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CATTLE AS POTENTIAL RESERVOIR FOR PARASITIC ZONOTIC DISEASES
IN THE LOWER MANYA KROBO DISTRICT IN THE EASTERN REGION OF
GHANA

BY

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DECLARATION

I do hereby declare that apart from references to the work of other investigators, which have been duly acknowledged, the work presented in this thesis is original and was carried out by me under the joint supervision of Dr. Fred Aboagye-Antwi and Dr. Isaac Frimpong Aboagye of the Department of Animal Biology and Conservation Science, University of Ghana.

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DEDICATION

This work is dedicated to my children Princess Adu, Prince Kelvin Adu and Aaron Adu.

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My greatest appreciation goes to the Almighty God who made it possible for this work to be accomplished. My sincere gratitude also goes to my supervisors; I am indebted to them for their guidance and excellent supervision.

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ABSTRACT

The transmission of zoonotic gastrointestinal parasites in cattle is dependent on the availability of intermediate hosts, poor sanitation and principally, the husbandry and management practices of cattle. The research work was aimed at studying cattle as reservoirs for parasitic zoonotic diseases in the Lower Manya Krobo District of southern Ghana. Microscopic examinations were done on stool samples to determine the prevalence and intensity of egg counts among cattle (N = 486) sampled at Akuse and Narhkorkpe cattle farms in the district. The overall prevalence of parasite egg infestation in Akuse was 14.2% while that of Narhkorkpe was 19.6 %. The observed difference in prevalence (5.4 % [-1.5 to 12.3]) of helminths in cattle sampled from Akuse and Narhkorkpe by Chi-squared test was not significant ($\chi^2 (1) = 2.52$; $P = 0.1123$). Using morphological characteristics, four genera of helminth eggs were identified. Also, four genera of helminths were identified as follows: *Cooperia* spp., *Heamonchus* spp., Hookworm and *Trichostrongylus* spp. Hookworm and *Trichostrongylus* spp. were the dominant helminth species in the farms of both study communities with prevalence's of 21.5 % and 14.2 % respectively for Akuse and 16.7 % and 19.6 % respectively for Narhkorkpe. The highest median hookworm egg intensity count of 250 eggs/gram in Akuse and 550 egg/gram in Narhkorkpe were observed. Significantly higher median egg intensity of hookworm was recorded in cattle from Narhkorkpe farms (550 eggs/gram) compared with that cattle (250 eggs/gram) from Akuse using Mood's median test [$P = 0.003$]. Quarterly anthelmintic treatment may be appropriately used to minimize the possibility of re-infection and development of resistance. This study provides some data that could serve as a basis for development of effective management and control guidelines of potential cattle zoonoses for stakeholders in the cattle industry, particularly farmers and the veterinary officers.

CHAPTER ONE

1.0 INTRODUCTION

Cattle are domestic animals that are kept in agricultural communities, which help to generate materials such as labour, food and fibre (Alemug, 2017). Cattle can produce milk, which can be processed into other food products like cheese, butter and yoghurt. Other parts of cattle like hooves, bones and horns are used to produce jewellery, pendants, or headgear. The waste from the cattle can be used as compost, which is spread on the farm-land to increase the production of crops (Abunyeya *et al.*, 2007). Cattle is one of the most frequent domestic livestock in the African continent, they are essential property in conventional and contemporary agricultural communities. Cattle also provide human with milk, meat, skin, and draught power for farming activities (Abunyeya *et al.*, 2007). In a good number of conventional settlements, cattle production also plays an imperative role in satisfying the economic needs of the cattle farmers (Fabiya, 1973). Meat is one of the most frequent and essential cattle products, despite the fact that there could be fatalities as a result of helminths infections and other communicable diseases. The value and amount of meat and the income obtained from livestock is not sufficient for most national demand as a result of death and illness as well as decreased productivity and high cost of treating the livestock (Adedipe *et al.*, 1996). Gastrointestinal helminths are a major disadvantage to the health status of cattle, which affect the production of the cattle (Keyyu *et al.*, 2005). Gastrointestinal helminths are frequent parasites of livestock and ruminants and have also been noted to decrease cattle production in many livestock production communities (Rajput *et al.*, 2006). Death of cattle which occur as a result of parasitic diseases may not be serious sometimes but their effects on livestock production and their zoonotic effect on the health of human are known to be very high (Ballweber, 2006). Other losses of livestock that may be linked

with helminth infections, may include weight loss, retarded growth rate, diarrhoea, anaemia and anorexia (Swai *et al.*, 2005). Some other factors influencing helminth infections include weather type, grazing habits, nutritional insufficiency, paddock management, immunological status, existence of intermediate host, the quantity of infective larvae and eggs in the surroundings and vector species involved in disease transmission (Radostits *et al.*, 1994). Helminth infections are facilitated by a number of conditions which include the susceptibility of the host species, the number of the parasite species, the host/parasite interaction, parasite infective dose as well as its pathogenicity (Food and Agricultural Organization, 2000). Zoonosis is an infectious disease of animals, which mostly include vertebrates that can infect humans through various routes (Garcia *et al.*, 2007). Most diseases such as Ebola virus disease, influenza and salmonellosis are zoonosis that are triggered by a range of disease causing organisms such as bacteria, fungi, parasites and virus. About 61% of disease-causing organisms known to infect humans are zoonotic. Numerous of human diseases were can be traced into animals, however, only Zoonotic diseases like rabies are frequently involved in animal to human transmission (Garcia *et al.*, 2007).

Zoonotic infections are worldwide and often spread to humans through a variety of routes (Robinson *et al.*, 2009). Zoonotic diseases are part of the most common diseases and have been shown to be responsible for over 60% of all infectious diseases affecting human (Robinson *et al.*, 2009). Some of the most important zoonosis of human are caused by helminth parasites, including some members of the cestodes, trematodes and nematodes (Robinson *et al.*, 2009). Tissue parasites that affect human are still known to be high in most “third world” countries of the world, with increased human travel playing a major role in disease dispersal (Garcia *et al.*, 2007). Infections like *Echinococcus* currently account for pulmonary and hepatic pathology. Cysticercosis is a major challenge that causes epilepsy. Fascioliasis may also produce a

significant liver problem. Human infection with these zoonotic parasites may result from ingesting contaminated food substances (Garcia *et al.*, 2007). This food substance may be meat that is infested with the parasite such as Taeniasis and trichinosis or consuming food infested with the infective stage of the worm with contaminated soil, water or salad plants (Fascioliasis). Skin contact with water containing active infective larvae contaminated soil and penetrated the skin (dermatitis) through direct contact with infected animals/intermediate hosts or because of insect vectors bites (Robinson *et al.*, 2009). The increasing effect of zoonotic helminth infections in man in any country is directly linked with the prevalence of infections in the animal inhabitants in that country. A research work conducted in Anse-la-Raye, St. Lucia which aims to estimate the prevalence and symptomatology of paediatric toxocariasis observed that seroprevalence of *Toxocara canis* among children was 86%. The prevalence of infection in dogs was not abnormally high in the community (Thompson *et al.*, 1986). Cattle Fascioliasis with prevalence 16.9% was reported from a research work conducted in helminthosis among cattle presented to the Fulani in Zaria in Nigeria state (Okaiyeto, 2008). Research conducted on dogs and cats at New Bussa revealed a massive high occurrence of *Ancylostoma* spp. of hookworm. Zoonosis has diverse transmission route. In the case of direct Zoonosis, the disease is transferred from animal carriers to humans using routes such as droplets/air-borne (influenza), bites, scratches and contact with bodily fluids like urine, blood and saliva leading to rabies infections. On the other hand, transmission of zoonotic diseases can also occur through an intermediate vector, which carries the disease-causing organism with or without being infected with the pathogen (Robinson *et al.*, 2009).

1.1 Problem statement and justification of the study

There is a wide range of zoonotic diseases that could be transmitted from cattle to humans (Clinton, 2014). Examples of these zoonosis include *Trichostrongylus*, and *Fasciola* species, *Clonorchis sinensis*, *Opisthorchis viverrini*. Zoonotic intestinal helminth parasite infections in cattle were the focus of this study. Cattle are among the most prominent domesticated livestock in Sub-Saharan African countries and represent a valuable asset in both traditional and modern agriculture. In addition, they also provide meat, milk, skin, and farm power for farming. In Netherlands, Borgsteede *et al.* (2000) examined cattle for zoonotic diseases and identified high prevalence of *Ostertagia* spp., larvae of *Trichostrongylus* spp., *Oesophagostomum* spp., *Cooperia punctate*, and *Haemonchus contortus*. In Zimbabwe, Moyo *et al.* (1996) reported a high prevalence in *Cooperia pectinata*, *Haemonchus placei*, *Trichostrongylus axei* and *Oesophagostomum radiatum* in some selected cattle farms. Similar work carried out by Komoin *et al.* (2000) in central Cote d'Ivoire also identified similar zoonotic helminths. In Ghana, Agyei (1997) revealed a high prevalence of zoonotic intestinal helminths. The Ghanaian Government, through its peri-urban dairy cattle development project, undertook a research work on zoonotic helminths infestation in cattle in 2002 to promote the use of milk and dairy products from local cattle in some selected districts of the (Bonsu *et al.*, 2000). Districts in which the work was done included the Dangme-West district of the Greater Accra region and a prevalence of 13.8% of zoonotic diseases in cattle was observed in the district. A high prevalence of 19.0% in the Ningo sub-district and 14% in Dodowa, which is the district capital, was reported. At two other sub-districts, Prampram and Osudoku, lower infection rates of 11.3% and 10.8% respectively were recorded. Work conducted by Bannerman (2013) on intestinal helminths in some parts of Greater

Accra Region also reported a high prevalence. These previous works underscore the potential of these cattle infections to impact on human health.

Additionally, meat production and its role as dietary protein has been extensively discussed (Smith *et al.*, 2000; Adzitey, 2012), highlighting the need to investigate and control endoparasitic infections in cattle towards ultimately boosting the health of the populace. In Ghana, the consumption rate of cow meat has increased over the past years (FAO, 2012). Most research works show that cow meat is one of the most common meat products consumed in Ghana (FAO, 2012). Due to this, some attempts are being made at specific abattoirs and livestock farming stations to minimise disease transmission from animals to humans and vice versa. However, the efficacy of such efforts is greatly in doubt, requiring supporting data hence the need for the current study. Most of these zoonotic haemoparasites and helminths could be passed onto persons in the beef production value chain as well as consumer of infected meat product. These infections are detrimental to the health of these at risk individual and negatively impact the already unstable economies of most developing countries, including Ghana. Indeed, the effects of potentially zoonotic infections on the most vulnerable in society, children, could be dire. Children tend to be the singular demographic group that have significantly the highest intensities of zoonotic helminths and therefore experience extreme physical and mental challenges (Fenwick, 2006) affecting the learning abilities of school-going children in particular. Such adverse consequences of potentially zoonotic and or zoonotic diseases emphasises the need to determine the role cattle could play as reservoirs of potentially zoonotic infection towards minimising their impact on human health.

Zoonotic diseases greatly contribute to increased morbidity and mortality rates in sub-Saharan Africa, including Ghana (Ehizibolo *et al.*, 2011). The increasing relevance of zoonotic infection

to public health in recent times (Meslin, 2016), necessitates further investigations into infections that could be easily contracted from livestock, and in the case of the current study, cattle because of the human/animal interactions involved in dairy products production. Such a study could shed light on the disease transmission dynamics of potential cattle zoonoses. Understanding disease transmission dynamics could contribute towards reducing the morbidity and mortality associated with cattle related zoonotic infections. This study therefore set out to examine the role of cattle as reservoirs for potentially zoonotic parasite diseases in the Lower Manya-Krobo district of the Eastern Region of Ghana. The data generated could serve as the basis for the development of management practices and control guidelines.

1.2 General objective of the study

The main aim of the study was to investigate the role of cattle as reservoirs for parasitic zoonotic diseases in the Lower Manya-Krobo district of the Eastern Region of Ghana.

1.2.1 Specific objectives

- 1.To determine the prevalence and intensity of helminthic infections in cattle in the study sites.
- 2.To determine the prevalence of haemoparasites in cattle at the study communities.
- 3.To identify and assess the range of human endoparasites potentially harboured by cattle in the Lower Manya-Krobo district of the Eastern Region of Ghana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global zoonotic disease burden

There is an increasing trend of zoonotic diseases globally with about 61% of infectious diseases known to be zoonotic (Cantas and Suer, 2014). However in Africa and Ghana, there is no much record on zoonotic infections (Cantas and Suer, 2014). Parasitic zoonotic infections are highly prevalent globally causing serious health problems, particularly in the tropics. Diseases due to parasitic zoonoses affect about one quarter of the world's inhabitants (Gilian *et al.*, 2006). Several zoonotic helminths are transmitted from livestock through consumption of their meat product and reside in the intestinal tract of humans. Those of major health importance and some of which are of zoonotic importance include nematodes such as *A. lumbricoides*, *T. trichiura*, hookworms and *S. stercoralis*, trematodes such as schistosomes, *Clonorchis sinensis*, *Opisthorchis viverrini* and *Fasciola* spp. as well as cestodes such as *Taenia solium*, and *T. saginata* (Harhey *et al.*, 2010). Cattle forms a central part of the livestock industry in Ghana. The focus of the livestock industry in Ghana has been the production of beef and to a lesser extent, milk production (Okanta 1992). However, reports indicate that the management systems of cattle production in Ghana, exposes the animals to several diseases (FAO, 2012), some of which could be parasitic zoonotic infections that can be transmitted from cattle to man.

2.2 Burden and geographic distribution of helminths in sub-Saharan Africa

Prevalence of helminth infections was first discovered by Stoll in 1962 (Beasley *et al.*, 2002). The total prevalence, which is the overall helminths infections, is known to be constant in Africa and sub-Saharan African countries. The number however declined in some parts of the

developing countries (De Silva *et al.*, 2003). Recent findings indicate that between a quarter and a third of sub-Saharan African countries inhabitants is at risk for more than one infection of helminths with school going age children dominating (De Silva *et al.*, 2003). With about 181 million school going aged pupils in sub-Saharan Africa examined, an estimate of about eighty-nine million of them are infected with Trichuriasis, Ascariasis, Hookworm, or a combination of these helminths (Brooker *et al.*, 2006). Children tend to harbour significantly high intensities of helminths compared to adults and as a result suffer from intense physical and mental challenges (Fenwick, 2006). These challenges in children in developing countries are very more common due to the increased disease burden in Sub-Saharan African countries. In Kenya for example, these effects also lead to high numbers of school dropout and reduction in school performance (Miguel *et al.*, 2003).

2.2.1 Hookworm infection

According to preceding survey conducted in the year 2002, an estimated number of one hundred and ninety eight million people in Sub-Saharan African countries are known to be infected with hookworm species. About 29% of the world's inhabitants, including (40 to 50 million) children of school going aged, are known to be infected (Brooker, 2006). About 1/3 of hookworm infections in the world occur in Sub-Saharan African countries with a high prevalence in Nigeria (38 million people), with the Democratic Republic of Congo being the next (31 million people), while Angola, Ethiopia and Cote d'Ivoire have a record of about (10 to 11 million people) (Molyneux *et al.*, 2005). Hookworm is the most largely known helminths in Sub-Saharan Africa countries and it is prevailing in most parts of the world, which includes rural and urban communities with the exception of few areas of Southern part of African continent (Brooker *et al.*, 2006).

