der Hoek, & Vellema, 2015). In Ghana, surveys of cattle demonstrated an 18% seroprevalence of the *C. burnetii* pathogen in coastal Ghana (Adu-Addai *et al.*, 2012). Also, a seroprevalence study in humans in Ghana's rural Ashanti Region revealed 17% of two-year-olds were seropositive(Kobbe *et al.*, 2008).

To reduce the rate of abortion and ultimately limited environmental contamination via bacterial shedding in ruminants, therapeutic and preventive measures are required. Although antibiotics are
used in the treatment of *C. burnetii*, it is difficult to determine its antibiotic susceptibility. Successfully used antibiotics include oxytetracycline (Angelakis & Raoult, 2010), although no desirable results were obtained when used on sheep. Another means to prevent abortion and bacteria shedding in animals is through vaccination. The vaccines differ based on their formulation methods for the organism strain that they harbour. Several efficient vaccines are made up of *C. burnetii* in phase I stage (Arricau-bouvery *et al.*, 2005). It has been observed that vaccinations are usually highly effective when used in non-infected animals prior to their initial conception (Brom *et al.*, 2013). Other means to prevent infections can be through the pasteurisation of milk from *C. burnetii* infected farms (Arricau-Bouvery & Odolakis, 2005).

**2.8.5 Rickettsial Infections**

The genus *Rickettsia* was classically classified into the typhus group (TGR) and spotted fever group (SFGR). It has however been further subdivided based on phylogenetic analysis with the scrub typhus group composed of *Orientia* spp. (formerly of the genus *Rickettsia*). Rickettsial infections are mostly transmitted by ticks during feeding or by scratching crushed ticks into the skin. Numerous human-pathogenic tick-borne *Rickettsia* species have been found in the African sub-region including Burkina Faso and the Ivory Coast, with human seroprevalence rates ranging from 17 to 36% (Mediannikov *et al.*, 2010; Philippe Parola, Inokuma, Camicas, Brouqui, & Raoult, 2001). In travellers, including military personnel, the most frequently diagnosed rickettsial diseases are the murine typhus or spotted fever (African tick-bite fever) but travellers may acquire a wide range of rickettsioses, including emerging and re-emerging species (Leshem, Meltzer, & Schwartz, 2011). American soldiers participating in a 10-day training exercise in Botswana had an attack rate of approximately 30% for African tick-bite fever (Smoak *et al.*, 1996). In 2009, a study
conducted in Nigeria reported infection rates of unfed ticks in vegetation were 3.1% for *Rickettsia* species, 0.1% for *Coxiella burnetii* and 0.4% for *Borrelia* species; for feeding ticks collected from cattle, the infection rates were 12.5% for *Rickettsia* species and 14% for *Coxiella burnetii* (Reye, Arinola, Hübschen, & Muller, 2012). Amongst the tick-borne spotted fever *Rickettsia* species found within Africa, two most common are *R. africae* and *R. aeschlimannii*. The African tick bite fever is caused by *R. africae*. *Amblyomma hebraeum*, also known as the bont tick in southern Africa is the focal reservoir and vector of *R. africae* whereas *Amblyomma variegatum*, a widely distributed vector is responsible for transmitting the bacteria in West, Central, and East Africa. The first tick infection by *R. aeschlimannii* was detected in *H. marginatum* in Morocco in 1997 and continues to spread across the continent (Beati, Meskini, Thiers, & Raoult, 1997). In recent times, *R aeschlimannii* has been identified in Northern and North-Eastern countries of African. An example is the detection of the bacteria in *Hyalomma aegyptium* and *Rhipicephalus bursa*, in tick samples collected from humans in Turkey (Gargili *et al*., 2012).

### 2.8.6 Babesiosis

*Babesia* spp are protozoans distributed around the world and usually infect the blood cells of many vertebrates (Telford, SR, Gorenflot, Brasseur, & Spielman, 1993), inducing the disease babesiosis. In dogs, four *Babesia* species including *Babesia canis*, *Babesia vogeli*, *Babesia gibsoni* and *Babesia vulpes* sp. nov. cause infections (Baneth, Florin-Christensen, Cardoso, & Schnittger, 2015; Matijatko, Torti, & Schetters, 2012). Factors that influence the gravity of the disease, as well as clinical signs, include the specific *Babesia* species infection and the immunity of the animal (Solano-Gallego & Baneth, 2011). Humans have been found to become susceptible to babesiosis in situations where they are otherwise immunocompromised with the common causal agents being...
Babesia divergens, a parasite of cattle, or B. microti, found in rodents (Gray, Zintl, Hildebrandt, Hunfeld, & Weiss, 2010). In the process of blood feeding on an infected host, ticks acquire Babesia spp., even though the transovarial transmission of B. canis has been detected through up to five tick generations (Chauvin, Moreau, Bonnet, Plantard, & Malandrin, 2009).

