KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

TOPIC: PREVALENCE OF SOIL-TRANSMITTED HELMINTHES AMONG PUPILS IN GIA AND KAJELO PRIMARY SCHOOLS IN THE KASSENA-NANKANA EAST AND WEST DISTRICTS IN THE UPPER EAST REGION OF GHANA.

THESIS PRESENTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY

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BY

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil(Clinical Microbiology) degree and that, to the best of my knowledge; it contains no material previously published by another person or material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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ABSTRACT

Soil-Transmitted helminthes is prevalent in many communities in the Kassena-Nankana district of the Upper East Region of Ghana. Gia is one community where the prevalence of soil-transmitted helminthes has been reported in 2007 to be 10% prevalent by the direct wet mount method. This study determined the current prevalence of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the formol-ether concentration techniques.

Three hundred and ninety-four stool samples were collected from subjects (pupils) between October 2010 and March 2011 for this study. Each stool sample was processed within two hours after collection using the direct wet mount and the formol-ether concentration techniques in accordance with standard protocols.

Formol-ether concentration technique, gave the highest overall prevalence of 9.40% of helminthes parasites made up of *Strongyloides stercoralis* (5.08%), Hookworm (3.30%), *Ascaris lumbricoides* (1.02%) and *Trichuris trichuira* (0.00%) in both Gia and Kajelo pupils. The direct wet mount was found to be 33.3% sensitive.

The Gia primary school had a population of 198 pupils, 6 positives were detected out of the total number sampled representing 3.54% of the stool samples that were detected by the direct wet mount method. The formol-ether concentration detected 21 positives of the total number sampled representing 10.61%.

The Kajelo primary school had a population of 196, 5 positives were detected out of the total number sampled giving a prevalence of 2.55% with the direct wet mount method. The formol-ether concentration detected 16 positives out of the total number sampled giving a prevalence of 8.16%.

This study supports the finding that stool samples that are negative for parasites by the direct wet mount method should be re-examined using the formol-ether concentration technique as the confirmatory test. This approach will improve the detection of helminthes from stool specimens for accurate diagnosis of soil-transmitted helminthes infections and ultimately improve the quality of life of individuals in these communities.

CHAPTER ONE

1.0 INTRODUCTION

1:1BACKGROUND TO THE STUDY.

Soil transmitted helminthes (STHs) are those that mostly come into contact with the soil through contamination with human feaces from an infected person (Lewis et al, 2004). Some of the species of soil transmitted helminthes that are important in terms of public health are roundworms (*Ascaris lumbricoides*) whipworms (*Trichuris trichuira*) and hookworms *Ancylostoma duodenale* and *Necatoramericanus* (Lewis et al., 2004).Soil transmitted helminthes are also known in many endemic communities as common intestinal worms (WHO, 2002).

Prevalence of helminthic infections exceeding 70% of the population has been reported in equatorial and tropical countries of West Africa (Brooker et al, 2000). In Ghana, Annan et al. (1986) have reported up to 63% infections among school age children (WHO, 2002).The major soil transmitted helminthes in Ghana are *Ascaris lumbricoides*, *Trichuris trichuira*,hookworms *Ancylostoma duodenale* and *Necator americanus* and *Strongyloides stercoralis* (GHS, 2010).

More than one billion people are infected with intestinal helminthes worldwide. They are the most prevalent of chronic human infection (WHO, 2002). The morbidity associated with these parasites is strongly related to parasite burden (WHO, 2002), these include pathological effects such as stunting growth,anemia and deficient cognitive functions. The disease burden is particularly high in developing countries (WHO, 2002).

Over 35.4 million school aged children in Africa are infected with *Ascaris lumbricoides*, 40.1 million with *Trichuris trichuira* and 41.1 million with hookworm (Lewis et al., 2004). Since many children have multiple infections, it is estimated that 89.9 million are infected with soil transmitted helminthes species.

About 44% of the infections are concentrated mainly in four African countries; Nigeria, the Democratic Republic of Congo, South Africa and Tanzania (de Silva et al, 2003).Previous estimates have suggested that 53 million school aged children (5-15years) are infected with *Ascaris lumbricoides*, 50 million with *Trichuris trichiura*and 47 million with hookworm (deSilva et al., 2003).