The littoral regions and places of very high temperatures where land surface temperatures going beyond 37 to 40°C, as well as those closer to Sahel like Cameroon (Brooker, 2000), Chad (Brooker *et al.*, 2002) as well as Mali (Behnke, 2000) are well known for their high occurrence of hookworm and egg intensity as related to other helminths infections (Mabaso *et al.*, 2004). *Ancylostoma duodenale* and *Necator americanus* have been recognized in Sub-Saharan Africa countries with the *N. americanus* representing the most prevalent species of Hookworm (Albonico *et al.*, 1998). The major starting point of iron deficiency and anaemia in the region includes Hookworm (Hotez *et al.*, 2004). Among children of school going age in Zanzibar, about thirty-three percent of deficiency in iron and seventy-three percent of severe anaemia was associated to hookworm infection (De Clercq *et al.*, 1997), while in Kenya and other sub-Saharan African countries; hookworm causes significant effect in anaemia in children in the pre-school (Brooker *et al.*, 2002). Hookworm has also been found to be a major cause of illness and other situations leading to anaemia in women who are in their reproductive age in Sub-Saharan Africa, predominantly among women who have conceived (Hotez *et al.*, 2004). At any specific time, about seven million of expectant mothers in the Sub-Saharan Africa countries (about 1/3 of expectant mothers in the region) have been infested with hookworm (Brooker *et al.*, 2002).

2.2.2 Trichuriasis and ascariasis infections

Trichuris and *Ascaris* infections are known to have a very high prevalence in children of school going age globally (Brooker *et al.*, 2002). Research work indicates that about one hundred and seventy-three million and one hundred and sixty-two million people are known to be infected in sub-Saharan African countries. From these findings, *Ascaris* has one hundred and seventy-three million and one hundred and sixty-two million people been infected while *Trichuris* has one hundred and sixty-two million people been infected. From these report, thirty-six million of the

number are school children who are infected with ascariasis while forty-four million of them are infested with *Trichuriasis* (Brooker *et al.*, 2002). With these infections, Nigeria has been known to have the largest number of infections, where co-infections with hookworm is very common (Dada-Adegbola, 2005). Millions of reported prevalence's are also recorded in Ethiopia, South Africa and Democratic Republic of Congo (DRC). In comparison to hookworm, Ascariasis and Trichuriasis show a remarkable equal distribution in sub-Saharan Africa. The highest prevalence of infections has been recorded in equatorial southeast Africa, Central Africa, West Africa and Eastern Madagascar. In comparison to hookworm, high prevalence of Trichuriasis and Ascariasis in South Africa is less frequent except in KwaZulu-Natal (Brooker *et al.*, 2002). In addition, Ascariasis and Trichuriasis have higher prevalence in most Africans urban communities than in rural communities, unlike hookworm, which is distributed more evenly (Brooker *et al.*, 2002). These results may indicate the ability of the eggs of *Trichuris* as well as *Ascaris* having the potential to live in urban communities which has facilitated the emergence of *Trichuris* as well as *Ascaris* in sub-Saharan African countries.

2.2.3 Strongyloidiasis and other infections

Strongyloidiasis gives symptoms of frequent diarrhoea and nutritional deficiency in sub-Saharan Africa, even though there is decreased statistics on the epidemiology of the disease, due to the difficulties in identifying the infection and the parasite. In a recent research work, Strongyloidiasis was known to be responsible for about 5.3 % of diarrhoea in malnutrition children in Nigerian (Ziem, 2006). *Oesophagostomum bifurcum* and *Ternidens deminutus* are focally endemic and prevalent in Northern Ghana and the Republic of Togo (Bradley, 1990).

2.3 Prevalence of helminths in cattle in some parts of Southern Ghana

Cattle are significant aspect of the livestock industry in Ghana for the production of beef as well as the production of milk (Okanta, 1992). Cattle are used in the Agricultural communities as labour in the farmland. Several metric tons of beef, milk and pieces of hide are obtained from cattle in the country (FAO, 2009). These produce from cattle are mostly not sufficient for the country. These have resulted in the importation of cattle meat products from other countries to supplement local supply (VSD, 2010).

The main management practice which is the extensive system depends primarily on extensive grazing by mostly smallholder herders. There are diverse levels of farming systems used by the large scale of various farms of the state that are higher than the extensive system of cattle production (Oppong-Anane, 2010). In this system of cattle production, cattle may feed on cultivated pastures and natural pastures. All these practices however increase the parasitic infections rate and diseases among cattle. Parasitic helminth infection adds significantly to a reduction of livestock production in sub-Saharan African countries (Harper and Penzhorn, 1999; Kagira, 2009). The helminths decrease animal production and may add to the death of the animal in Ghana (Lebbie *et al.*, 1994). In tropics and sub-tropical countries where marginal levels of nutrition exacerbate the negative effects of infection, most animals die through nematodes (Ademola and Eloff, 2010).

The trematodes, protozoans and cestodes are also significant helminths that cause damage to cattle in Ghana. Some of these are zoonotic and are known to pose danger to consumers. In Ghana, helminthic parasites are part of the increasing death in cattle production. A research work conducted on the health conditions of ruminates of various ages for more than six years' period on the Agricultural Research Station of the University of Ghana, confirmed that most death rates

of cattle of about 67 % happens in the age group of one to twelve months. The research also proved that pneumonia, parasitic gastroenteritis and tapeworms, were diagnosed as the main symptom of these parasitic infection (Oppong, 1972).

In Southern Ghana, cattle are often infected with diverse types of helminth parasites like protozoa, nematodes, cestodes, and trematodes (Pam *et al.*, 2013). The overall infection rate of helminths in the southern part of Ghana is high in all groups of livestock, which also includes the various sexes. Taken the sexes into consideration, the infection rate of *Schistosoma* and *Fasciola*, is significantly higher in the females, while *Strongyloides* genera is marginally higher in the male cattle (Animal Health and Food Safety Division, 2013). The overall prevalence among the native Ndama is marginally higher than that of the Sanga and Friesian-Sanga in Pokuase cattle (Health and Food Safety Division, 2013). The overall infection rate among the local Ndama is marginally higher. *Fasciola* spp. is highly prevalent in the Ndama while *Dicrocoelium* and *Schistosomes* is highly prevalent in the Sanga Torgerson and Claxton (1999). On the other hand, there is no significant difference in the overall infection rate that was noted at Pokuase and Frafraha even though the Ndama is mostly found at Pokuase while the other breeds at Frafraha in the Adenta District (Squire *et al.*, 2013). *Trichostrongylus* spp., *Heamonchus* spp. and *Oesophagostomum* spp. are the frequent *Strongyle* genera found in cattle in Ghana (Agyei, 1991). *Strongyles* have been recognized in Ghana and the tropics to be the most observed and economically significant gastrointestinal helminth (Odoi *et al.*, 2007).

2.4 Discovery of the helminth worms

The identification of helminth worms and their diseases can be linked historically to the Bible. Inside the medical Egyptian papyri, the Ebers papyrus was recognized as the intestinal worms. These facts can be authenticated by the identification of calcified helminth eggs in mummies discovered in 1200 BC. Roman physicians researched on human roundworms known as *Ascaris lumbricoides*, *Enterobius vermicularis* and tape worms which were recognized to be part of the species *Taenia*. (Spencer, 1953). Paulus Aegineta gave a better medical explanation of the effect they can cause in ruminants (Grove, 1990). Due to the fall of the Roman Empire, the study of medical associated programmes was changed by Arabic physicians who recognized *Ascaris*, tapeworms and *Enterobius* as well as the guinea worm (*Dracunculus medinensis*).

Six helminth worms namely, *Taenia lata*, *Ascaris vermicularis*, *Fasciola hepatica*, *Ascaris lumbricoides*, *Taenia solium* and *Gordius medinensis* was identified and explained by Linnaeus recognized and explained (Linnaeus, 1758). At the onset of the 20th century, more species were recognized and explained after 28 species had been noted in human inhabitants. This number has now increased to about 300 species which may involve accidental and very rare species (Cox, 1998). About 280 species of helminths were recognized by Crewe and Ashford (Ashford, 1979). The worm that was identified by Linnaeus was named as *A. lumbricoides* and was part of the six helminths identified (Linnaeus, 1758). About One billion inhabitants of the world are now estimated to be infected with this *A. lumbricoides* (Ashford, 1998).

Ascariasis is known to be an ancient age infection and *A. lumbricoides* eggs have been recognized in human coprolites from Peru (Horne, 1985). In the ancient World, there have been reports of *A. lumbricoides* in the Middle Kingdom of Egyptian mummy (Cockburn *et al.*, 1998) as well as China in the Ming Dynasty (Dexiang *et al.*, 1981). There are detailed records that

captures the Egyptian medical papyri, the investigations of Hippocrates in the fifth century (Hoeppli, 1956), and texts of physicians from Arabic and Roman (Grove, 1990). In the later part of the 17th century, Edward Tyson described the frame work of the worm in more details (Tyson, 1683). Shortly afterwards the worm was described by Redi (1684). These two journals, with that published by Tyson on species of tapeworms of humans can be said to have marked the initial stages of helminthology, which moved to the peak in the 19th century (Tyson, 1683). Research work conducted by (Elliotson, 1833) also revealed that, during this period, the first effort was made to understand the infections triggered by *Ascaris* and other intestinal helminth and in what way they ought to be cured.

The major difficulty for this research work on *Ascaris* and other parasitic nematodes was on the manner in which the parasite's eggs infect a fresh host after migrating from the main host. In the year 1862, the transmission caused by ingesting eggs was explained by Casimir Joseph Davaine (Kean *et al.*, 1978) and later by Grassi (1990), who infected himself with the eggs of *A. lumbricoides* and found eggs of the parasite in his faeces. The life cycle in humans, comprising the relocation of the larval stages from one place to another in the body, was exposed in 1922 by a paediatrician from Japan called Shimesu Koino, who infected a volunteer and himself and detected what was happening when he found huge quantities of larvae in his sputum (Kean *et al.*, 1978).

Another important component of helminths is the hookworm. Hookworm that infects human is caused by *N. americanus* and *A. duodenale*. *Ancylostoma duodenale* originates from Asia and *N. americanus* originates from Africa (Hoeppli, 1956). *Necator americanus* and *A. duodenale* species of the Hookworm have similar life cycles. Mature male and female worms reside in the small intestine and cause serious loss of blood. Soil becomes contaminated with egg when eggs

pass out with faeces into the soil. The larvae emerge and transform into larvae that become infectious and penetrate the skin of another host. In humans there is migration of the larvae to the lungs as well as the trachea. They are then swallowed before they mature and migrate into the small intestine of human (Hoepli, 1956). Human hookworm infestation can be traced to humans for over (5,000) years in the Old World (Hoepli, 1956). There have been a lot of questions about the existence of hookworm infections in the pre-Columbian America. Robert Desowitz agree that hookworms were in human before the Europeans arrived (Desowitz, 1997). Kathleen Fuller proposed that, hookworms were found in Americas after 1492 (Fuller, 1997). Parasitological facts appear to back Desowitz's ideas since ova recognized as *Ancylostoma* species, which have been present in a human coprolite since 3350 BC and AD 480 (Ferreira *et al.*, 2000).

Larval of nematodes that was recognized to be hookworms, have been present in faecal samples from 200 BC around the Plateau of Colorado (Faulkner *et al.*, 1989). The greenish pallor colour of the Egyptian chlorosis was initially associated with hookworm infections during the 19th century. It has been suggested that the enigmatic condition that happens in several papyri which may include the Ebers papyrus could refer to hookworms (Ebbell, 1937), but no scientific fact was made available (Nunn *et al.*, 2000).

Information is also available from the third century BC in China to a yellow disease era (Hoepli, 1956). During the 18th to 19th century's era, information on hookworm from the West Indies and Central America increased drastically (Grove, 1990). Hookworms were present in human inhabitants during 1838 (Kean *et al.*, 1978). The linkage between the worms and disease was established by Wilhelm Griesinger finally (Kean *et al.*, 1978). Edoardo Perroncito in the year 1879 established the real connection while researching on the diseases of mining workers in

the tunnel of St. Gothard (Perroncito, 1877). There were favourable Conditions in mining communities that facilitated the development of larvae from hookworms. When Arthur Looss infected himself accidentally during the 19th century he established the fact that hookworm larvae enter the human system by boring through the skin (Looss, 1905). Hookworm disease became a very serious problem in the United States in the 20th century. The Rockefeller Foundation devised control measures to deal with the infection. The foundation led to the establishment of Public Health schools and led to the creation of the World Health Organization (Ettling, 1990).

Stroglyoides: *Stroglyoides stercoralis* and *S. fuelleborni* are the common sub-species of human. *Stroglyoides elleborni* is recurrent in Africa while *S. Kellyi* are also common in Papua New Guinea. *Stroglyoides stercoralis* is the more frequent and important species as far as human disease is concerned (Grove, 1990). Its life cycle is more complex than that of any of the other nematodes discussed so far and involves both parasitic and free-living generations. The adult female worm, which is pathogenic, is located in the small intestine where it lay eggs which will later hatch in the host to form the first-stage larvae. The larvae are therefore released into the faeces, which later found itself in the soil when the egg comes into contact with the soil from the faeces. At this stage moulting occurs which lead to the production of larvae that is the infective stage and then penetrate the skin. The larvae then move to the lungs where they are swallowed. It then migrates to the gut just as in the case of hookworms. The larvae stage may sometimes develop into the infective stage without escaping from the gut and penetrate the wall of the gut walls (Grove, 1990).

Flukes: More than hundred species of flukes infect human's inhabitants in both the adult's stage and the larvae stage. The first significant ones that is of zoonotic importance here are *Paragonimus westermani* which is the lung fluke that causes paragonimiasis. The second one that will be considered is the *Clonorchis sinensis* that causes clonorchiasis. The last one is the *Opisthorchiasis* spp. that is responsible for Opisthorchiasis (Nieto, 1982). Almost all the significant discoveries about the parasites were recognized from 1874–1918 (Nieto, 1982).

2.5 Hookworm classification and history of discovery

Hookworm is a parasite that belongs to the species of *A. duodenale* and *N. americanus*. It is caused by a nematode that is a blood-feeding parasite. The parasites infect about 576 to 740 million people globally. About eighty million people being infected severely (Gasser *et al.*, 2009). The morbidity that is linked with intense infection includes intestinal blood loss, anaemia and protein malnutrition. The burden of infection is found mostly among the world's poorest countries that depend on not more than two dollars a day (Gasser *et al.*, 2009). Children in low and middle-income countries are more prone to the parasite (Bethony *et al.*, 2006). Infection with hookworm can cause stunted growth and impair intellectual and cognitive development. Hookworm is classified as followed (Bethony *et al.*, 2006).

Kingdom: Animalia

Phylum: Nematoda

Class: Secernentea

Order: Strongiloidae

Family: Ancylostomatidae

Genus: Necator/Ancylostoma

The documentation on hookworm can be traced from the third-century B.C. when the authors of the Hippocratic Corpus recognized a disease that has a characteristic intestinal discomfort. (Bethony *et al.*, 2006). The first definitive observations of hookworm were discovered by Angelo Dubini during an autopsy in 1838 (Bethony *et al.*, 2006). Research work on hookworm infection began to rise globally, in 1846 in Egypt and then in Brazil in the year 1865. Giovanni Grassi and others had announced a technique of diagnosis through microscopic examination of the faecal sample for the eggs of hookworm in the year 1878 (Hotez *et al.*, 2005).

Edoardo Perroncito observed a significant correlation between hookworms and anaemia among mining workers at the St. Gotthard tunnel in 1880 (Hotez *et al.*, 2005). In 1881, the first anthelmintic drug was developed and helped in the treatment of hookworm infestation. Arthur Looss discovered the life cycle of hookworm. Charles Stiles also discovered *Necator Americana* as another species of hookworm that is very harmful to man (Hotez *et al.*, 2005). Stiles also facilitated in the Rockefeller Foundation to set up one million dollars in the treatment of hookworm in the United States (Hotez *et al.*, 2005), which was successful in getting rid of hookworm from the United States.