In the tropics and sub-tropics, Babesiosis is an economically important tick-borne protozoan disease which affects cattle (Bhat, Singh, Singh, & Rath, 2017). The genus of Babesia known to cause infection in cattle includes B. bigemina, which is responsible for most infections and B. bovis. These are transmitted by cattle fever tick, Rhipicephalus microplus (Murrell, Campbell, & Barker, 2001). In Ghana, a study conducted to determine the sera-prevalence of tick pathogens across the country’s three ecological zones showed the sera-prevalence rate of B. bigemina was 2% for a sample size of 397 cattle (Beckley, Shaban, Palmer, Hudak, & Noh, 2016). To identify Babesia parasites in an individual, a peripheral blood smear is prepared and stained with Giemsa. However, when the level of parasitaemia is too low, latent infections are likely not to be detected. Other methods employed involve detecting antibodies against Babesia infection in cattle using Indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) (Bhat et al., 2017). Recently, in the study of the epidemiology of babesiosis, PCR-based assays which are sensitive and specific have been developed to detect infections in both vertebrates and tick species. (Tavassoli, Tabatabaei, Mohammadi, Esmaeilnejad, & Mohamadpour, 2013). Drugs such as imidocarb or diminazene aceturate have been used in the treatment of babesiosis. Due to various carried out research, new pharmacological compounds are being developed and evaluated, offering new alternatives to control the disease (Mosqueda et al., 2012).
2.8.7 Tick-borne Encephalitis (TBE)

Tick-borne encephalitis (TBE), is a significant viral infection of the central nervous system affecting Europe and many locations in Asia. The tick-borne encephalitis virus (TBEV) is estimated to cause infections in about 11,000 human cases annually, mostly in Russia (Gritsun, Nuttall, & Gould, 2003). Even though there are vaccines to prevent TBE infections, the incident rates continue to rise causing a major public health concern in almost all endemic European and Asian countries. Countries outside endemic regions are at risk of exposure due to an increase in tourism (Süss, 2011). The main reservoirs and hosts of TBEV are small rodents with humans serving only as accidental hosts. The virus is spread to humans primarily through the bites of hard ticks (Bogovic & Strle, 2015). There is currently no specific antiviral treatment for TBE. Patients may be hospitalized and supportive care given depending on the severity of the infection. Preventive measures to avoid becoming infected with the virus include the pasteurization of milk, limiting the tick population and adopting the use of personal protective procedures and equipment. The best way to avoid infection is active immunization. The disease is zoonotic in nature with no chance of human to human transmission. This means that vaccination is for an individual protection and not the entire population.

2.9 The Control of Ticks and Tick-borne Diseases

An extensive diversity of prevention and control approaches have been put in place to reduce tick infestation, the prevalence of tick-borne pathogens and the risk of human vulnerability to tick-borne pathogens (Eisen & Dolan, 2016). These measures are usually split into personal protection strategies such as the use of repellents, protective clothing and tick vector control procedures. These are with the aim of reducing contact between ticks and humans, reducing tick infestations
or reducing the rate of tick-borne pathogens in the ticks. Some methods in the tick vector control include acaricides and biological control agents usage (Stafford, Williams, & Molaei, 2017).

Over the past decades, the application of acaricides has become the main technique for controlling tick infestation on domestic animals as well as in the environment. Semiochemicals; pheromone, kairomones, and attractants, may be utilized to improve acaricide potency or reduce pesticide concentrations (Sonenshine, 2006). The use of acaricides has however caused the contamination of the environment, milk and meat products and the development of acaricide-resistance in ticks.

Globally, there are vaccines aimed at controlling tick-borne infections. There is also a need to create anti-tick vaccines that are suitable for a more global protection against the main species of economic and epidemiological interest (Domingos et al., 2013).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

This study was carried out with a descriptive cross-sectional design. The study was aimed at gathering preliminary information on tick species, their distribution and their preferred sites of attachment on cattle hosts in the study sites. This was done by collecting ticks from the study sites and collating quantitative data on the different tick species in the study sites and their abundance.