1.2 ROUTINE DIAGNOSIS OF STOOL

In the diagnosis of intestinal parasites a wide variety of laboratory methods can be employed (Ahmadi et al., 2007). The choice of a particular technique for routine use is influenced by its affordability, simplicity, sensitivity and level of technical skills involved (CLSI 1997; Melvin and Brooke, 1985; WHO, 2000).

Stool microscopy using direct wet mounts and the formol-ether concentration technique offer many relative advantages over other diagnostic methods for attracting intestinal parasites (Watson et al 1988., Bogoch et al., 2006;Pariga and Srinivasa, 1999). Direct saline wet mount provides economical and rapid diagnosis for intestinal parasites when they are present in sufficient density in the faecal samples (Ukaga et al., 2002).The formol-ether concentration method described by Allen and Ridley (1970) increases the sensitivity and specificity of stool microscopy to allow the detection of low numbers of organisms recovers most ova, cysts and larvae and retains their morphology, thereby facilitating identification (Ahmadi et al., 2007).

It is well established that the direct wet mount technique is low in sensitivity but most hospital laboratories in developing countries rely on this method for routine stool examinations as a result of its simplicity and affordability (Ahmadi et al., 2007; Ebrahim et al., 1997; Ukaja et al., 2002; Wirkom et al., 2007). Most laboratories in Ghana perform routine stool examination by the direct

wet mount only; standard operating procedures' for stool examination. Direct wet mount technique is rapid, simple and inexpensive, but it can miss low intensity infections or if too much debris is present in the preparation (Akujobi et al., 2005). Therefore, there is the need for more accurate diagnosis of intestinal helminthes infections in hospitals and health centers in Ghana (Annan et al., 1986).

Soil-Transmitted helminthes cause malnutrition, anemia, growth retardation, cognitive impairment as well as lowering of resistance to other infections (Chan et al., 1997). *Ascarislumbricoides* can cause intestinal obstruction in children and other complications when adult worms migrate from the small intestines to other parts of the body (Lewis et al., 2004). Intestinal soil transmitted helminthes contribute to iron deficiency anemia as they feed on blood and cause further bleeding by releasing anticoagulant compounds (Lewis et al., 2004). They also affect the supply of nutrients and cause vomiting and diarrhoea (Chan et al., 1997). Pregnancy complications by maternal hookworm infection pose a serious threat to the health of mothers and their babies, especially in developing countries. Women who are anemic during pregnancy are more likely to have ill health and low birth weight babies with low iron reserves (Chan et al., 1997).

It is difficult to eliminate the factors that make individuals vulnerable to infection with intestinal helminthes, such as lack of sanitation, poor hygiene and overcrowding. Mass treatment with broad spectrum anthelminthic drugs is considered to be the key strategy for controlling morbidity among endemic populations (Chan et al., 1997).

Based on the demonstrable impact on child development, there is global commitment to finance and implement control strategies with focus on school – based programmes (WHO, 2002).

1.3JUSTIFICATION FOR THE STUDY

According to the strategic plan for Integrated Neglected Tropical Diseases (2007- 2008) of the Ghana Health Services, there is inadequate prevalence data on soil transmitted helminthes in Ghana. The World Health Organization has emphasized the need for epidemiological study where up- to – date information on soil-transmitted helminthes is not available (WHO, 1993). The study will thus provide useful data that will inform policy makers particularly the District Health Management Team (DHMT) to roll out programmes and policies towards the deworming of the pupils in order to eradicate soil-transmitted helminthes. This study of the prevalence of soil-transmitted helminthes is therefore important for the control and elimination of soil-transmitted helminthes in Ghana as a whole.

1.4 AIM OF THE STUDY

The aim of the study is to determine the prevalence rate of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena- Nankana district in the Upper East Region of Ghana, after six years of community treatment.