2.5.1 Epidemiology of hookworm.

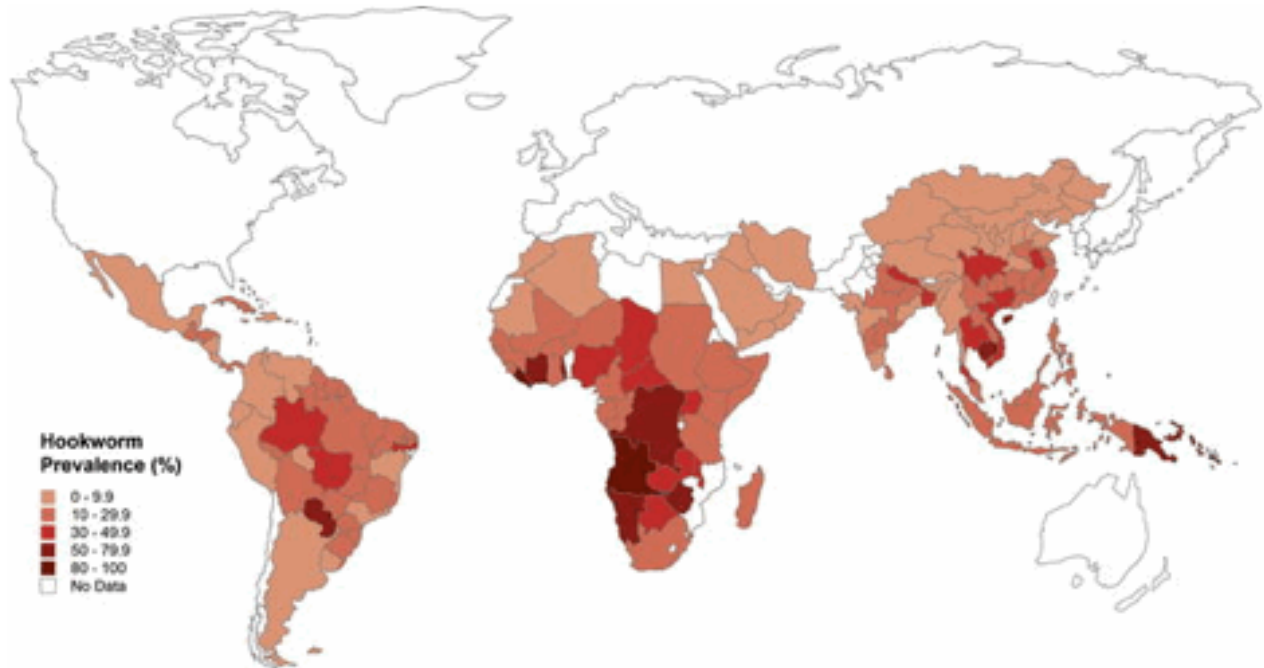


Figure 2.1: Map of global hookworm prevalence (Hotez *et al.*, 2005)

There is an estimation of 576 to 740 million people been infected with Hookworm worldwide (Gasser *et al.*, 2009). With these individuals who are infected, close to eighty million people have severe infection (Gasser *et al.*, 2009). *Necator americanus* is very frequent in the sub-Saharan African countries, America and Asia (Hotez *et al.*, 2005). Species of *A. Duodenale* is highly prevalent in Europe and the Mediterranean. Most people who show symptoms of the infection are found in sub-Saharan African countries, East Asia and the Pacific Islands with each area having an average number of one hundred and ninety-eight million and one hundred and forty-nine million people showing symptoms of the parasite (Hotez *et al.*, 2005). Other areas with significant rate of infection include South Asia with about fifty million people, Latin

America and the Caribbean with both recording fifty million. South Asia has a record of about fifty-nine million, Middle East/North Africa also recording ten million cases (Bethony *et al.*, 2006). Most of these infected people reside in developing countries with poor sanitary conditions. Hookworm infection is mostly present in under developed countries that depend on less than two dollars a day for survival (Hotez *et al.*, 2005).

2.5.2 Life cycle of hookworm

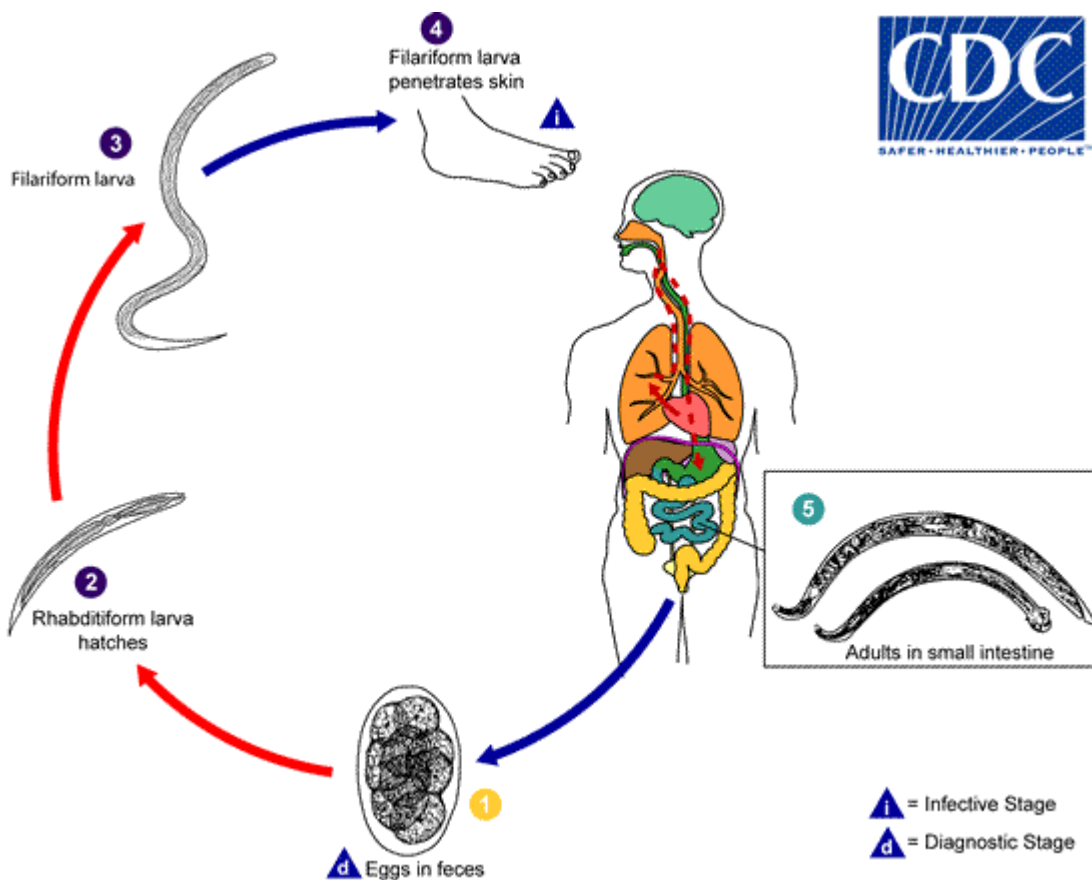


Figure 2.2: Hookworm life cycle showing the various developmental stages of the parasite (CDC, 2000).

Ancylostoma duodenale and *N. americanus* eggs are common in humid, damp soil where they will develop into first stage larvae. The first stage larvae are also known as the non-infective

rhabditiform stage. The first stage feed on microbes in the soil and then transform into next stage larvae also known as the L₂. This stage is also in the rhabditiform stage. It feed for about seven days and then develops into the third stage larvae (L₃). The third stage is the filariform stage of the lifecycle of the parasite. This is also known as the non-feeding infective form of the larvae stage (Hawdon and Hotez, 1996). The third stage larvae form is motile. They can survive for about two weeks even when not present in a host. The only route for penetration for *N. americanus* larvae is the skin, with *A. duodenale* finding its way through its host by ingesting it orally (Hawdon and Hotez, 1996).

The next stage of the cycle allows L₃ larvae to enter the host, from where they move through the subcutaneous venules and lymphatic vessels of the infected human host. The L₃ larvae then enter the lungs through the pulmonary capillaries and then come out into the alveoli. They then move up the trachea to be coughed and swallowed by the host. The L₃ larvae then migrate to the small intestine and then develop into the L₄ stage. It takes about 5 to 9 weeks to complete the entire process. The female adult worms then release about 9,000 to 10,000 eggs per (*N. americanus*) *A. duodenale* also releasing 25,000 to 30,000 eggs per day which are released in the faeces of the human host. The eggs then hatch into the environment after some days for the cycle to continue (Hawdon and Hotez, 1996).



Figure 2.3: A typical hookworm egg and larvae commonly found in infected stool of vertebrate organisms. A: egg of hookworm, B: rhabditiform larvae and C: hookworm filariform larvae (CDC, 2011)

2.5.3 Morphology of hookworm.

Ancylostoma duodenale worms have grimy white colour or sometimes have pink colour. The head is bent somewhat in relation to the rest of the body. The curvature shape forms a hook shape at the frontal end. This was why it was given the name hookworm; their mouth-parts are well-developed and possess two pair of teeth. The male hookworm is about one centimetre by 0.5 millimetres, with the females mostly longer and stouter. The males can be differentiated from female hookworm by the established posterior copulatory *bursa* (John *et al.*, 2006). *Necator americanus* is similar to *A. duodenale* when using the morphological characteristics. *N.*

americanus has usually smaller structure than *A. duodenale*. *Ancylostoma duodenale* has two pairs of teeth, while *N. americanus* has a pair of cutting plates in the buccal capsule. In addition, the structure of the hook is much clearer in *N. americanus* than in *A. duodenale* (John *et al.*, 2006).

2.5.4 Pathology of hookworm infection

Infection of Hookworm is well thought-out to be asymptomatic. It is a very harmful infection due to its negative effect on its host (Stoll, 1962). Infected person's experience general symptoms of the parasite after a short time of infection. The symptoms are accompanied by Ground-itch sensitive reaction at the point where the parasite enters the skin. Such symptoms are common with *N. americanus* penetration into the skin (John *et al.*, 2006) and could lead into coughing and subsequently, pneumonitis may occur when the larvae commence to split into the alveoli and moves into the trachea. The larvae then travel to the small intestine of the host where maturation begins and as a result, the patient suffers from diarrhoea and gastrointestinal discomfort (John *et al.*, 2006). Most deaths relating to hookworm infection happen as a result of intestinal blood loss, protein malnutrition, anaemia and iron insufficiency (Bethony *et al.*, 2006). This happens because the mature hookworms in the small intestine ingesting blood, rupturing erythrocyte, and destroying haemoglobin in the infected person. This prolonged blood loss can manifest itself through facial and peripheral oedema; eosinophilia and pica caused by iron insufficiency anaemia is also observed by a number of hookworm-infected individuals (Bethony *et al.*, 2006).

In recent times, more consideration has been given to other significant results of hookworm infection that play a great role in public health. It is now a fact that children who experience chronic hookworm infection can undergo growth retardation as well as rational and cognitive

challenges (Hotez *et al.*, 1995). Symptoms include swelling in the gut, inspired by the feeding ability of the hookworms, nausea, abdominal pain and discontinuous diarrhoea, progressive anaemia in protracted disease, impulsive appetite, pica, continual constipation followed by diarrhoea, palpitations, thread pulse, chilliness of the skin, paleness of the mucous membranes, exhaustion and weakness, shortness of breath and in cases running a fatal course, dysentery, haemorrhages, and oedema (John *et al.*, 2006). Blood examination in premature infection frequently show an increase in the figures of eosinophilia, a type of white blood cell that is stimulated by worm infections in tissues. Declining blood haemoglobin levels will be observed in cases of protracted infection with anaemia (Hotez *et al.*, 1995). Hookworm occurrence and concentration can be advanced among adult males. This is why hookworm infection tends to be work-related, which means that co-workers and other close groups sustain a high occurrence of infection amid themselves by contaminating their working surroundings. On the other hand, in most widespread areas, adult women are violently affected by anaemia largely because they have to a large extent, higher physiological needs for iron (menstruation, repeated pregnancy) (Hotez *et al.*, 1995). In pregnant women, after childbirth a number of or all of the larvae re-enter the circulatory system and then shift into the mammary glands. This will cause the new-born baby to obtain a large quantity of infective larvae all the way through their mother's milk (Hotez *et al.*, 1995).

2.5.5 Diagnosis of hookworm infection

The eggs of the parasite have oval shape and measures about 60 μm by 40 μm . It has colourless and a thin clear hyaline shell membrane. When the worm migrates into the host's intestine, it expels eggs which consists of an unsegmented ovum. As the egg moves down the intestine, the ovum develops leading to the development of the segmented ovum of the egg. The eggs of both

Ancylostoma and *Necator* and other hookworm species are impossible to differentiate and must be cultured in the lab to allow larvae to hatch out before the species can be recognized (John *et al.*, 2006). If the faecal sample is left for a day or more under humid environment, the larvae will have hatched out, so eggs might no longer be visible in the stool. In such circumstances, it is important to differentiate hookworms from *Strongyloides* larvae. The larvae of the two hookworm species can also be differentiated microscopically (John *et al.*, 2006).

Mature worms are not often seen but if found, would allow definitive identification of the species. Classification can be done by using the distance end to end of the buccal cavity, the space amid the oral aperture and the oesophagus. The rhabditiform of hookworm larvae have extended buccal cavities while *Strongyloides* rhabditiform larvae have short buccal cavities (John *et al.*, 2006). In Recent times there has been the development of DNA-based tools for diagnosis of infection, specific identification of hookworm, and analysis of genetic inconsistency contained by population of hookworm (Yong *et al.*, 2007). Because hookworm eggs are often not varied from other parasitic eggs, PCR techniques could be used as molecular method for more precise identification of hookworm in stool samples (Yong *et al.*, 2007).

2.5.6 Prevention of hookworm infection

The appropriate condition for infective larvae to develop and continue to exist in its environment is a damp dirt loamy and sandy soil. The larvae are unable to continue to exist in clay soil. The major way of prevention is to practice good personal hygiene. This includes the avoidance of defecating in an open space and the use of human excreta or raw sewage as fertilizer in agriculture (CDC, 2015).

2.6 *Haemonchus* species

2.6.1 Classification and history of discovery

Haemonchus species are nematodes of abomasum's and are mostly important pathogens that occur among a diverse group of artiodactyls hosts including species among 46 genera of *Camelidae* and *Pecora* (Gibbs, 1986). *Haemonchus* represent the most important helminth parasites in goat, sheep and cattle throughout the world (Gibbs, 1986). A considerable research has been conducted on the species of *Haemonchus*. *Haemonchus contortus*, *H. placei* and *H. similis* are distributed around the globe in domesticated Bovinae and Caprinae (Gibbs, 1986). Another seven species include *H. bedfordi dinniki* Sachs, *H. horaki lichtenfels*, *H. kruger*, *H. lawrencei* and *H. Mitchellii* are primary parasites among respective host groups and species of wild Bovinae (Hoberg, 1997). *Cephalophinae*, *Reduncinae*, *Aepycerotinae*, *Antilopinae*, *Alcelaphinae*, and *Caprinae* are frequently observed in sub-Saharan Africa. *Haemonchus okapiaevan* are frequent in *Giraffidae* and *H. Longistipes* are common infections in *Camelidae* (Gibbons, 1979) domestic sheep, goats and cattle across a broader range extending from Africa to southern Eurasia.