3.2 Selection and Description of Study Sites

The study sites were selected based on inputs from the US NAMRU-3 and the Ghana Armed Forces as part of the collaboration between the two institutions. Ticks samples were collected from cattle from within two ecological zones of Ghana – the Coastal grassland and the Guinea savanna, as the distribution of arthropods is generally known to be influenced by environmental factors such as temperature and vegetation. The tick samples were collected from military unit-owned kraals in the Greater Accra (Burma Camp, Afienya and Shai Hills) and Northern (Tamale Airforce Base, Air-born Force and Kamina Barracks) Regions.

3.2.1 Burma Camp (5BN)

The 5BN Cattle Kraal (N 5° 43' 57.673'' W 0° 2' 30.832'') is located at and owned by the Fifth Infantry Battalion unit in Burma Camp, Greater Accra Region. The kraal has one roofed holding area, two unroofed holding areas and a crush pen. The kraal houses about 50 cattle.
3.2.2 Michel Camp (1BN)

The 1BN Cattle Kraal (N 5° 43' 57.673" W 0° 2' 30.832") is located at and owned by the First Infantry Battalion unit in Michel Camp, Greater Accra Region. The 1BN kraal has a large enclosed diary house type of kraal but has no crush pen. The 1BN kraal houses about 55 cattle.

3.2.3 Shai Hills (ARTS)

The ARTS Cattle Kraal (N 5° 52' 46" E 0° 1' 58") is located at and owned by the Army Recruit Training School in Shai Hills, Greater Accra Region. The ARTS kraal has a large unroofed fences area split into two sections that are used in rotation. The ARTS kraal houses about 35 cattle and has no crush pen.

3.2.4 Tamale Air Force Base (AFB)

The Air Force Base Kraal (N 9° 32' 48.746" W 0° 51' 20.458") is located at and owned by the Tamale Air Force Station, Northern Region. The Air Force station kraal has one unroofed enclosure that houses about 60 cattle and has no crush pen.

3.2.5 Airborne Force (ABF)

The Air Borne Force Kraal (N 9° 32' 32.924" W 0° 51' 23.461") is located at the Air Borne Force unit in Barwah Barracks-Tamale, Northern Region. The Airborne Force kraal has one large unroofed enclosure that houses about 112 cattle and has no crush pen.
3.2.6 Kamina Barracks (6BN)

The 6BN Kraal (N 9° 28' 6.312" W 0° 51' 14.256") is located at the Sixth Infantry Battalion in Kamina Barracks-Tamale, Northern Region. The Kamina kraal has one large unroofed enclosure that houses about 60 cattle and has no crush pen.

3.3 Tick Sample Collection

The study did not involve experimentation procedures on the cattle. At each sample collection visit, ticks were directly removed from as many conveniently sampled cattle. The body of the cattle was divided into seven regions, the head, dewlap, back, udder/scrotum, limbs, anal and tail regions. The sampling process involved a physical examination of the cattle and collection of ticks off the animals with the assistance of cattle handlers. Research assistants/cattle handlers who had undergone training on tick removal, sample labelling were supervised by the officer in charge of the kraal and a senior research assistant. The blunt forceps were used to find and hold the mouthparts of the ticks, then using applying a gentle tug the ticks were pulled away from the animal till they detached (Figure 3.1). The ticks were grouped and stored in different sample vials appropriately labelled to correspond with the different regions.

3.4 Storage and Transport of samples

The tick samples were stored in labelled vials containing RNA-later to prevent decomposition and desiccation that will change morphological characteristics; possibly rendering the samples unidentifiable, as well as keep the integrity of the genetic material of the ticks. The ticks were then transported to the Entomology laboratory of NMIMR within 48 hours for further processing.
Figure 3.1 Procedure for Picking of a Tick Using Blunt Forceps (Charlesworth, 2008b)
3.5 Tick Identification

Tick identification was carried out in the entomology laboratory of NMIMR using a dissecting microscope at 4x-10x magnification and using “Ticks of Domestic Animals in Africa: a Guide to Identification of Species” as the reference key for morphological identification of the different genera and species (Walker et al., 2003).