1.5 SPECIFIC OBJECTIVES

i. To determine the prevalence of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena- Nankana District using the direct wet mount method and the formol- ether concentration technique.

ii. To estimate the effect of the previous treatment of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena- Nankana District.

iii. To determine the accuracy/sensitivity of the wet mount method relative to the formol- ether concentration technique as the diagnostic “gold standard” test.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1GLOBAL DISTRIBUTION AND EPIDEMILOGY OF INTESTINAL HELMINTH PARASITES.

Soil-transmitted helminthes are important cause of morbidity and mortality throughout the world, particularly in under developed countries (Guyatt and Bundy, 1991; de Silva et al., 1997). There are about twenty major helminthes infections of human, affecting more than one third of the world population (Awasthi et al., 2003). The major soil- transmitted helminthes infections, namely roundworm infection or *ascariasis* (*Ascaris lumbricoides*), whipworm infection or *trichuriasis* (*Trichuris trichuira*), and hookworm *(Necatoramericanus and Ancylostoma duodenale*) and infection caused by these soil-transmitted helminthes account for most of the global helminthes disease burden (Bethony et al., 2006; de Silva et al., 2003).

2.1.1SOCIO-ECONOMIC EFFECTS OF INTESTINAL HELMINTH INFESTATIONS

Soil-transmitted helminthes (STHs) infections are common throughout communities of poor and socio-economically deprived people of the tropics and sub tropics (Kightlinger et al., 1998), due to lack of adequate private and public latrines and poor environmental sanitation (Kightlinger et al., 1998; Appleton et al., 1999; Beasley et al., 2000). Children are the most vulnerable to soil-transmitted helminthes infections (Crompton and Stephenson, 1990).

Children as young as six months can be infected with helminthes (Goodman et al., 2007) which influence their nutritional status leading to growth retardation (Adam et al., 1994) and reduce learning ability with increased absenteeism from school (Beasley et al., 2000; Nokes et al., 1992).

For *Trichuris trichuira* and *Ascaris lumbricoides*, convex age intensity profiles, in which the incidence peaks in childhood, are observed (Woolhouse, 1998). Infection prevalence and intensities rise with increasing age to a peak around 5 – 10 years old and decline in adults (Batchta et al., 1990).

Unlike other soil-transmitted helminthes, hookworm infestation appears to increase throughout childhood until it reaches a peak in adulthood with highest prevalence occurring among the middle aged or even individuals over the age of sixty years (Brooker et al., 2004; Behnke et al., 2000).

Strongyloidiasis, has been shown to occur in all age groups, although acquisition is more common during childhood and has no predilection for either sex (Pearson, 2002).

On sex- dependency, helminthes infections have been reported in some cases to infect males more than females (Anderson and May, 1991).

Hookworm shows significantly higher prevalence in males than females (Behnke et al., 2000) but prevalence of *Ascaris lumbricoides* and *Trichuris trichuira* show no significant difference between males and females (Guyatt and Bundy, 1991).

2.1.2 MEDICAL INDICATIONS OF SOIL-TRANSMITTED HELMINTHES INFECTIONS.

Majority of intestinal helminthes infections are chronic and mild, and are usually subclinical (Udonsi, 1984). However, several clinical signs and symptoms can occur in patients with moderate and heavy infections (Neva and Brown, 1994).

During the first one or two weeks after a cutaneous infection, helminthiasis can produce an intensely pruritic dermatitis at the site of infection termed ground itch, and larval invasion of the

lungs may produce respiratory symptoms called Loeffleror Loeffler-like syndrome (Neva and Brown, 1994). This syndrome is characterized by pneumonitis which can be accompanied by paroxysmal attacks of cough, coughing with blood-tinged sputum, wheezing, dyspnea, pleurisy, low grade fever, substernal pain, urticaria, asthma and eosinophilia (Crompton and Coombs, 1991).

Adult worms in the intestine commonly cause abdominal pain (Spurchler, 1987). Other enteric symptoms reported include abdominal cramps, intestinal blockage, nausea and or vomiting (rarely), tenesmus, diarrhoea, occasionally constipation and dysentery (Vadlamudi et al., 2006; Spurchler 1987).