2.6.2 Epidemiology of *Haemonchus*

Haemonchus contortus occurs in almost all regions where small ruminants are raised, with the potential for out breaks of Haemonchosis, irrespective of the climatic zone (Dorny *et al.*, 1995). As a result of clinical and economic importance, *H. contortus* is probably the most studied of ruminant helminths. Epidemiological studies constitute a vast literature that defines its ecological adaptability across many different environments (Dorny *et al.*, 1995). The distribution of *H. contortus* is in relation to the availability of moisture (rainfall) and the typical temperature range

in different types of environments, namely tropical, sub-tropical, temperate (warm and cool) and arid region. Although information is not available from some regions, taken as a whole, the studies gives an indication of the prevalence of *H. contortus* and the severity of Haemonchosis in some communities (Dorny *et al.*, 1995). Due to continually high temperatures, most locations within these climatic zones consistently support the larval development of *H. contortus*, and the presence of this parasite relates almost entirely to rainfall. In the wet tropics and equatorial zones, infective larvae are found on pasture throughout the year, and Haemonchosis an important limitation to the raising of small ruminants and cattle (Barger *et al.*, 1994). These regions include Sub-Saharan tropical Africa, South East Asia, tropical Pacific Island countries, Central America and countries in the northern parts of South America and the Caribbean. In the sub-tropics, seasonal variations in rainfall largely determine whether *H. contortus* is a continual or, alternatively, a routine seasonal threat, as generally high temperatures maintain the potential for rapid population development. However, there is extensive variation in the risk for haemonchosis throughout this zone, depending on the relative length of dry seasons, and in some cases, on the effects of altitude and temperature control (Githigia *et al.*, 2001). In markedly seasonal climates with long and hot dry seasons, during which there is minimal external survival of the infective larvae, hypobiosis of the fourth-stage larvae allows *H. contortus* to survive until more favourable conditions resume (Gibbs, 1986). Regions of high prevalence include the north and south of the true tropics in Africa, Asia and the Americas, and some southern regions of the USA, Central and Southern India and the North of Australia. *Haemonchus contortus* is an important seasonal threat in the warmer temperate climatic zones, as temperatures are sufficiently high to permit development of the parasite for several months of the year. Seasonally dry conditions or droughts, limit egg development for part of the year, particularly when combined with altitude

(Gibbs, 1986). These verities are greatest in summer rainfall regions and whether larval development is constant or sporadic throughout summer depends mostly on the pattern of rainfall (Swan, 1970). In predominately winter rainfall areas as in this zone, Haemonchosis usually occurs more sporadically but is still a seasonally endemic threat, also depending on the distribution of summer rainfall (Swan, 1970). Where significant small ruminant populations occur, affected regions extend from the tropics to around 35 ° N and 35 ° S, including southern Africa, Eastern Australia, parts of southern USA, mid-regions of, southern Asia, Mediterranean Climatic zones and South America (Altaif *et al.*, 1983).

2.6.3 Life cycle of *Haemonchus*

The mature worms attach to the abomasal mucosa and feed on the blood supplying these tissues (Okon and Enyenihi, 1975). The female then lay over 10,000 eggs a day which are expelled from the host animal through their faeces. After egg hatching, *H. contortus* larvae moult three times, leading to the formation L₃ form that is communicable for the animals. The host consumes these larvae when feeding and the L₄ larvae are produced after an extra moult (Okon and Enyenihi, 1975).

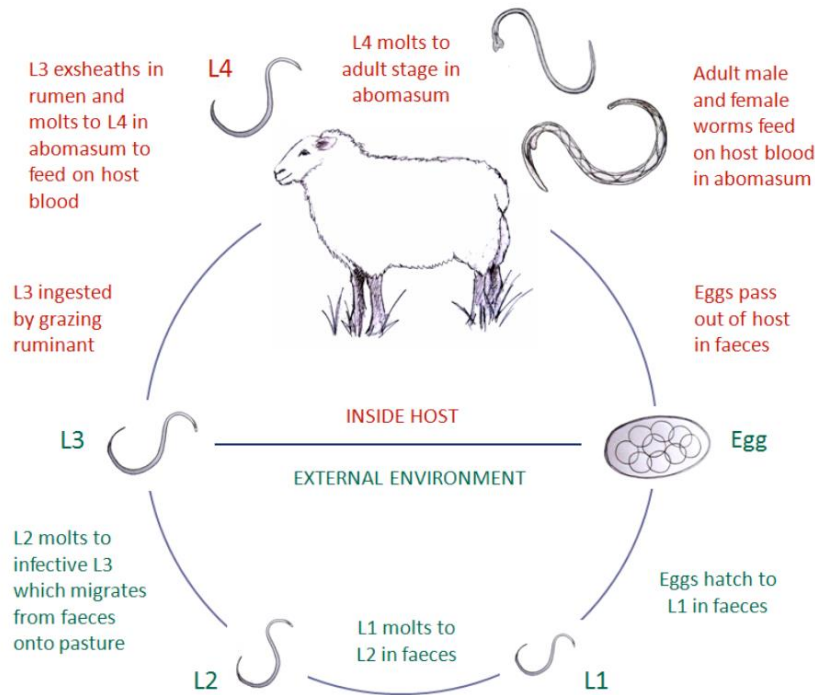


Figure 2.4: Life cycle of *Haemonchus* showing the various developmental stages (CDC, 2002)

2.6.4 Morphology of *Haemonchus*

The oocyte of *H. contortus* is yellowish in colour with eggs of about 70–85µm long by 44µm broad, with an initial stage of cleavage containing about 16 and 32 cells. The adult female is about 18–30 mm long and can be identified by its mark "barber pole" colouration. The red and white colour manifestation is as a result of the fact that *H. contortus* feed on blood, and the white ovaries can be observed as curves in the region of the blood-filled intestine. The male adult worm is to a great extent smaller, which is about 10–20 mm long, and portrays a unique characteristic of a copulatory bursa which is well developed and contains a dorsal lobe which is asymmetrical and a Y-shaped dorsal ray (Chiejina *et al.*, 2015).

2.6.5 Pathology of *Haemonchus* infection

Haemonchus contortus is the most pathogenic of the common nematodes of small ruminants. This is due to its blood-feeding activity and its capacity for rapid population increases during favourable conditions that lead to the development of the free-living stages. The pathology of Haemonchosis and associated clinical signs are mostly associated with the anaemia that develops as a consequence of the blood-feeding activity of the parasite (Dunn, 1978; Urquhart *et al.*, 1996). Blood loss begins with the development of the fourth larval stage (Veglia, 1915), with anaemia being the first to be detected some few days after infection has started (Hunter and McKenzie, 1982). Depending on the extent and the intensity of infection and the response of the host, Haemonchosis has been categorized into three broad syndromes: hyper acute, acute and chronic (Dunn, 1978; Urquhart *et al.*, 1996). In the relatively rare hyper acute form, massive blood loss from infection with about 30,000 *H. contortus* causes a haemorrhagic terminal anaemia and gastritis (Dunn, 1978). Deaths occur suddenly with no symptoms of the disease, but with symptoms of anaemia in many of the survivors. The diagnosis is obvious necropsy due to very large numbers of worm for different developmental stages, and numerous haemorrhages on the mucosal surface (Dunn, 1978). In acute Haemonchosis, anaemia develops over a longer period, but deaths may occur within few days of infection, depending on the rate of larval infection. *Haemonchus contortus* burdens of 20,000 worms per sheep may be present (Urquhart *et al.* 1996), with faecal worm egg counts of 50,000 eggs per gram (Dunn, 1978). At necropsy, the carcass becomes pale with marked ascites and submandibular oedema, reflecting the hypoproteinaemia which also results from the blood-feeding activity of *H. contortus*. The blood may be watery and fail to clot, and the abomasal mucosa is often oedematous with blood-flecked mucous with signs of parasite attachment (Dunn, 1978). The histopathological changes that are

linked with acute Haemonchosis include traumatic damage to the mucosal surface and cellular immunological response (Hunter and McKenzie, 1982; Silverman and Paterson, 1960). Infections with smaller but persistent *H. contortus* burden have been characterized as chronic Haemonchosis which may pass unnoticed or become obvious only when larval intake and, hence, worm burdens increase, or when poor nutritional conditions reduce the capacity of the host to tolerate the pathogenic effects (Urquhart,1975; Dunn,1978). Chronic Haemonchosis is most common in environments, which are marginal for the development of the free-living stages, or during less favourable periods in seasonally endemic zones, and is usually accompanied by infections with other helminths (Urquhart, 1975; Dunn, 1978).

2.6.6 Diagnosis of *Haemonchus* infection

Faecal worm egg counts are used as a monitoring tool to show the extent of pasture contamination with *H. contortus* eggs (Barger *et al.*, 1972). With nematode species the faecal egg worm count is not a sensitive measure of infection, as a result of variable affiliation between the number of worms in the gastrointestinal tract of the host and the number of eggs in the faeces, and the large variation in worm burdens among diverse groups of animals (Barger *et al.*, 1972). Faecal worm egg count does not account for the presence of immature worms and is influenced by the density of faeces due to variations in the water content (Le Jambre *et al.*, 2006). Faecal worm egg count has advantages of low cost with simple technique, and is an effective diagnostic indicator, provided that sufficient animals from a group are sampled, the laboratory procedures are appropriate (Le Jambre *et al.*, 2006).

In addition, the diagnostic value of faecal worm egg count is greater for *H. contortus* than for other *Trichostrongylus* spp., because there is a relatively strong affiliation between the biomass

and the output of worm egg (Coadwell and Ward, 1982), and between the total *H. contortus* egg count and faecal worm egg count in sheep (Roberts and Swan, 1981). Most ruminant nematodes like the *H. contortus* is a prolific egg-producer (Gordon, 1948), and the high faecal egg worm counts typically seen in acute Haemonchosis regularly allow this disease to be distinguished from other helminthiasis. The interpretation of nematode faecal worm egg counts is regularly based on the mean for a flock or herd, predominantly if processed by a composite (bulked) laboratory method; this value is noticeably far lower than the highest extreme counts, but the latter must be taken into consideration. There is no point in withholding treatment on the basis of a moderate mean faecal worm egg count if some individuals are likely to be at significant risk, and where (as usual) it is not possible to individually identify them. Faecal worm egg counts are less definitive when intended to indicate the presence of *H. contortus* before significant burdens develop, or the degree of pasture contamination with *H. contortus* eggs, predominantly if no diversification of counts to species or species is available. In such situations, considerably lower faecal worm egg counts become significant, and to prevent excessive pasture contamination, treatment may be recommended at mean values as low as 1000 egg per gram. Research works indicate that faecal worm egg count for *H. contortus* are moderately repeatable between individuals, indicating that the same animals tend to have a consistently high or low ranking of counts inside the group (Barger, 1985). Other sources of variation also affect the mean egg count result, including variability inherent to the technique and operator proficiency, each of which follow diverse distributions statistically (Van Burgel *et al.*, 2014), and regularly results in wide confidence intervals around the true mean count. The variation expected for egg distribution inside faecal suspensions as a Poisson distribution can be predicted, and inappropriate variation decreased by increasing the sample number and to a degree by more sensitive method of

detection (Torgerson *et al.*, 2012). The minimum sample size of 10 from a flock, which has been accepted (Brundson, 1970), is considered reasonable for the majority of situations, and is supported by modelling of sample sizes for use in a composite method (Morgan *et al.*, 2005). However, this statement is made with the important qualification that more samples may be required if there is significant aggregation (over dispersion) inside the group. Noticeable, the degree of inside-flock variation cannot be easily estimated from a small sample, but it is predominantly frequent for *H. contortus*, where individual animals with high burdens may have massively higher faecal worm egg count than the mean of the group; to this extent, a sample number larger than 10 is generally warranted when a sensitive indication of *H. contortus* burden or an estimate of *H. contortus* egg output is required (Morgan *et al.*, 2005).

The most frequently used method in the research laboratory remains variations on the McMaster procedure where helminthic faecal eggs are counted by faecal salt and zinc sulphate flotation (Urquhart *et al.*, 1996). The Christie inside-laboratory sensitivity can be increased by counting more chambers. If the cost of faecal egg count techniques is a limitation to wide adoption for routine nematode monitoring, this can be decreased by the use of a composite counting technique. This entails counting a smaller number of chambers, but there is no loss of precision of the mean count in comparison with that from individual faecal egg counts (Morgan *et al.*, 2005), allowing the sampling and testing of a large number of animals at a decreased cost. Irrespective of the method used, its apparent simplicity ought not to be overestimated, as evaluations have shown considerable potential for operator-error when conducted in a research laboratory setting (Van Burgel *et al.*, 2014) by inadequately trained operators (McCoy *et al.*, 2005)

2.6.6.1 Genetic selection strategies against *Haemonchus contortus*

Genetic techniques for *H. contortus* have largely centred on resistance, as a reduction in worm burdens decreases both the risk of Haemonchosis and pasture worm egg contamination. The relatively high heritability gives significant potential for genetic selection techniques (Van Wyk, 2009), and although some investigations with Merino sheep indicate that selection for low *H. contortus* faecal worm egg counts may result in lower animal production (Kelly *et al.*, 2013). It has also been shown that sheep that survive heavy *H. contortus* challenge have lower faecal worm egg counts and higher haematocrits and body weights (Kelly *et al.*, 2013). Taken together, it appears that selection based on either faecal worm egg counts or body weight when under significant *H. contortus* challenge will identify sheep that are both resistant and resilient, and hence most suited to haemonchosis-endemic situations, although possibly with a minor compromise in wool production (Kelly *et al.*, 2013). The greater natural resistance to *H. contortus* of hair-breed sheep compared with European breeds was well described from observations on Red Masai sheep in Kenya and has since been confirmed for both sheep and goats in numerous reports from a wide range of communities (Amarante *et al.*, 1999). Variation of breeds reflects the diverse evolutionary environments and is consistent with demonstrated differences in immunological responses (Amarante *et al.*, 2005).

A key factor influencing the uptake of genetic strategies by livestock owners is the practicality and accuracy of selection markers for worm resistance. Currently, selection is generally based on an FWEC index, and significant genetic progress towards increased flock resistance has been achieved where this has been pursued over some years. The potential for more precise and easily utilized genetic markers has been the subject of much research work (Amarante *et al.*, 2005) including quantitative trait based on haematological parameters (Andronicos *et al.*, 2014), immunological indicators (Amarante *et al.*, 2005) and molecular markers. Direct genomic

assessment is likely to prove challenging, given the multiplicity of processes contributing to the immunological recognition and response mechanisms, but at least one genomic test that explains a proportion of the resistance variation between individuals is available on a large scale (Van Wyk, 2002).

2.6.6.2 Biological control of *Haemonchus*

The potential for biological control technologies to supplement the use of anthelmintic has led to a considerable volume of investigation over some decades, especially into the possible roles for nematophagous fungi and bioactive pasture plants (Waller *et al.*, 2001). The effect of naturally occurring fungi which inhabit the soil and pasture, and form hyphae which trap and destroy nematode larvae, has been exploited by dosing sheep with fungal spores, so that these pass into the faeces, where they mature and prey on infective larvae (Waller *et al.*, 2001). A number of fungal species have activity against the larvae of ruminant nematode parasites, with investigations chiefly involving *Duddingto flagrans*, although the search for additional candidate species continues. It is envisaged that by continuous feeding of the predacious fungi to grazing animals in feed supplements over periods of weeks or months, an epidemiological effect will be achieved due to the reduction in their larval intake. Some promising, though variable, results have been shown in small-scale grazing studies in diverse environment with sheep (Waller *et al.*, 2001) and goats but it this approach is yet to be translated into routine control programmes for ruminants.