Hard ticks are identified by the presence of a scutum or shield on the dorsal side and by having their mouthparts that can be seen from above. In males, the scutum extends across most of the dorsal side, whiles in the females, it usually covers up between a third to half of the dorsal area. Fundamental features in identifying hard ticks to the genus level include the length of the mouthparts, presence or absence of eyes, presence or absence of festoons, colour or markings on the scutum, and shape and positioning of the anal groove. (Mathison & Pritt, 2014). Figure 3.2.

Following identification, the ticks were grouped into pools of two or three by species, sex, and point of collection on cattle and stored for further molecular studies.
Figure 3.2 External Structure of Adult Ixodid Ticks (The example is Hyalomma)(Walker et al., 2003, p. 25)
3.6 Data Processing and Analysis

3.6.1 Data Processing
Data on morphological identification, Global Positioning System coordinates, site of attachment were recorded and collated in Microsoft Excel 2013 and exported as CSV files to STATA 15 for cleaning and analysis preparation.

3.6.2 Data Analysis
Univariate analysis was conducted using the variables: genera, species and sites of attachments. Descriptive statistics were such as frequency distribution tables and percentages were used to present the results of the species distribution and abundance as well as the preferred sites of attachments in the tick species.

3.7 Geographic information system analysis
The data on the distribution and abundance of tick species collected in the six study sites were analyzed with the GPS coordinates of the corresponding study sites. Spatial maps of the two ecological zones were constructed using the version 10 of ArcView geographical information system (GIS) software package.
3.8 Quality Control

3.8.1 Field Sampling
During the collection of the tick samples, research assistants and the animal handlers were assigned specific regions of the cattle and they were given corresponding labelled vials to prevent mismatching of tick and site of attachment.

3.8.2 Tick Identification
Three research assistants were involved in morphological identification. Each tick was morphologically identified by one research assistant and confirmed by another. In the event that a second identification was not sufficient to confirm species identification, a third research assistant would observe the morphological features to confirm the species identification.

3.8.3 Data Entry and Analysis
Data entry was done by two research assistants who crossed checked the sample collection forms and laboratory notebooks to prevent omission and double entry of data.

3.9 Study Challenges
Most of the kraals at the study sites did not have crush pens so the animal handlers had to immobilize the cattle by roping them down for tick sampling. This made sampling procedure quiet cumbersome and although efforts were made to minimize discomfort to the cattle and ensure the safety of the animal handlers and research assistants. Built-in crush pens that restrict movement in the cattle would have made the sampling process less cumbersome.
CHAPTER FOUR

4.0 RESULTS

4.1 Ticks Collected

4.1.1 Overview of Genera and Species Composition Of Ticks in The Six Study Sites

A total of 1625 ticks comprising three genera were collected from cattle across all the study sites. The genera included *Amblyomma* (73.66%), *Hyalomma* (10.83%) and *Rhipicephalus* (15.51%). Out of the three tick genera, the species identified were 1197 (73.66%) *Amblyomma variegatum*, 52 (3.2%) *Hyalomma rufipes*, 124 (7.5%) *Hyalomma truncatum*, 203 (12.5%) *Rhipicephalus* spp., 41 (2.5%) *Rhipicephalus sanguineus*, 1 (0.06%) *Rhipicephalus boophilus* and 7 (0.43%) *Rhipicephalus evertsi* (Figure 4.1). Among the species mentioned, *Amblyomma variegatum* (73.66%) was the most common species in all the study sites whilst *Rhipicephalus boophilus* (0.05%) was the least collected. All the three genera were prevalent in the three sites of the coastal savannah while two, *Amblyomma* and *Hyalomma* were prevalent in the three Guinea savannah sites.
Figure 4.1 Distribution of Ticks Species Collected in the Study Sites
4.1.2 Distribution and Abundance of Tick Species in the Coastal Grassland Study Sites