Commonly observed complications in heavy helminthiasis include situations in which large and tangled worms of *Ascaris lumbricoides* may cause intestinal usually ileal, common duct, pancreatic, or appendiceal obstruction (de Silva et al., 1997). Less common features include ascending cholangitis, acute pancreatitis, and, rarely, obstructive jaundice (Khuroo, 2001; Bahu et al., 2001)

In heavy *Trichuriasis*, infected people may show mild anemia, eosinophilia, bloody diarrhea, prolapsed rectum (especially in children), and impaired physical and mental growth (Drake et al., 2000).The major manifestation of hookworm disease is iron deficiency anemia, and patients with severe anemia may have fatigue, syncope, or external dyspnea (Stoltzfus et al., 1997; Albonico et al., 1998).*Strongyloides stercoralis* infection produces burning or colicky abdominal pain, often epigastric, and is associated with diarrhoea and the passage of mucus (Oduntan, 1974; Udonsi, 1984; Adams et al., 1994; Drake et al., 2000; Stephenson et al, 2000).Non-specific clinical

manifestation includes restlessness, irritability, anorexia, chronic protein energy malnutrition, malabsorption and weight loss (Spurchler, 1987).

2.2AETIOLOGY OF SOIL-TRANSMITTED HELMINTHS (STHS).

Intestinal helminthes infections that remain prevalent in sub-Sahara Africa, Ghana in particular include *trichuriasi*s, *strongyloidiasis*, *ascariasis*, intestinal *schistosomiasis*; and infections caused by hookworm (Feacham et al., 1983; Annan et al., 1986; Brooker et al., 2000; PCD, 1998). A record of spurious and of genuine *dicrocoeliosis*; *Dicrocoelium* infections in man have been reported in Sierra Leone (King, 1971), and in Ghana (Odei, 1966; Wolfe, 1966).

Kassena-Nankana district in the Upper East Region of Ghana, is where the Centre for Neglected Tropical Diseases in 2007, has reported the prevalence of soil-transmitted helminthes.

2.2.1*Ascaris lumbricoides*

*Ascaris lumbricoides*isthe most common and important soil-transmitted helminthes (Cheesbrough, 2005).This parasite is cosmopolitan and its distribution is largely determined lack of proper disposal of human faeces, because its eggs reach the soil in human feaces and so contaminate the human environment (Kightlinger et al., 1998).

The parasite is one of the major public health problems in sub-Saharan Africa. In communities where the prevailing social environment is characterized by poverty, poor housing, inadequate sanitary practices and overcrowding the organism is mostly seen in stools of people (Chan et al., 1997; Chan et al., 1994; Pearson, 2002; O'Lorcain and Holland 2000; Crompton and Savioli, 2007).

Prevalence of *ascariasis* worldwide is estimated to be more than 1.3billion, approximately one-quarter of the world population and over two fifty million suffer from associated morbidity (Crompton and Savioli, 2007).

It is estimated that two hundred and four million cases of *ascariasis* occur elsewhere in East Asia and the pacific, One hundred and forty million in India, ninety-seven million elsewhere in South Asia, Eighty-four in Latin America and the Caribbean, twenty three million in the Middle East and North Africa and one hundred and seventy-three million in Sub-Saharan Africa (Pearson, 2002; de Silva et al., 2003; Peters et al., 2005).

Ascaris lumbricoides have been shown to play a significant role in children malnutrition, which leads to growth retardation, cognitive impairment, and poor academic performance, resulting in a poorer quality of life and less ability to contribute to society (Drake et al., 2000; Adams et al., 1994; O'Lorcain and Holland, 2000).

2.2.2*Strongyloides stercoralis*

Worldwide, an estimated thirty to hundred million people are infected with *strongyloidesstercoralis*. *Strongyloidiasis* is endemic in tropical and sub-tropical countries and more frequently found in rural areas, institutional settings, and lower socio economic groups (Pearson, 2002).*Strongyloides* is important for its ability to auto infect and disseminate throughout the organ system to cause life-threatening infection (hyper infection syndrome, disseminated *Strongyloidiasis*) in immune suppressed host (Vadlamudi and Krishnaswany, 2006). Human infection is acquired through penetration of intact skin by filariform larvae during contact with contaminated soil or other material contaminated with human faeces (Cheesbrough, 2005).