A large number of pasture plant species are known to contain bioactive chemicals, especially the condensed tannin phenolic compounds, which are associated with decreased nematode burdens and improved animal production performance (Hoste *et al.*, 2016). These compounds, especially the condensed tannins, bind to proteins and prevent their degradation in the rumen, and can have

a positive nutritive value, although in excessive concentrations or when protein intake is low, they can also have detrimental nutritional effects (Waghorn, 2008). There is some contention regarding the mode of anthelmintic action of condensed tannins: whether this is a direct effect of pharmaceutical-like of various polyphenolic compounds on nematode at various life-cycle stages of the parasite (Hoste *et al.*, 2012) or an indirect effect through an improved host immune response due to the protein-binding properties of tannins (Waghorn, 2008). Positive, but variable effects against worm infections have been demonstrated in pen feeding and grazing studies for a number of candidate species. (Waghorn, 2008).

2.6.6.3 Alternative anthelmintic compounds

Observations of the use of traditional plant-based remedies against parasitic disease have underpinned a considerable body of research into alternative anthelmintic, initially for economic reasons, and, as a response to increasing anthelmintic resistance (Vatta *et al.*, 2009). In many cases, the putative beneficial effects of ethno- veterinary preparations, as extracts or whole plant material, are anecdotal and are not supported when objectively researched, but a number does appear to have some potential (Vatta *et al.*, 2009). Some widely used traditional remedies have been shown to be inactive against *H. contortus* (Burke *et al.*, 2006), while for others the results are positively proven to be effective (Irum *et al.*, 2015). In some instances, positive effects from *in vitro* laboratory investigations of plant extracts have not translated to useful activity in animals. Natural compounds found to have activity would require the development of practical deployment systems, predominantly regarding the occurrence of administration (most are less effective than anthelmintic), and format as plant material or an extract. The known effect of the element copper against nematodes, used in various forms as an anthelmintic until the development of modern synthetic compounds, has been the subject of numerous researches as an

alternative treatment when used as a copper oxide wire particle bolus product (COWP). Encouraging anthelmintic effects with copper oxide wire have been demonstrated, especially against *H. contortus* (Vatta *et al.*, 2009), although clarification is needed as to whether this effect is largely against adult worm burdens or whether there is also a persistent effect against infective larvae (Galindo-Barboza *et al.*, 2001). However, as it appears that there is no significant toxicity risk when COWPs are used at the recommended dose (Burke and Miller, 2006), there could be a role for this form of therapy to augment conventional anthelmintic, predominantly when used in conjunction with other forms of non-anthelmintic control (Burke *et al.*, 2005).

The investigation for alternatives to synthetic anthelmintic raises the query of how effective they must be to justify their development as widely applicable control techniques. In contrast to anthelmintic-based control, no single biological control approach is generally expected to provide total efficacy, and they will be best used in conjunction with other natural approaches or existing strategies (Torres-Acosta and Hoste, 2008). However, inside these limits, the individual methodologies require objective evaluation, including across diverse environments and animal management systems, before acceptance for wide recommendation (Ketzis *et al.*, 2006).

2.7 *Trichostrongylus*

2.7.1 Classification and history of discovery of *Trichostrongylus*

Six taxonomy levels for “*Trichostrongylus colubriformis*” from kingdom to species is as follows

Kingdom	<i>Animalia</i>
Phylum	<i>Nematoda</i>
Class:	<i>Secernenea</i>
Order:	<i>Strongylida</i>
Family:	<i>Trichostrongylidae</i>
Species:	<i>Trichostrongylus</i>

Trichostrongylid nematodes are parasites found in the digestive tract of most ruminants with diverse types existing in ruminants in diverse regions. The damage caused by the parasites on ruminant’s health and production has been marked as one of the significant veterinary significance. Some species can also contaminate human, mainly people living in close association with animals (Mowlavi *et al.*, 2008). Diverse species of *Trichostrongylus* have been recognized from diverse hosts of ruminants (Esiami *et al.*, 1978), domestic animals and human (Ghadirian, 1975). A good number of species exist in domestic ruminant, which are also found in the human inhabitants (Ghadirian, 1977). Southwest Iran has officially been found with high occurrence of *Trichostrongylus* species in both human (Ghadirian, 1977) and in ruminants. Compared to the preceding years, the rate of infectivity in human is now much lower; however, there is no much information about the species of *Trichostrongylus* infecting human inhabitants and cross-spread of these parasites among human and diverse animal hosts (Mowlavi *et al.*, 2008).

2.7.2 Epidemiology of *Trichostrongylus*

Epidemiological studies of *Trichostrongylus* have a global allocation and its occurrence has been low among humans (Cancrini *et al.*, 1982). Accidental infections by *T. orientalis* have been recorded in Japan, China, Korea, and Armenia (John and William, 2006). Some species of *Trichostrongylus* associated with infections in humans have also been described, such as *T. colubriformis*, *T. capricola*, *T. vitrinus*, *T. axei*, *T. probolurus* and *T. skrjabin*. Nevertheless, the species *T. axei*, *T. colubriformis* and *T. orientalis* are the most frequent parasites in human infections; most frequently acquired through contact with ruminants (Ghadirian and Arfaa, 1975).

Weather condition is one of the major factors affecting the transmission of the parasite is affected by weather situation which occurs in areas of humid temperatures between 22°C to 26°C and high humidity of about 80 to 100%. The inter-tropical humid areas give the most favourable conditions, with arid environments, which present the least favourable circumstances for larval development. Sandy soil and grasses of large-size also add to the increase of *Trichostrongylus*, by enhancing the movement of larvae and their survival (Morgan *et al.*, 2006). The spread of the parasite to humans is mostly associated to the intake of water or vegetables that has not been washed properly and contaminated with infective larvae, predominantly where compost is used for soil fertilization (CDC, 2001).

2.7.3 Life cycle of *Trichostrongylus*

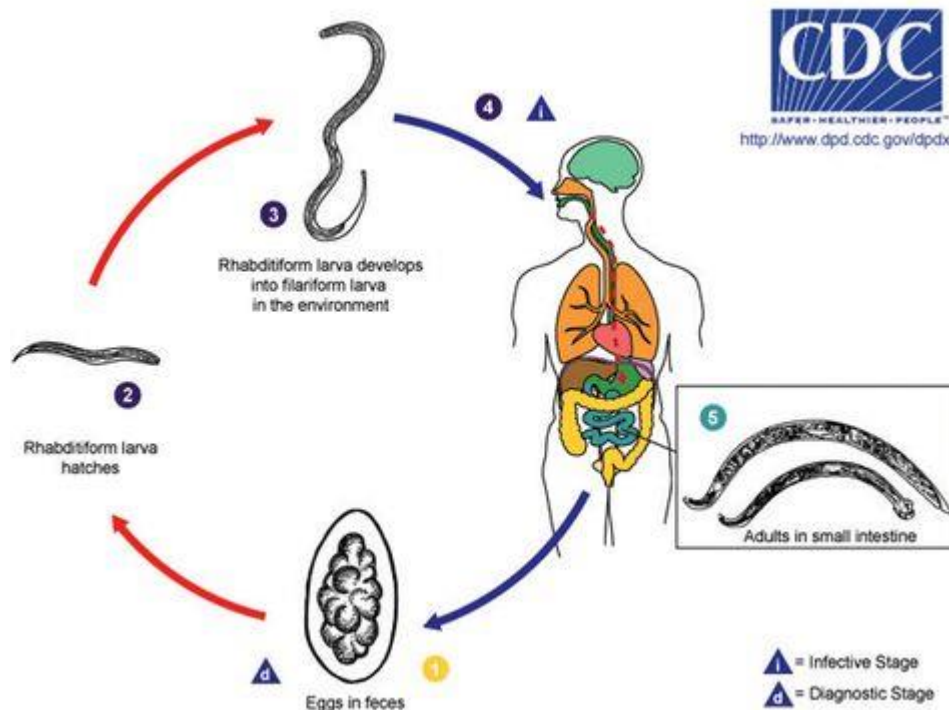


Figure 2.5: Life cycle of *Trichostrongylus* showing the various developmental stages of the parasite (CDC, 2001)

Eggs are laid and are released into the faeces where they hatch in the external environment when faeces are ejected into the environment. They then develop in six days into an infective larvae stage. The stages of L₁ and L₂ of the larvae are microbivorous. In the digestive tract of the host, the L₃, L₄ and L₅ mature and develop. The cycle is direct and does not require an intermediary host (CDD, 2001).

2.7.4 Morphology of *Trichostrongylus*

There is segmentation of the egg when laid, which later develops into infective larvae. The parasites are slender with small frontal ends with no buccal cavity. The male worms have

asymmetrical dorsal ray and two short nearly equal spicules, which help in their identification. There is the presence of vulva in the female, which measures about one millimetre close to the tip of the tail. Frequently eggs can be seen in the body of the female (CDC, 2001).

2.7.5 Pathology of *Trichostrongylus* infection

Trichostrongylosis has symptoms of weight loss, poor bodily state and more often than not diarrhoea. Symptoms of weight loss and diarrhoea are usually linked with *Trichostrongylus* infections of veterinary significance (Ritchie *et al.*, 1966). There are a number of different changes in blood components, the most prominent being hypoalbuminemia and a depression in the serum total protein concentration. In severe cases of hypoalbuminemia, oedema in the face might be seen (Ritchie *et al.*, 1966).

Abomasal parasitism is coupled with a raise in the concentration of pepsinogen in the blood. Most of the pathological consequences reflect the medical symptoms of abnormal bodily condition and weight loss, while others are associated to exact changes within the gastrointestinal tract. In the circumstances of abomasal parasitism caused by *Ostertagia* spp., the main outcome happens when the fourth stage larvae appear from the gastric glands (Ritchie *et al.*, 1966). As a result of the loss of purposeful parietal cells, acid emission is decreased and pH values are superior (Ritchie *et al.*, 1966). The lesions connected with *H. contortus* are largely connected to other *Trichostrongylus* infections of the Abomasum, even though it is the haematophagic activities of the fourth stage larvae and adults follow their appearance from gastric glands which are most disadvantageous to the host. In *Trichostrongylus* infections of the small intestine, the main harm results from activity of the adult worms. About ninety percent of worms are usually present in the small intestine and this is where the maximum injury occurs. Heavy infections have the distinctiveness of enteritis, which is rigorous (Ritchie *et al.*, 1966). There is widespread

villous atrophy, mucosal thickening and stunting of the microvilli, erosion of the epithelia and infiltration of lymphocytes and neutrophils into the injured areas. Parasites are situated in tunnels in the epithelium of villi and intestinal crypts in all the developmental stages.

2.7.6 Diagnosis of *Trichostrongylus* infection

The mature worms survive in the small intestines. The diagnosis is dependent on the identification of eggs in the stool. Different methods like floatation, sedimentation and larvae culture can be used to identify the eggs. The eggs can then be identified microscopically. The eggs are about 85 to 115 μm . It is elongated and either pointed at one point or the two points (Strickland, 2000). *Trichostrongylus* eggs must be distinguished from hookworm eggs by their smaller size and do not have sharp end (Garcia, 2007).

2.7.7 Control of *Trichostrongylus* infection

The use of herbivore compost as manure is a common practice following infection. Comprehensive clean-up and cooking of vegetables is necessary for the prevention of infection (Garcia, 2007). Treating the infection with pyrantel pamoate is extremely good (El-Shazly *et al.*, 2006). Other treatment includes the use of albendazole and mebendazole. Managing the parasite with Ivermectin has also been reported to be efficient (Raph *et al.*, 2006).

2.8 *Babesia*: classification and history of discovery

Babesia is also known as *Nuttallia* which belongs to the Apicomplexan parasite and infects red blood cells which causes a disease called Babesiosis (Nowell, 1969). The parasite was discovered by a Romanian bacteriologist who revealed the vector known as Babes. More than

100 species of *Babesia* have been identified, but only a few have been recognized to cause disease in humans (Nowell, 1969).

In the United States, *B. microti* is the most frequent strain that infects humans. Some other species infects cattle, livestock, and domestic animals (Ristic *et al.*, 1984). People who become infected with Babesiosis go through malaria related signs. In some cases, malaria is frequently misdiagnosed for the disease. *Babesia* is a protozoan parasite of which *B. microti* and *B. divergens* are the two species most commonly found to infect humans. Infections from other species of *Babesia* have been documented in humans but are not seen frequently. Babesiosis has also been recognized as piroplasmiasis (Ristic *et al.*, 1984). Due to chronological misclassifications, this protozoan was labelled with many names that are no longer in use. Common names of the ailment include Texas cattle fever, red water fever, and tick fever. For centuries, babesiosis was known to be a serious illness for wild and domestic animals, particularly cattle. Victor Babeş, who first recognized the disease in Romania in 1888, described symptoms of a severe haemolytic illness observed in sheep and cattle (Vannier, 2012). Even though he recognized the causative agent in 1888, he wrongly thought it could be due to the bacterium he named as *Haematococcus bovis*. An American scientist called Theobald Smith and Fred Kilborne recognized the parasite in 1893, as the cause of Texas cattle fever. Smith and Kilborne also accepted the tick as the agent of transmission of the parasite, a discovery that first introduced the concept of arthropods performance as the vectors of the disease (Schultz, 2008).

2.8.1 Epidemiology of *Babesia*

With the species that infects humans, *B. microti* is recurrent in the United States whereas *B. divergens* is the frequent strain located in Europe. Prevalent areas are regions of tick habitat, as

well as the forest regions of the north-eastern United States and temperate regions of Europe (Karbowski, 2004). Ixodidae is the tick vectors of *B. microti*, which transmits *Borrelia burgdorferi*, (the causative agent of Lyme disease). For some reasons that cannot be explained, in areas of high prevalence of both Lyme disease and babesiosis, Lyme disease transmission is dominant in the region (Ristic *et al.*, 1984). Occurrence of babesiosis in malaria prevalent regions remains unknown as a result of the possibility of misdiagnosis as malaria parasite (Karbowski, 2004). As the infection leads in a high number of asymptomatic persons, many people can harbour high seroprevalence without much observation of illness. Occurrence of babesiosis is mostly recorded throughout the months of May to September when tick activity in high prevalence region (Karbowski, 2004). Bovine babesiosis can be observed wherever the tick vectors survive, but it is most frequent in humid and sub-tropical regions (Karbowski, 2004).

2.8.2 Life cycle of *Babesia* spp.

The life cycle of *B. microti*, needs a deer host or a rodent host before it is transmitted by the Ixodidae family type of ticks. The tick deposits sporozoites into the host when it feeds on a meal of blood (CDC, 2009). The sporozoites go through the erythrocytes in the blood and start the cyclical growth amid the trophozoites and merozoites. The definitive host takes up the gametocytes when it is involved in blood meal for survival. The gametes go through fertilization in the gut of the tick and enlarge into sporozoites in the salivary glands (CDC, 2009). The sporozoites then enter into a human upon injection at the gnawing of a contaminated tick. The changes of phase which take place in the parasite are the same within humans as in the biological hosts (CDC, 2009). *Babesia* can be identified at the trophozoites stage, and can be transmitted from one person to another, through the vector or the transfer of the blood sample (CDC, 2009).