A total of 742 ticks were collected from the three study sites in the coastal grassland. Of the 742 ticks collected, 349 were from the 5BN kraal in Burma Camp with the proportions being *Amblyomma variegatum* (61.32%), *Hyalomma rufipes* (2.29%), *Rhipicephalus spp* (31.81%) and *Rhipicephalus sanguineus* (4.58%). A total of 187 ticks were collected from the 1BN kraal in Michel Camp with the proportions being *Amblyomma variegatum* (28.34%), *Hyalomma rufipes* (12.83%), *Hyalomma truncatum* (0.5%), *Rhipicephalus spp* (49.2%), *Rhipicephalus sanguineus* (4.81%), *Rhipicephalus boophilus* (0.54%) and *Rhipicephalus evertsi* (3.74%). A total of 206 ticks were collected from the ARTS kraal in Shai Hills with the proportions being *Amblyomma variegatum* (88.35%), *Hyalomma rufipes* (3.88%) and *Rhipicephalus sanguineus* (7.77%) (Figure 4.2). *Amblyomma variegatum* was the most predominant species collected from the 5BN kraal at Burma camp and the ARTS Kraal Shai Hills. The most predominant species collected from the 1BN kraal at Michel Camp was *Rhipicephalus spp*. 
Figure 4.2 Proportion of the Different Tick Species Collected in the Coastal Grassland
4.1.3 Distribution and Abundance of Tick Species in the Guinea Savannah Study Sites

A total of 883 ticks were collected from the three study sites in the Guinea savannah. Of the 742 ticks collected, 280 were from the AFB kraal at the Tamale Airforce Base and the proportions were *Amblyomma variegatum* (77.14%), *Hyalomma rufipes* (1.07%), *Hyalomma truncatum* (21.79%). A total of 364 ticks were collected from the AFB Kraal at Barwah Barracks with the proportions *Amblyomma variegatum* (82.69%), *Hyalomma rufipes* (2.47%) and *Hyalomma truncatum* (14.84%). At the 6BN kraal at Kamina Barracks, 239 ticks were collected and the proportions were *Amblyomma variegatum* (96.65%) and *Hyalomma truncatum* (3.35%) (Figure 4.3). *Amblyomma variegatum* constitute the most predominant species collected in all three of the Guinea savannah sites.
Figure 4.3 Proportion of the Different Tick Species Collected in the Guinea Savannah
4.2 Spatial Distribution of Tick Species in the Study Sites

The data of the different tick species and their relative abundance with respect to the GPS coordinates of their corresponding study sites was used to construct two maps due to the differences in proximities of the sites to each other in the two ecological zones.

4.2.1 Map of Tick Species Densities in the Coastal Grassland Sites

The map of the coastal grassland sites was drawn at a scale of 1 inch: 20 km to clearly show the different study sites. The densities of the different tick species at each study site was pictorially shown in a pie chart at the corresponding GPS coordinates (Figure 4.4).

4.2.2 Map of Tick Densities in the Guinea Savannah Sites

The map of the Guinea savannah sites was drawn at a scale of 1 inch: 5 km due to the close proximity of the study sites to each other. The scale used made it possible to clearly show the different study sites. The densities of the different tick species at each study site was pictorially shown in a pie chart at the corresponding GPS coordinates (Figure 4.5).
Figure 4.4 Spatial Distribution of Tick Species in the Coastal Grasslands
Figure 4.5 Spatial Distribution of Tick Species in the Guinea Savannah Sites
4.3 Tick Attachment Preferences

The sites of attachment of 209 ticks were unaccounted for due to compromised labels on some of the collection vials thus a total of 1416 ticks were assessed for the preferential sites of attachment. The results of the preferred sites of attachments showed that overall, the most preferred site of attachment was the udder/scrotum area where 49.72% of ticks were collected from. The dewlap region was the next preferred site of attachment where 32.77% of the ticks were collected from. This order of preference was consistent for all the tick species found except for the Hyalomma rufipes which had the most preferred site of attachment to be the anal region with 70.00% of ticks of that species collected from.

4.3.1 Amblyomma Species

The *Amblyomma variegatum* species mostly preferred in the udder/scrotum (48.98%) and the dewlap (40.20%) regions and the least preferred region was the back (0.59%) region.

4.3.2 Hyalomma Species

The *Hyalomma rufipes* species mostly preferred the anal (70.00%) region and least preferred the head (0%) and limb (0%) regions. The *Hyalomma truncatum* species mostly preferred in the udder/scrotum region and least preferred the head (0%) and back (0%) regions.