Severe *Strongyloidiasis* carries a high mortality rate (up to 80%) because the diagnosis is often delayed (Vadlamudi and Krishnaswany, 2006).Prevalence of *strongyloides stercoralis* infection in tropical Africa is high (Peters and Pasvol, 2005). Yelifari et al., (2005), reported high prevalence rates of*Strongyloidiasis* in northern Ghana.

2.2.3*Trichuris trichuira*

Infection with *Trichuris trichuira (trichuriasis)* is one of the most common helminthes infection of humans (Peters and Pasvol, 2005). Adult *Trichuris trichuira* are approximately 40-50mm in length, the posterior end is thick and the anterior two-third of the body is slender, giving a ‘’whiplike’’ shape to the worm; hence the name whipworm (Neva and Brown, 1994).

Trichuriasis afflicts about one billion people throughout the world (Pearson, 2002). It is spread through faecal-oral transmission and high prevalence occurs in areas with tropical weather and poor environmental sanitation (Bethony et al., 2006). Children are especially vulnerable because of the high exposure risk (Matsubayashi et al., 1965).Infection rates of up to 75% were found in young school children in Nigeria (Oduntan, 1974).

2.2.4*Hymenolepis nana*

Hymenolepis nana, the dwarf tapeworm, is the smallest tapeworm to infect humans, and has a cosmopolitan distribution. (Stoll, 1999). The infection is more frequently seen in children and institutionalized groups, although adults are also infected, causing *hymenolepiasis* (Matsubayashi et al., 1965).

The life cycle of *Hymenolepis nana* does not require an intermediate host. The eggs are immediately infective when passed with the stool. The parasite exhibits auto-infection where

complete egg development occurs within the villi of a single host, resulting in an internal autoinfection, thus allowing the infection to persist for years (Bogitsh and Cheng, 1999).

Like *Taeniasis* infections, prevalence of *Hymenolepis nana* infection in most communities in Ghana have been relatively low (Annan et al., 1986; PCD, 1998).

2.2.5*Dicrocoelium species*

Dicrocoeliasis, also known as small liver fluke disease is caused by *Dicrocoelium dendriticum* and *Dicrocoelium hospes* which are parasites of the liver, gall bladder, pancreas, and intestine of amphibians, reptiles, birds, and mammals, but can infect the bile ducts of humans (Markell et al., 1999; Manga-Gonzalez et al., 2001).

Whilst *Dicrocoelium dentricum* is found throughout Europe, the Middle East, and Asia (Manga-Gonzalez et al., 2001).*Dicrocelium hospes* is distributed in Ghana, Nigeria, Chad, Togo, Central African republic, Cameroon, Niger, Sierra Leone, Senegal and several Sub-Saharan countries (Odei, 1966; Wolfe, 1966; King, 1971).

It is reported that up to 50% of oxen and sheep in La Cote d'ivoire (Ivory Coast), about 80% of oxen in Uganda and up to 94% of small ruminants in Niger harbors' *Dicrocoelium horpes* infection (Manga-Gonzalez et al., 2001). Human infections are generally rare and are attributed to accidental ingestion of either infected ants or raw or under cooked animal liver (Crompton, 1999).

The presence of *Dicrocoelium* species provides clues about the interactions among humans and animals (Crompton and Savioli, 2007).

Spurious and of genuine *Dicrocoelium* infections in man have been reported in Sierra Leone (King, 1971) and elsewhere in Ghana (Wolfe, 1966; Odei, 1966).

2.2.6 Hookworms

Hookworm, like any parasitic worms, takes their name from the hook-like appendages that surround their mouths (Silva et al., 2003). An estimated 1.2 billion people worldwide have been infected (Chan et al., 1997), mainly in the tropics and subtropics (de Silva et al., 2003), with the foci predominantly in areas of rural poverty within Asia, Sub-Saharan Africa, and Latin America (de Silva et al., 2003).

Reports indicate high prevalence of human hookworm infections in Kenya (Adams et al., 1994), Nigeria (Adenusi, 1997), Mali (Behnke et al., 2000), Cote d'ivoire (Booth et al., 2003), Zambia (Hira and Patel, 1984) and Ghana (Annan et al., 1986; de Gruijter et al., 2005).