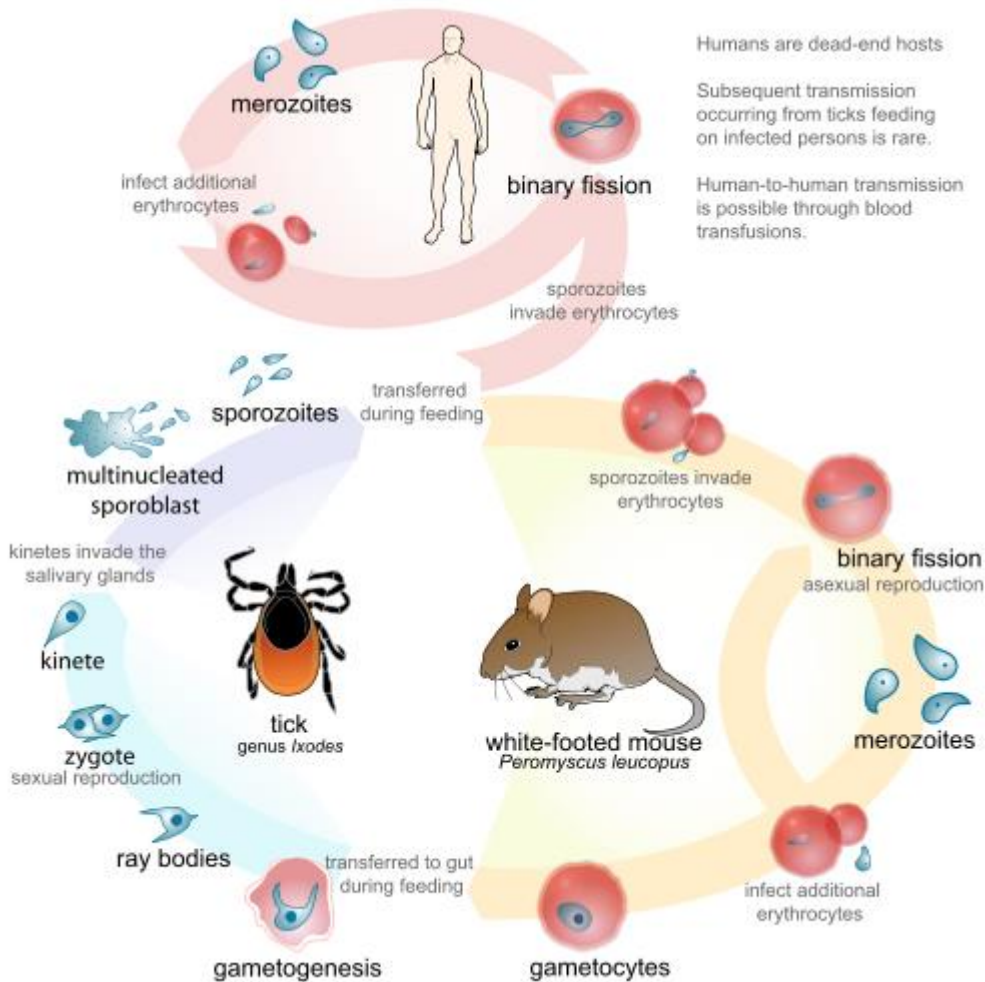


Figure 2.6: A generalised life cycle of *Babesia* spp. (CDC, 2009)

2.8.3 Morphology of *Babesia* spp.

Babesia species penetrates the red cells of the blood at the sporozoites stage. Inside the red blood cell, the protozoa turn out to be cyclical and grow into a trophozoites ring. The trophozoites change into merozoites, which have a tetrad formation (Herwaldt *et al.*, 2003). The tetrad morphology, which can be observed by staining the blood smear with Giemsa, is exceptional to *Babesia*. Trophozoites and merozoites mature and rupture the host erythrocyte, leading to the

emergence of vermicules, the infectious parasitic bodies, which swiftly multiply the protozoa all the way through the blood (Ristic *et al.*, 1984).

2.8.4 Pathology of *Babesia* spp.

Post-mortem lesions comprise intravascular haemolysis, anaemia and jaundice. The mucous membranes are frequently pale and may be icteric, and the blood is commonly thin and watery. Icterus may also be seen in the omasum, abdominal fat and subcutaneous tissues. The spleen becomes engorged with a dark, pulpy, friable uniformity (Noskoviak, 2008). The liver may be distended and pitch-black or icteric, with a bloated gallbladder with thick grainy bile. The kidneys are recurrently reddish purple or black, and the urinary bladder often consists of rosy-brown urine; though, in some cases, the urine may be usual. The lung sporadically shows signs of pulmonary oedema. Other organs as well as the heart and brain may have petechial or ecchymosis or be crowded with crimson surface (Noskoviak, 2008).

2.8.5 Diagnosis of *Babesia* spp. infection

Babesia can be located in blood and tissues. Both thin blood films and organ smears ought to be taken at necropsy. Tissues that are ideal consist of brain (cerebral cortex), kidney, liver, spleen and bone marrow (CDC, 2011). Analysis is defective in animals that have been lifeless for more than 24 hours; on the other hand, parasites can for a moment or two be seen after this time in blood from the lower leg. For high-quality stain description, blood films ought to be stained as almost immediately as possible. Slides ought to be air-dried, fixed in absolute methanol for 5 minutes for organ smears and one minute for thin blood smears and stained for 20-30 minutes with 10 % Giemsa.

Both thick and thin blood films can be obtained from animals on the field. At every time probable, blood ought to be taken from the capillaries in the ear. *Babesia bovis* is greatly easier to find in capillary blood than in the all-purpose circulation. *Babesia bigemina* and *B. divergens* can be located all the way through the vasculature. Thick blood films are not fixed before staining, which enabled the red blood cell to be lysed for the parasites to be concentrated. These films ought to be air-dried, warmed and fixed at 80°C for about five minutes, and stained with 10% Giemsa for 15-20 minutes. If capillary blood is not obtainable, jugular blood may be obtained into an anticoagulant tube. Blood samples ought to be kept cool, if possible at 5 °C, and at any time possible ought to be sent to the laboratory for analysis (CDC, 2011).

2.8.6 Treatment of *Babesia* infection

A number of methods are accessible to administer and treat babesiosis in animals. In many cases, patients impulsively get better, having only experienced placid signs undiagnosed as the disease. This incidence is more or less always seen in *B. microti* infections, which are normally more frequent in the United States. For *B. divergens* and *B. microti* infections, the average treatment in the past, for symptomatic persons was oral or intravenous clindamycin with oral quinine (Beaver, 1984). With the outcome of investigation concluded in 2000, though, treatment has been more and more leaning towards the administering of oral atovaquone with oral azithromycin. Azithromycin are preferential as they are similarly effective in all but the most ruthless cases but gives unfavourable symptoms (Krause *et al.*, 2000). In ruthless situations, blood replacing transfusions have been done to reduce the parasitic load in the person (Ristic *et al.*, 1984). Supplementary basic treatment measures comprise checking and controlling irregular clinical signals. Global prevalence of Babesiosis.

2.8.7 Global prevalence of *Babesia* sp. infection

Countless animals are infected by over 100 *Babesia* species but only a small number of species have been recognized to infect humans (Spielman, 1976; Vannier *et al.*, 2008). *Babesia microti* is responsible for most cases in the United States and such cases occur in the Northeast and upper Midwest, mostly from May through October (Vannier *et al.*, 2008). The occurrence of human 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). A few other cases caused by *B. duncani* and *B. duncani* were identified on the Pacific Coast from northern California to Washington State (Kjemtrup, 2000). Accidental cases of infection with *B. divergens* 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, 1992). Most of these cases were found in normo-splenic patients who resided in rural areas. Over (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. Majority of the reported cases in Europe have been attributed to *B. divergens*, and a few of the cases are caused by *B. venatorum* and *B. microti* (Gray *et al.*, 2010). *Babesia microti* have been shown to cause illness in Japan and Taiwan, although a new *Babesia* agent (KO1 strain) has been identified in South Korea (Kim *et al.*, 2007).

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

2.9 Global prevalence of Human fasciolosis

Even though fasciolosis is regarded as a disease of livestock, it is now recognized as an important emerging zoonotic disease of humans. The total number of reported human cases of fasciolosis was estimated to be less than 3000. Recent figures suggest that between 2.4 and 17 million people are currently infected with an additional 91.1 million living at risk of infection (Keiser, 2005). Human infections mostly occur in areas where animal fasciolosis is endemic. Transmission occurs where rural farming communities regularly share the same water source as their animals or consume water-based vegetation growing in endemic areas.

The highest prevalence of human fasciolosis is found in the Altiplano region of Northern Bolivia. Infection is highest in children and females, and prevalence can reach over 40 per cent in certain communities (Mas-Coma *et al.* 2005). The prevalence of animal and human fasciolosis corresponds to snail distribution, which is restricted to the northwest of the Altiplano. Snail infection is facilitated by animal reservoirs such as sheep, cattle and pigs. In Europe, larval development within the snail is halted in winter as low temperatures influence the development of the free living and intra-molluscan stages of the life cycle and outbreaks occur in early spring as daytime temperature increases to above 9 °C. In contrast, temperature is not a significant limiting constraint in disease transmission in the Bolivian Altiplano as all-year average night-time and day-time temperatures range from 0 to 6 °C and 18 to 22 °C, respectively. Furthermore, unlike the European mud-snail, snails in the Altiplano reside almost wholly sub-aqua and are observed on aquatic plants during the dry season at a time when animals and humans collect around the shrinking water sources. During the height of the rainy season, Lake Titicaca and its

tributaries overflow causing extensive flooding, optimizing conditions for transmission. A recent meta-analysis of 10 epidemiological surveys from 38 communities in the region showed that human fasciolosis has been endemic there since at least 1984 and is a significant zoonosis in rural communities (Parkinson *et al.* 2007). Infection levels as high as 67 per cent of the human population were reported in areas adjacent to tributaries of Lake Titicaca where corresponding high levels of animal infection was also observed. As *F. hepatica* can survive for up to 13 years in humans, producing large numbers of eggs (up to 5000 per gram of faeces in some Bolivian children) that are infective to other hosts, humans are likely to play an important role in transmission of the disease.

Transmission in humans is linked to their dietary habits since individuals, particularly children, supplement their diet with aquatic plants during daily animal husbandry. The main types of aquatic plants are 'berro berro' (watercress), 'algas' (algae), kjosco and totora (Mas-Coma *et al.*, 2005). Drinking untreated water may be a source of infection due to the presence of free-floating metacercarial cysts. Vegetables washed in contaminated water may also become a source of infection.

Hyperendemic human fasciolosis has also been reported in the Nile Delta region between Cairo and Alexandria (Esteban *et al.* 2003). A recent report shows that selective chemotherapy of humans over a 4-year period has been effective in reducing the prevalence of human fasciolosis in the Nile Delta (Curtale *et al.*, 2005).

Significant levels of human *F. hepatica* infections also occur, with regular outbreaks involving up to 10 000 infections, in the Gilan and Mazandaran provinces that border the Caspian Sea of northern Iran (Rokni *et al.*, 2002). In this region high animal fasciolosis also exists and, like the

Altiplano, animals are free to roam among arable land. Outbreaks in relatively large towns, such as Rasht, have been associated with the consumption of aquatic plants sold by farmers of the surrounding countryside at local markets. Since reports of human fasciolosis are increasing from Turkey it is also plausible that problems of human fascioliasis are widespread around the Caspian Sea (Mas-Coma *et al.*, 2005).

In Europe human fluke infections occur more periodically, however substantial and recurrent outbreaks of the disease occur in France, Portugal and Spain causing 50–100 infections per year (Mas-Coma *et al.* 2005). Human infection is mostly due to consumption of aquatic plants, such as watercress, on which *Fasciola* metacercarial have settled. While farm-management practices, including the culture of edible aquatic plants in greenhouses, reduce the risk of human infection, the disease remains an important human health problem in these countries. Sporadic cases of human fasciolosis have been diagnosed in the USA, but these are usually imported through migrants or returning tourists (Graham *et al.*, 2001).

To date, the majority of reported human cases of fasciolosis are due to infections of *F. hepatica*. However, some reports indicate a rise in human infections due to *F. gigantica* in Vietnam (Hien *et al.*, 2001; Mas-Coma *et al.*, 2005)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Description of study area

The study was carried out at Akuse and Narhkorkpe communities in the Lower Manya Krobo district (Figure 3.1). There are twenty-six (26) municipalities and districts in the Eastern Region of Ghana. The geographical coordinates of Akuse are Latitude $6^{\circ} 02.34' N$ and Longitude $0^{\circ} 25.89' E$, while that of Narhkorkpe are Latitude $6^{\circ} 16.28' N$ and Longitude $0^{\circ} 25.89' E$. The district covers an area of 1,476 km, which constitute about 8.1% of the overall land area within the Region (18,310 km), with Odumase serving as the administrative capital. Its major towns includes Odumase the township (which incorporates Atua, Agormanya and Nuaso), Akuse and Kpong in the Lower Manya area. Minor villages in the district include Teye Kwame and Narhkorkpe. Lower Manya Krobo district shares boundaries with Upper Manya Krobo district to the north, to the south with Dangme West and Yilo Krobo, to the west with Yilo Krobo Municipal and to the east with Asuogyaman District. Human population of the municipality according to the 2010 population and housing census stands at 89,246 with 41,470 males and 47,776 females (GSS, 2013).

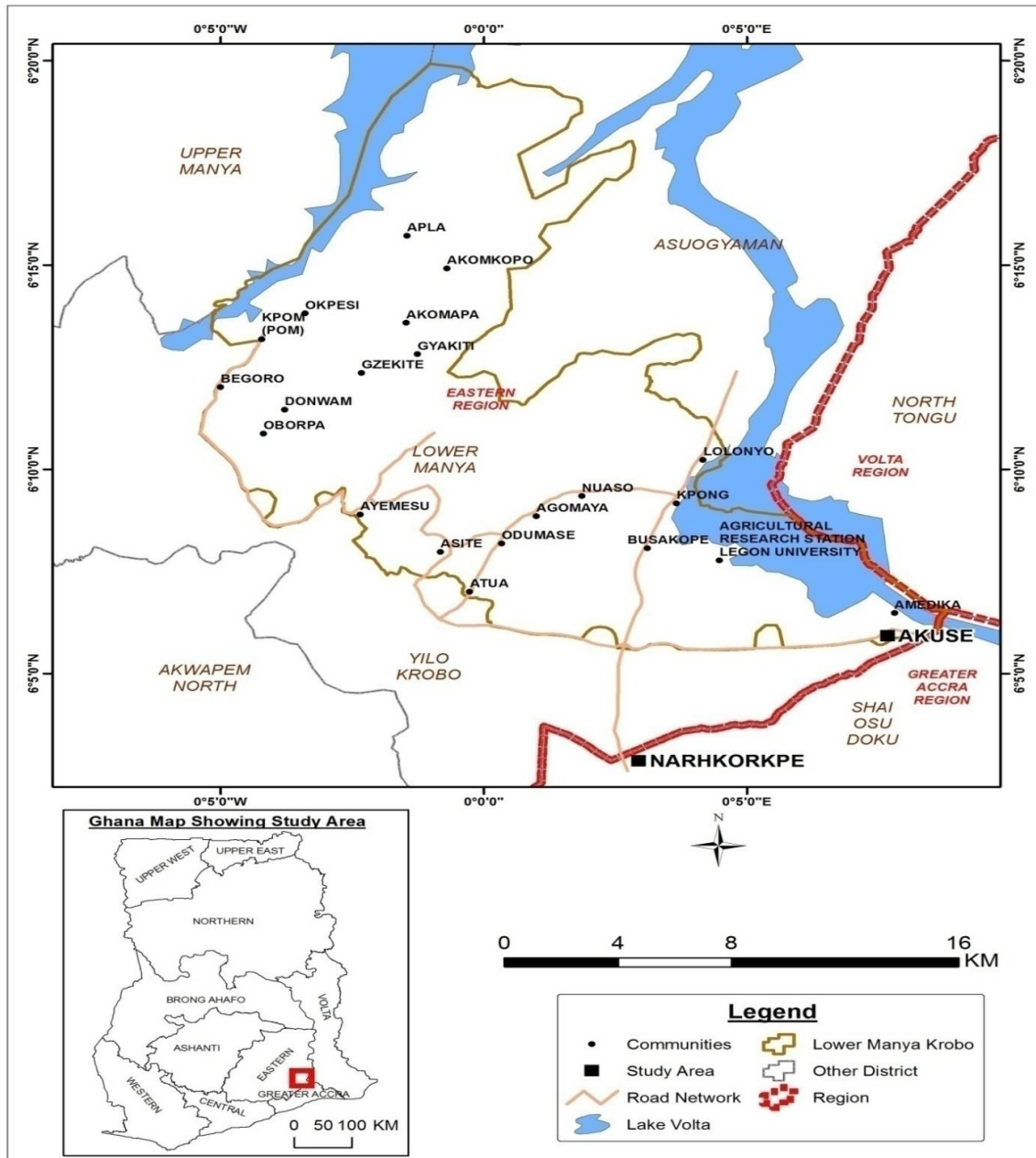


Figure 3.1: Map of lower Manya Krobo district showing the study areas. Akuse and Narhkorkpe communities are located around the southern banks of the Volta Lake. The Lower Manya Krobo district is just about 80-85 km east of Accra, the capital of Ghana. This district is found within the coastal savannah zone of the Accra plains, with a bimodal rainfall pattern. (Geography department, University of Ghana)