4.3.3 Rhipicephalus species

Rhipicephalus spp., Rhipicephalus sanguineus, Rhipicephalus boophilus and Rhipicephalus evertsi were all found to mostly prefer the udder/scrotum region (55.06%, 56.60%, 100%, and 100% respectively). For Rhipicephalus spp., the least preferred site of attachment was the back...
(0.56%). For the other three, the least preferred site of attachment was the dewlap (0%), limbs (0%) and tail (0%) regions (Table 2)
Table 1. Distribution of ticks on the different parts of the body of cattle

<table>
<thead>
<tr>
<th>Morphological ID</th>
<th>Head (%)</th>
<th>Dewlap (%)</th>
<th>Back (%)</th>
<th>Udder/Scrotum (%)</th>
<th>Limbs (%)</th>
<th>Anal (%)</th>
<th>Tail (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma variegatum</td>
<td>20 (1.95)</td>
<td>412 (40.20)</td>
<td>6 (0.59)</td>
<td>502 (48.98)</td>
<td>26 (2.54)</td>
<td>45 (4.39)</td>
<td>14 (1.36)</td>
<td>1,025</td>
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<td>Hyalomma rufipes</td>
<td>0</td>
<td>2 (5.00)</td>
<td>1 (2.50)</td>
<td>2 (5.00)</td>
<td>0</td>
<td>28 (70.00)</td>
<td>7 (17.50)</td>
<td>40</td>
</tr>
<tr>
<td>Hyalomma truncatum</td>
<td>0</td>
<td>17 (13.71)</td>
<td>0</td>
<td>70 (56.45)</td>
<td>8 (6.45)</td>
<td>14 (11.29)</td>
<td>15 (12.10)</td>
<td>124</td>
</tr>
<tr>
<td>Rhipicephalus spp</td>
<td>9 (5.06)</td>
<td>37 (20.79)</td>
<td>1 (0.56)</td>
<td>98 (55.06)</td>
<td>16 (8.99)</td>
<td>2 (1.12)</td>
<td>15 (8.43)</td>
<td>178</td>
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<tr>
<td>Rhipicephalus sanguineus</td>
<td>10 (26.42)</td>
<td>0</td>
<td>3 (5.66)</td>
<td>23 (56.60)</td>
<td>0</td>
<td>5 (11.32)</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>Rhipicephalus boophilus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rhipicephalus evertsi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (100.00)</td>
<td>0</td>
<td>0</td>
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<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>39 (3.01)</td>
<td>468 (32.77)</td>
<td>11 (0.77)</td>
<td>703 (49.72)</td>
<td>50 (3.50)</td>
<td>94 (6.65)</td>
<td>51 (3.57)</td>
<td>1,416</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION

In the tropical and subtropical regions, ticks serve as important disease vectors and cause significant economic losses by influencing animal health and productivity (Rajput, Hu, Chen, Arijo, & Xiao, 2006). The highest impact is on livestock health and this is usually caused by species belonging to only three genera, namely, *Amblyomma*, *Hyalomma*, and *Rhipicephalus* (Rajput, Hu, Chen, Arijo, & Xiao, 2006). Ticks are also capable of transmitting infectious pathogens that affect both livestock and humans (P. Parola & Raoult, 2001). For a tick to survive in an environment, some conditions including an optimal temperature and humidity as well as the availability of a suitable host are required (Uspensky, 2014). Pathogens transmitted by ticks can be pathogenic fungi, protozoa, viruses and bacteria (Brites-Neto et al., 2015).

A study carried out in Kumasi revealed the presence of CCHFV in ticks with a seroprevalence of 5.7% in abattoir workers (Akuffo et al., 2016). This finding suggests that the virus may have been transmitted by the reservoir vector tick species to the livestock increasing the risk of exposure to the animal handlers. More recently, Dugbe virus has been successfully isolated for the first time in Ghana from collected ticks (Kobayashi et al., 2017). These studies indicate the need to understand the dynamics of tick populations to formulate efficient control strategies and potentially prevent future outbreaks.
5.1 Species Composition of Ticks across the Ecological Sites