3.2 Study design, sample size and inclusion criteria

This study was a multi-site observational study that involves cross-sectional field survey to set up the current baseline dominance of the most important endoparasitic species that are of significance to the smallholder livestock production systems as well as posing a threat to human health in eastern Ghana. The two sampling sites of Akuse and Narhkorkpe are both located in the Lower Manya Krobo district. Animals from six farms in Akuse and seven in Narhkorkpe were sampled. Faecal and blood samples were taken from cattle aged between one and two years. Not less than five animals per herd were sampled. During each sampling period, faecal and blood samples were taken from one hundred and twenty (120) cattle for helminths and haemoparasites infection status determination. The number of animals sampled was based on the number of animals that could be examined during one sampling day. Four to five animals were sampled per hour (24 animals per day) resulting in 120 animals per five-day sampling period. This translates into a total of 240 animals being sampled from each of the two different sampling localities (Site1 and Site2). Each sampling site was sampled twice and this gave a total sample of 480. An excess of six samples were added to take care of any loss of sample that might occur.

Cattle that were included in this survey ranged from 1 to 2 years old and were males and females. They were not treated with a topical or systemic acaricide, insecticide or anthelmintic two weeks before sample collection and were not excessively fractious to reduce danger they might pose to the researcher. All cattle that did not meet these criteria were not included in the study. Figure 3.1 shows a section of one of the cattle farms from where samples were taken.



Figure 3.2: A section of one cattle farm from where samples were taken at Narhkorkpe



Figure 3.3: A section of a cattle farm sampled in Akuse



Figure 3.4: Researcher taking samples from cattle on the field

3.3 Sampling method

Simple random sampling technique was used to sample cattle to represent all the study population at various farms and sites. In this sampling technique, a maximum of ten cattle was selected in each farm where each cattle had equal chance of been selected. Cattle between the ages of one to two years were identified by the farmer. There were average of twenty-five cattle that fell within these ages. An average of ten cattle was chosen at random from these groups and sampled. The study was conducted on 486 cattle traditionally managed under large holder system. The age of the sampled animals was determined based on the owner's information.

3.3.1 Faecal sample collection procedure

A clean glove was worn. A nickel size amount of water or water-based lubricant was applied to the glove to allow easy penetration of the hand into the rectum. Hand was inserted into the rectum of the animal to remove faecal matter. The gloves were removed from the hand while the faecal sample was encased within it. The wrist portion of the glove was twisted and fastened with a label. Each stool sample was labelled with the farm code, the number and sex of the cattle. The sample was stored in an ice chest container to preserve the eggs as well as preventing the larvae from hatching out and then transported to the laboratory for storage and analysis.

3.3.2 Faecal sample analysis (flotation method)

About 4 g of faeces was weighed into a sterile labelled faecal cup. A flotation solution of saline of 6.9% concentration and of volume of about 10 ml was added to the sample. It was then mixed completely with the stool sample and sieved to remove any particles from the mixture. The solution was then transferred into an Eppendorf tube and spun in a centrifuge at 300 revolutions per minute for five minutes. The supernatant was then discarded leaving the pellet behind. It was then mixed with 33% of zinc sulphate in 15 ml Eppendorf tube and spun again for two minutes. The two chambers of the McMaster chamber were filled by using a syringe by placing the syringe tip at the edge of the slide and discharging sufficient sample between the upper and lower slides to fill the areas under the grid. The grid was focused with the light microscope using 40 times objective lens. The eggs inside and under the grid lines were counted. The number of eggs for each grid was identified and counted as described by Hansen (1956).

3.3.3 Blood collection for examination

Blood was drawn from the jugular vein of the cattle. Prior to blood collection, the site was disinfected with cotton swap containing 70% ethanol and a 5 ml sterile syringe was then inserted into the vein to collect about 2 ml of blood. A thick and thin film was then prepared instantly and was air dried for about five minutes. Staining was done immediately after close of days sampling using 10% Giemsa stain. The stain was gently poured onto the slides until they were totally covered. Each slide contained approximately 3 ml of stain. The stain was left on the slides for 45-60 minutes. The slide was then washed with water and then allowed to dry. The slides were then packaged and sent to the lab for examination.

3.3.4 Microscopic examination of stained blood

Each slide was observed under oil immersion using the light microscope. The 100 X objective lens was used to focus the parasite and any parasite that was observed was recorded as positive

3.4 Sampling of internal organs from the Somanya abattoir

3.4.1 Sampling procedure

About 20 individual cattle internal organs liver, abomasum, small and large intestine were bought from the abattoir just after the cattle has been slathered. It was then preserved in an ice chest containing ice packs to prevent deterioration and was transported to the lab for analysis for possible zoonotic helminths.

3.4.2 Examination of the organs

A section of the liver was cut and placed in a Petri dish. The specimen was then placed under the dissecting microscope for examination. The abomasa were cut opened and observed physically

for possible helminths. Both the large and small intestines were also cut opened and examined physically for any possible helminths

3.5 Data analyses

Data on individual animals were entered into MS-excel spread sheet to create a data base. Prevalence were calculated and Chi-squared (χ^2) test carried out using the MEDCALC statistical software as recommended by Campbell (2007), Richardson (2011) and Altman *et al.* (2000). The prevalence of infestation was calculated by dividing the proportion of positive animals with helminths infestation by the animals sampled and all multiplied by 100%. Pairwise comparisons of the prevalence for the various helminth species identified were carried out using unconditional Fishers exact test. Mood's median test in Quantitative Parasitology 3.0 (Rozsa *et al.*, 2000) was used to assess the parasite egg intensities in both communities. The use of the Mood's median test to assess the median intensity of infection was to show whether the typical level of infection differs significantly between the infected proportions of the two independent samples.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of helminth infection

A total of 486 cattle, aged between one to two years, were sampled from the two study communities in the Manya Krobo District of the Eastern Region of Ghana. The number of cattle sampled in Akuse was 246, with 94 being males and 152 females. At Narhkorkpe, 240 cattle were also sampled of which 92 were males and 148 females. Overall prevalence of general parasite egg infection in Akuse was 14.2% while that of Narhkorkpe was 19.6% as shown in Table 4.1. The observed difference in prevalence of helminths infection (5.4 % [-1.5 to 12.3]) in cattle sampled from Akuse and Narhkorkpe was not significant (Chi-squared test: $df= 1$; χ^2 statistic = 2.52; $P = 0.1123$).

Table 4.1: Prevalence of helminths infection in cattle sampled from Akuse and Narhkorkpe

Community	Number of cattle	Number infected (NHES)	Percentage prevalence (95% CI)	χ^2 (df); p-value*
Akuse	246	35 (211)	14.2(10.3, 19.3)	2.52 (1); 0.1123
NarhKorkpe	240	47 (193)	19.6 (15.0, 25.2)	

*p -value for observed difference in prevalence (5.4 % [-1.5 to 12.3]) of helminths infection in cattle sampled from Akuse and Narhkorkpe by Chi-squared test (χ^2). CI: Confidence interval, NHES: No helminth egg seen.

In all, four species of helminth eggs were identified microscopically, using morphological characteristics. These are *Cooperia* spp., *Haemonchus* spp., Hookworm and *Trichostrongylus* spp. Hookworm and *Trichostrongylus* spp. were the dominant helminth species in the farms of both study communities with prevalence of 21.5% and 14.2% respectively for Akuse and 16.7%

and 19.6% respectively for Narhkorkpe. In Akuse cattle farms, hookworm infestation was significantly higher compared with *Trichostrongylus* spp. infections by unconditional exact test ($P = 0.0382$).

However, no significant difference was observed between hookworm and *Trichostrongylus* spp. infections by a similar analysis in Narhkorkpe cattle farms ($P = 0.5317$). Hookworm infection also differed significantly from *Cooperia* sp. infection ($P < 0.001$) Narhkorkpe cattle farms. Interestingly, in Narhkorkpe farms, no egg was identified in cattle for *Haemonchus* spp. Table 4.2 shows pairwise comparisons of prevalence of various helminth infections in cattle sampled from Akuse and Narhkorkpe farms. Figure 4.1 and 4.2 show eggs of various helminth parasites identified.

Table 4.2: Comparison of prevalence of helminth infection in cattle sampled from Akuse and Narhkorkpe farms

Community	Type of parasite	Number of cattle infected (NHS)	Percentage prevalence (95%CI)	* <i>p</i> -value
Akuse	<i>Cooperia</i> spp.	5 (241)	2.0 (0.81, 4.61)	0.331 (b,c)
	<i>Haemonchus</i> spp.	9 (237)	3.70 (1.84, 6.84)	<0.001 (c,d)
	Hookworm spp.	53 (193)	21.50 (16.82, 27.20)	
	<i>Trichostrongylus</i> spp.	35 (211)	14.2 (10.32, 19.26)	0.0382 (d,e)
NarhKorkpe	<i>Cooperia</i> sp.	3 (237)	1.30 (0.35, 3.66)	<0.001 (b,d)
	Hookworm spp.	40 (200)	16.70 (12.47, 22.02)	0.5317 (d,e)
	<i>Trichostrongylus</i> spp.	47 (193)	19.60 (14.96, 25.17)	

AKUSE: *Annotations for pairwise comparisons (n = 246); **b, c:** *Cooperia* spp. vs. *Haemonchus* spp.; **c, d:** *Haemonchus* spp. vs. Hookworm spp.; **d, e:** Hookworm spp. vs. *Trichostrongylus* spp.; CI, confidence interval.

NARHKORKPE: **b, d:** *Cooperia* spp. vs. Hookworm spp.; **d, e:** Hookworm spp. vs. *Trichostrongylus* spp.

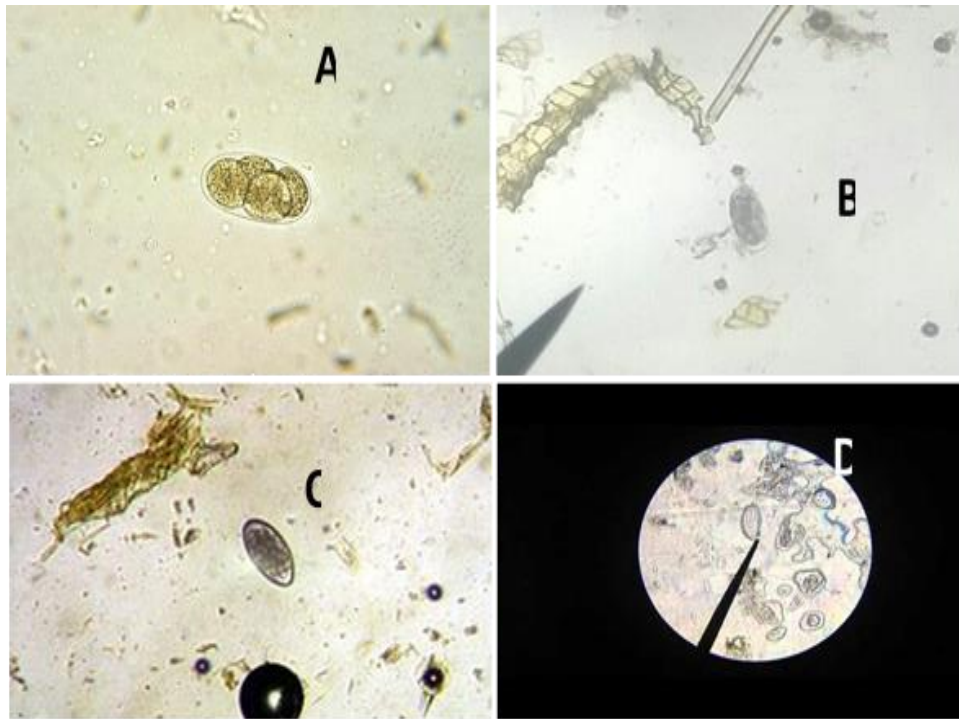


Figure 4.1: Eggs of helminth parasites commonly observed after stool examination of some cattle from Akuse and Narhkorkpe. A: Egg of hookworm spp.; B: Egg of *Trichostrongylus* spp.; C: Egg of *Haemonchus* spp.; D: Egg of *Cooperia* spp.

4.2 Comparison of median egg intensity in cattle sampled from Narhkorkpe and Akuse

Median intensity of infections was assessed by Mood's median test to show whether the typical level of infection differs significantly between the infected proportions of cattle from the two independent samples obtained from the two study communities. No infections of *Haemonchus* spp. were identified in Narhkorkpe cattle farms. However, *Haemonchus* spp. had median parasite egg intensities of 50 eggs/gram in cattle sampled from both Akuse and Narhkorkpe.

Interestingly, hookworm spp. had the highest median parasite egg intensity count of 250eggs/gram in Akuse and 550egg/gram in Narhkorkpe cattle farms respectively. Hookworm had significantly higher median egg intensity in cattle of Narhkorkpe farms (550 eggs/gram)

compared with that of Akuse cattle (250eggs/gram) by Mood’s median test [$P = 0.003$]. Table2.3 shows a summary of the Median helminth egg intensities in cattle sampled from Akuse and Narhkorkpe.