In this study, ticks were collected from six sites located in the Coastal grasslands and Guinea savannah zones. The use of hand picking of ticks from cattle was employed in the sample collection. This method had previously been used to collect tick species in a survey of domestic dogs and cattle at the Kumasi abattoir in the Ashanti Region of Ghana. The study yielded various tick species belonging to the genera *Rhipicephalus, Amblyomma* and *Haemaphysalis* (Akuffo et al., 2016). A similar method of tick collection used in a study conducted in Accra yielded diverse tick species (Kobayashi et al., 2017). Tick species identified from the Coastal grassland and Guinea savannah in this study were predominantly of the genera *Amblyomma* followed by *Rhipicephalus* and the least being *Hyalomma*. It was further observed that *Amblyomma variegatum* (73.66%) was the most collected species of ticks whereas *Rhipicephalus boophilus* (0.06%) was the least collected. The high percentage of *Amblyomma variegatum* species collected from all the study sites indicate that this species is common and poses a threat to public health due to their role in the transmission of tick-borne pathogens (Ogo et al., 2012). This species has been implicated in the transmission of CCHF (Akuffo et al., 2016) and Dugbe virus (Kobayashi et al., 2017) in Ghana. It should also be noted that other tick species identified in the study sites are capable of spreading various pathogens of veterinary and zoonotic importance (Reye, Arinola, Hübschen, & Muller, 2012). *Rhipicephalus* genera were completely absent in the dry Guinea savannah area. It is known that the occurrence of some species of *Rhipicephalus* such as *appendiculatus* becomes seasonal when there is a prolonged dry season (Randolph, 1994). The northern sector where the ticks were collected has a prolonged dry season with very little vegetation and this may be the reason why they may be absent in those areas.
5.2 Abundance of Ticks at the Various Study Sites

The density of ticks must be over a certain threshold to support the transmission cycles of tick-borne pathogens. This means that a higher density of ticks will mostly result in more efficient pathogen transmission cycle (Ogden et al., 2007). Different entomological indices have been used to quantitatively express vector density, however, because of the limited data of tick abundance the thresholds of different pathogen transmission of the different tick species in Ghana is still undefined. Determining the species abundance is a good first step in determining the vector density in a geographical area for subsequent studies into pathogen transmission cycles.

In the Greater Accra region, *Amblyomma variegatum* was the predominant species collected in both Burma Camp and Shai Hills. *Rhipicephalus* spp were the highest number collected from Michel Camp. It was observed that *Hyalomma truncatum* were only identified in samples collected from Michel Camp. The highest percentage of *Hyalomma rufipes* were identified in Michel Camp followed by Shai Hills and then Burma camp. *Rhipicephalus sanguineus* were predominant in Shai Hills and almost of equal population in both Michel and Burma camps.

In the Northern region, *Amblyomma variegatum* was also identified as the most predominant tick species. The second highest tick species was *Hyalomma truncatum* with *Hyalomma rufipes* present in both the Tamale Air Force Base and Barwah Barracks but not in Kamina Barracks. However, it was observed that no *Rhipicephalus* spp were identified in any of the collecting sites within the Northern region. This could be due to the fact that the study sites have a prolonged dry season or that the acaricides used in treating the cattle in the kraals were effective
against the *Rhipicephalus* spp.

The high density of *Amblyomma variegatum* suggests that they may be involved in the transmission of pathogens. This has further been confirmed by the detection of viral pathogens in *Amblyomma variegatum* in Ghana (Akuffo *et al.*, 2016; Kobayashi *et al.*, 2017), making it a very important species and therefore the need to understand its ecology and distribution across the country. Although *Hyalomma* species were of lower densities, their role in the spread of disease pathogens cannot be ignored as some studies suggest that they can be vectors of CCHF (Spengler & Estrada-Peña, 2018). Finding the above-mentioned species in Ghana indicates the need to conduct a nationwide surveillance to fully access their population structure and furthermore the burden of pathogens they carry. With the import of livestock from countries such as Burkina Faso, there is a possibility of invading tick species and consequently pathogens which would otherwise not be originally found in Ghana.

### 5.3 Preferred Sites for Attachment of Different Tick Species on Cattle

From this study, it was observed that a high percentage of ticks had a preference for the udder or scrotum of the cattle with the most predominant species being *Amblyomma variegatum*. This could be due to the fact that most tick species will usually seek a suitable attachment point on the host where maximum blood feeding can be achieved. Host body part characteristics such as skin thickness, humidity, blood circulation and de-ticking by grooming behaviour of the host play an important role in the selection of attachment sites by ticks (Ogden, Hailes, & Nuttall, 1998). The least number of ticks were identified from the back of cattle probably due to the fact that they would be more exposed to extreme temperatures and potential predators. High temperatures can cause dehydration in ticks leading to death.
*Amblyomma variegatum* was found at each sampling site on the cattle and had a high preference for the udder or scrotum and the sternum. These species are hunters which go in search of a suitable host, climb it and locate a safe spot where it will attach and feed without any form of external interference. Although *Rhipicephalus* spp were found at each sampled site on the cattle hosts, they were observed to have a high preference for the udder or scrotum. It was observed during the study that the animal handlers had used some insecticides to control the tick populations. This may have affected the proportion of ticks collected from the sites. However, the fact that many adult ticks were collected even three to four weeks after the application of the acaricides indicates that the tick may be resistance to the acaricides being used. Further studies are needed to understand the acaricide resistance pattern of the ticks in the areas of study. The insecticides used may have also had an influence on the proportions of ticks and ultimately their site of preference on the cattle host. This may account for the low numbers of *Rhipicephalus* spp at the points of attachment on the hosts. Even though there are many ways to control ticks, each method of control may have a limitation. An example is the use of chemicals with acaricides which has resulted in the development of resistance in tick species (Martins Joao, Correa, Cereser, & Arteche C, 1995). Problems arising from the use of acaricides which are toxic and expensive, increase in resistant tick species, have gone a long way to influence the formulation of more effective control measures such as the use of vaccines (Rajput, Hu, Chen, Arijo, & Xiao, 2006).
CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The present study has confirmed the presence of different species of ticks with a predominance of three genera of ticks, *Amblyomma* (73.7%), *Rhipicephalus* (15.5%) and *Hyalomma* (10.8%). *Amblyomma variegatum* ticks were found to be the most commonly occurring ticks in all the ecological zones whereas *Rhipicephalus* species were found to be absent in the three northern Sahel sites. The high numbers of *Amblyomma variegatum* pose a public health concern as they are said to be capable of harbouring and transmitting infectious pathogens to both humans and animals. Such pathogens including Crimean-Congo haemorrhagic fever virus and Dugbe virus have been detected and isolated in Ghana. A high proportion of tick species identified from this study were observed to have a preference for the udder or scrotum of the cattle with the most predominant species being *Amblyomma variegatum*. The prevalence of these three genera of ticks known to be vectors of various infectious pathogens in the study sites highlight the need for continuous tick surveillance to improve the knowledge of vector dynamics, ultimately leading to the formulation of effective control strategies.
6.2 Recommendations

Based on findings from this study, it is recommended that;

1. There should be a continuous tick surveillance to cover the entire country to identify all tick species present in order to formulate effective control measures.

2. Acaricide susceptibility and resistance levels in the tick vectors should be studied to determine the best insecticides needed to control ticks in the country.

3. Circulating pathogens within the tick populations, especially in Amblyomma variegatum, must be determined in order to assess the risk of infections and their role in pathogen transmission and outbreaks.


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Spengler, J. R., & Estrada-Peña, A. (2018). Host preferences support the prominent role of
Hyalomma ticks in the ecology of Crimean-Congo hemorrhagic fever. *PLOS Neglected Tropical Diseases, 12*(2), e0006248. https://doi.org/10.1371/journal.pntd.0006248


APPENDICES

Appendix I: Genera of ticks identified within selected sites

<table>
<thead>
<tr>
<th>Morphological ID</th>
<th>5BN, Burma Camp</th>
<th>IBN, Michel Camp</th>
<th>ARTS, Shai Hills</th>
<th>AFB, Tamale Air Force</th>
<th>ABF Airborne Barwah Barracks</th>
<th>6BN, Kamina Barracks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma spp.</td>
<td>214</td>
<td>53</td>
<td>182</td>
<td>216</td>
<td>301</td>
<td>231</td>
</tr>
<tr>
<td>Hyalomma spp.</td>
<td>8</td>
<td>25</td>
<td>8</td>
<td>64</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>Rhipicephalus spp.</td>
<td>127</td>
<td>109</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>349</td>
<td>187</td>
<td>206</td>
<td>280</td>
<td>364</td>
<td>239</td>
</tr>
</tbody>
</table>
Appendix II: Tick Sample Form

TICK SAMPLE FORM

<table>
<thead>
<tr>
<th>Date</th>
<th>Pool ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Morphological ID</td>
</tr>
<tr>
<td>GPS coordinates</td>
<td>Pool sex</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>Pool count</td>
</tr>
<tr>
<td>Host animal</td>
<td>The site of tick attachment</td>
</tr>
</tbody>
</table>

![Diagram of a cow highlighting various body parts](image)

Notes
Appendix III: Collection of ticks at selected sites

Plate 1. Collection of ticks from cattle