Table 4.3: Median helminth egg intensities in cattle sampled from Akuse and Narhkorkpe

Type of parasite	Akuse: Median intensity of parasite eggs	Narhkorkpe: Median intensity of parasite eggs	P-value for Mood’s median test
<i>Cooperia</i> sp.	50	50	1.00
<i>Haemonchus</i> sp.	50	0	1.00
Hookworm sp.	250	550	0.003
<i>Trichostrongylus</i> sp.	100	100	0.179

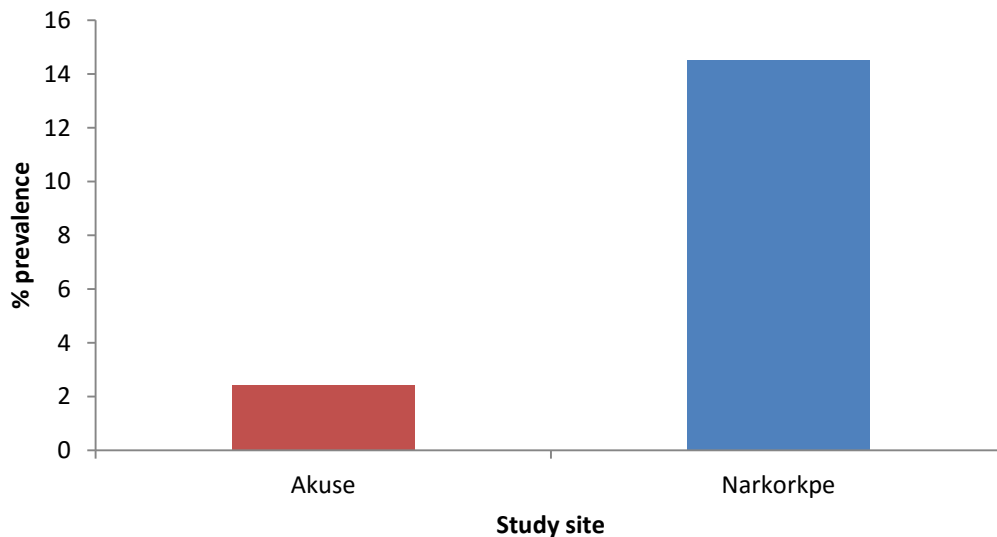


Figure 4.2: Prevalence of *Babesia* sp. infection in cattle from Akuse and Narkorkpe

4.3 Prevalence of *Babesia* spp. infections in cattle

Out of 246 cattle blood samples taken from Akuse farms, 6 (2.4%) of the cattle were infected with *Babesia* spp. However, 35 (14.6%) of the cattle sampled from Narhkorkpe (240) were infected with *Babesia* spp. as shown in Figure 4.1. The observed difference in prevalence of *Babesia* spp. infection (12.2 [7.2, 17.5 %]) is significantly higher in cattle sampled from Narhkorkpe farms by Chi-squared test ($\chi^2 (1) = 23.39; P < 0.0001$).

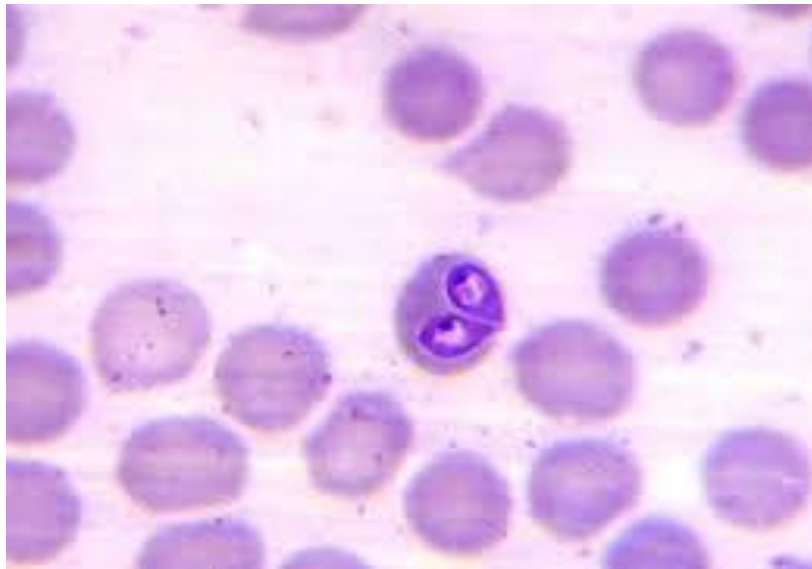


Figure 4.3: *Babesia* spp. identified under the light microscope using 100X objective

4.4 Prevalence of *Fasciola* at the Somanya abattoir

Additionally, examination of internal organs of twenty (20) abattoir cattle samples revealed 7 (35 %) of *Fasciola* spp. in the liver and 6 (30 %) of *Haemonchus* spp. in the Abomasum.

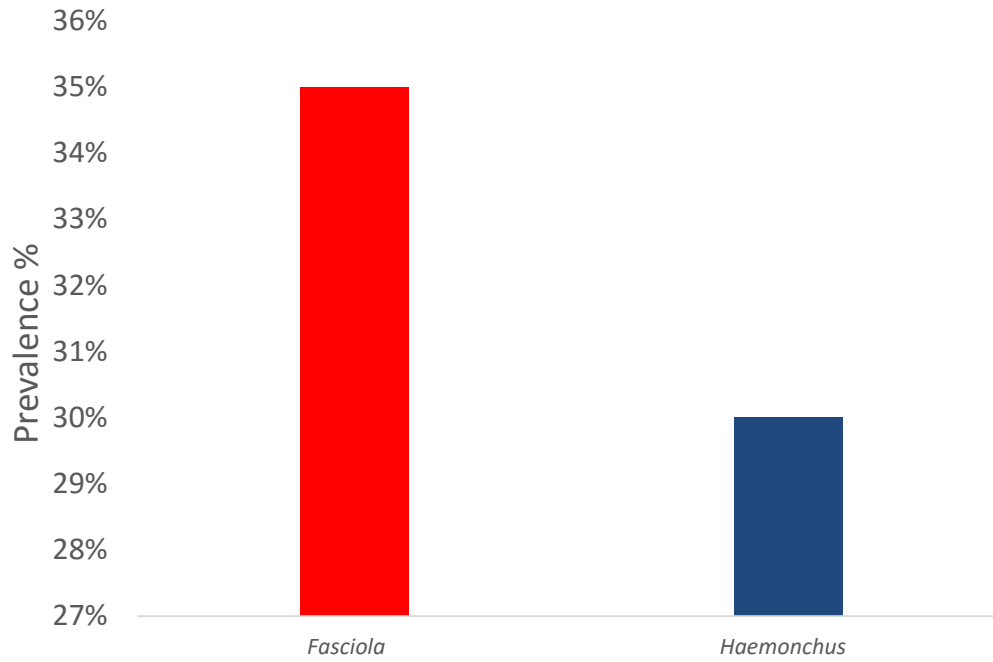


Figure 4.4: The prevalence of *Fasciola* spp. in the liver and of *Haemonchus* spp. in the Abomasum of cattle sampled from Somanya Abattoir

CHAPTER FIVE

5.0 DISCUSSION

The transmission of gastrointestinal parasites, to either humans or other cattle, is dependent on the incidence of infected cattle, intermediate host's availability, poor sanitation environment and principally, the husbandry and management practices of cattle (Islam *et al.*, 2014). In this study, hookworm and *Trichostrongylus* spp. with the potential of infecting humans were identified to be the dominant parasite species in both Akuse and Narhkorkpe cattle farms. Hookworm sp. infections were found to have the highest prevalence (21.5%) in cattle on farms at Akuse. This finding was similar to that of Ajanusi and Chiezey (2005), albeit relatively lower prevalence, who reported hookworm prevalence to be the highest (89.1%) of all helminth infections in livestock (small ruminants) in Zaria, Nigeria.

The high prevalence of hookworm in this study is suggestive of a link to ineffective treatment and poor management practices including poor sanitation and the keeping of large numbers of cattle together, which was the case in most of the farms where the studied animals were sourced from. It could also be due to differences in management systems of the animals as well as the age group of cattle that were sampled. Some livestock owners practice extensive pasture feeding which large numbers of the animals are kept together and this could increase the degree of pasture contamination. An overlap of land use activities could be putting both cattle and humans at risk of hookworm infections. Land used for grazing could be serving other purposes such as farming and open defecation, a common practice in the study area. Most cattle watering holes are also shared with humans serving as source for cross-species infections. The observed high hookworm prevalence underscores the important of these cattle infections as potential zoonotic infections. Global estimates of the health and economic cost of hookworm as at 2010 was

estimated at over 4 million dollars and productivity losses of between \$7.5 billion to almost \$140 billion respectively (Bartsch *et al.*, 2016). Ideally, samples should have been collected from farmers and other persons in the cattle meat production industry to ascertain whether the parasites of the cattle are also found in humans. However, this could not be carried out in this study due to its rather limited scope as well as time and logistical constraints.

Trichostrongylus spp. infection was observed to have a lower prevalence of 14.20% compared with hookworm infection in the Akuse cattle farms. The result conforms to the findings of the work done by Yimer *et al.* (2015) on the prevalence of gastrointestinal nematode of cattle in Dawa Districts of Eastern Ethiopia.

The different prevalence recorded in the two communities observed might be due to differences in sanitary conditions, irregular deworming of the cattle and the different places of grazing the farm animals. It could also be due to the differences in the ages of the cattle sampled. The prevalence of Hookworm and *Trichostrongylus* spp. stands out in areas of Narhkorkpe and Akuse. This could be due to the fact that conditions that facilitated the growth of these two helminths in these two communities might be similar. Even though the prevalence of *Trichostrongylus* spp. observed was not as high as that of hookworm but the percentage recorded is significant enough to pose threat to the cattle farmers and human population at large. The infective larvae could be transmitted to human through the water bodies the cattle contaminate in the community. The poor sanitary condition in the community can easily transmit the infective larvae to the inhabitants. There has been a reported worldwide estimated prevalence of 5.5 million people infected with zoonosis *Trichostrongylus* spp. (Garcia, 2007), and this situation is alarming. The observed high *Trichostrongylus* spp. prevalence underscores the importance of these cattle infections as potential zoonotic infections.

Factors that affect faecal worm egg shedding are numerous and as a result of that, a number of these factors need to be considered every time an analysis is made to make a fair assessment of the worm egg counts. These are the age of the animal, the season of the year, the amount of exposure to pasture, the stocking rate of the animals on pasture, the amount of rainfall or moisture on the pasture, the number of days and temperature requirements to promote parasite development on pasture, the health of the animals, the stage of gestation and lactation and the numbers and type of parasites present at each examination (Bliss and Kvasnicka, 1997).

Egg count greater than 25 egg/gram for Hookworms is considered to be high (Bliss and Kvasnicka, 1997). *Trichostrongylus* spp. intensity of infection with an egg count of 50 egg/gram and above is considered to be high, while *Haemonchus* spp. is considered to be high when the egg count is 200 egg/gram (NSW DPI, 2007). *Cooperia* spp. is considered to be high when the egg count is 500 egg/gram (NSW DPI, 2007). A research work conducted by (Moralesi *et al.*, 1995) on adult worm burden and faecal egg count in cattle in Venezuela established a high egg count in *Cooperia* spp. and *Haemonchus* spp. which is not in agreement with this research work which recorded a low egg count. This might be due to the season of the year that the animals were sampled, the stocking rate of the animals on pasture, the amount of rainfall or moisture on the pasture and the number days with temperatures sufficient to promote parasite develop on pasture. The egg count of *Trichostrongylus* spp. was observed to be high but do not conform to the previous work done by (Moralesi *et al.*, 1995) which recorded a low egg count on a work done on relationship between adult worm burden and faecal egg count in naturally infected cattle. The high egg count of *Trichostrongylus* spp. might be due to the numbers and type of parasites present at each examination and the ages of the cattle that were sampled. The average egg count of Hookworm was high and correspond with a work done by (Mabaso *et al.*,

2004) which gave high prevalence of hookworm and intensity of egg count in South Africa. Interestingly, comparison of median egg intensities by mood's median analyses revealed a significantly higher parasite burden of Hookworm in cattle sampled at Akuse and Narhkorkpe. In addition, the significantly higher levels of Hookworm might be due to the number of days and temperatures sufficient to promote the parasites develop on pasture. The high egg count recorded in hookworm and *Trichostrongylus* spp. could serve as potential zoonotic infection due to the poor sanitary condition in the community which can easily facilitate the transmission of the infective larvae to the inhabitants. The use of the common source of water for watering vegetable farms in the communities can also transmit the infective larvae of the parasites to the vegetables for consumption hence posing zoonotic danger.

Fascioliasis is an infection caused by the liver fluke. *Fasciola* infection has traditionally been shown to be an important veterinary disease because of the substantial production and economic losses it causes in livestock, particularly sheep and cattle. In addition, some internal organs were analysed from the Somanya abattoir which recorded a high prevalence of 35% of *Fasciola* spp. in the liver which also correspond to a previous work done by Ardo *et al.* (2013). The work established a high prevalence of bovine Fascioliasis in major abattoirs of Adamawa state, Nigeria. According to WHO report in 2015, about 2.4 million people are infected with Fascioliasis in more than 70 countries worldwide. There is the possibility of cross transmission from the cattle to human in the community, since the inhabitants and the cattle depend on the same source of water, as such their waste can contaminate their source of water.

Babesia causes a disease called Babesiosis which causes an important economic loss in cattle in and different countries (Dinçer, 1999). The prevalence of *Babesia* spp. by the microscopic examination of Giemsa stained blood smears was found to be 2.4% in Akuse and 14.6% in

Narhkorkpe. The low prevalence recorded in Akuse corresponds with the previous work done by (Ugachukwu and Sidney, 2014) on the prevalence of Haemoparasites of cattle from three abattoirs in Ibadan Metropolis of Nigeria in cattle in the various regions of Turkey. This might be due to the periodic spraying of the cattle to reduce the number of ticks on their body. The high prevalence of 14.6% recorded in Narhkorkpe however contradict the work by (Ugachukwu and Sidney, 2014) on the prevalence of Haemoparasites in cattle at Ibadan state of Nigeria the various. There are different types of *Babesia* species but the species that is of zoonotic importance is the *Babesia microti*. Most countries in the Sub Saharan Africa countries have reported significant prevalence's of *Babesia microti* (Vannier and Krause, 2012). Due to logistic constrains species identification was not performed. *Babesia microti* can infect the inhabitants of the two communities since most of the tick vectors which transmit the parasite were found to be attached to the body of the cattle. The close association between the cattle and the inhabitants of the community can lead to the transfer of the tick vectors to human, which a bite of the vector can transmit the parasite to human.

CHAPTER SIX

6.0 CONCLUSION AND RECOMENDATIONS

Parasites identified to be potentially of zoonotic importance were hookworm species, *Trichostrongylus* spp., *Babesia* spp. and *Fasciola* spp. Other parasites, not necessarily zoonotic, like *Cooperia* spp. and *Haemonchus* spp. were also identified. Hookworm species was the predominant parasitic infection likely to be a zoonosis. The high prevalence and intensity of average egg count of hookworm species could be due to the fact that the number of days with optimum temperatures was sufficient to promote parasite developing on pasture. Additionally, the absence of designated grazing grounds, as well as the commonness of the practice of open defecation in the study communities facilitate the overlap of niches between humans and cattle, thus enhancing the spread of potential zoonosis. The results revealed a significant difference in hookworm spp. infection prevalence between Akuse and Narhkorkpe, suggesting a difference in animal husbandry practices and at risk human behaviour. Further investigations are required to shed more light on these observations.

6.1 RECOMMENDATIONS

It would have been ideal to carry out the study throughout the year so as to get a complete picture of zoonotic parasites whose incidence and prevalence are likely to be influenced by the seasons to enable effective control of the parasites. Future work should aim at identifying the various parasites herein observed to the species level to give more insight into their role as zoonotic parasites. To further confirm the observed parasitic infections as zoonotic, persons within the study communities, particularly those in the cattle meat production value chain such as cattle herders, persons working in slaughterhouses and butchers should have their stool and blood samples examined. The study showed that there is urgent need for general control measures to be implemented to reduce the severity of the parasitic infections in cattle in the study communities towards reducing the likelihood of the transmission of, not only zoonotic infection but also infections in general. By so doing, livestock production could also be boosted and the livelihood of farmers improved. In this regard, it is suggested that the practice of separate grazing of animals at designated grazing grounds with low stocking rate be adopted. It is also recommended that farmers frequently administer anthelmintic to their cattle for treatment on quarterly basis may be employed to minimize the possibility of re-infection; however, resistance to these drugs has been reported recently and hence this should only be done under the supervision of veterinary officers. The provision of veterinary extension services by the veterinary services department to farmers in the study areas should thus be intensified since farmers complained of the non-existence of such services.

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