The two principal species of hookworm infecting humans are *Necator americanus* and *Ancylostoma duodenale* (Hotez et al., 2004). They produce morphologically identical eggs, so they cannot be distinguished on stool microscopy (WHO, 1991). Therefore, they are traditionally reported as hookworm and have been considered identical for treatment purposes (WHO, 1991; Pillai and Kain, 2003).

The life cycles of *Ancylostoma duodenale* and *Necator americanus* follow a general pattern (Hotez et al., 2004). The adult parasite stages inhabit the gastrointestinal tract especially in the small intestine, reproduce sexually, and produce eggs, which are passed in human faeces and deposited in the external environment. Soil-transmitted helminthes rarely cause death. Instead, the burden of disease is related less to mortality than to the chronic and insidious effects on the hosts' health and nutritional status (Stephenson et al., 2000; Soltzfus et al., 1997).

Whilst *Ancylostoma duodenale* is more geographically restricted, *Necator americanus* has global distribution and is the dominant species in most parts of Nigeria (Adenusi, 1997; Oyerinde, 1978), Togo (de Gruijter, 2005), Mali (Behnke et al., 2000) and Ghana (Yelifari et al., 2005).

2.3 MODE OF TRANSMISSION OF SOIL-TRANSMITTED HELMINTHES(STHS)

Soil-transmitted helminthes depend for transmission on environments contaminated with egg-carrying faeces (Crompton et al., 1993). Consequently, helminthes are intimately associated with poverty, poor sanitation and lack of clean water (Crompton and Savioli, 1993).

A person infected with soil transmitted helminthes has parasite eggs in their faeces. In areas where there are no latrines, the soil (and waters) around the human settlement or communities become contaminated with faeces containing worm eggs. In the soil, the eggs mature; a process that takes between two and four weeks, depending on the type of worm, about two weeks for roundworms and hookworms; and about three weeks for whipworm.

Soil-transmitted helminthes can infect humans in several ways, which include ingestion of eggs from water sources, or food which has become contaminated as well as larval penetration through the skin, usually between the toes for other hookworm infections (Chan et al., 1997).

2.3.1 SYMPTOMS OF SOIL-TRANSMITTED HELMINTHES (STHS) INFECTIONS.

The symptoms of soil-transmitted helminthes infections are nonspecific and only become evident when the infection is particularly severe. The nonspecific symptoms include nausea, tiredness, abdominal pain and loss of appetite. Soil-transmitted helminthes cause morbidity through various transmission mechanisms;

Hookworms live in the intestine; they attach and re-attach themselves to the intestine wall every few hours as they feed on blood from the cut vessels and mucosal tissues. This blood loss contributes to anemia, especially in countries where the dietary intake of iron is already marginal and malaria is widespread.

Soil-transmitted helminthes infections can also affect vitamin A deficiency (Crompton and Savioli, 1993). In many countries, already marginal and in these situations, soil-transmitted helminthes infection can compete for the limited amount of vitamin A which is absorbed (Crompton and Savioli 1993).

One of the most noticeable changes after a child has been dewormed is that the child losses his or her appetite (Lewis et al., 2004).

2.3.2 CONSEQUENCES OF SOIL-TRANSMITTED HELMINTHES (STHS)

The impact of soil-transmitted helminthes on an infected person's life can be significant leading to loss of appetite, decreased levels of vitamin A deficiency and higher anemia levels all interfere with the ones ability to grow healthy (Chan et al., 1997). Moreover, there is evidence to suggest that a child with heavy worm infections is less resistant to other infections. Altogether, this means a child infected with soil transmitted helminthes will be sickly and, if untreated, will grow up to be an unhealthy adult (Chan et al., 1997).

When the number of worms in the body becomes extremely high, they build up in the intestines, stopping absorption and eventually blocking the intestine entirely (Chan et al., 1997). The only solution in this situation is surgical intervention and in many cases, this is not possible in remote areas and the individual can die (Brooker et al., 2000).

Worm-infected children are less able to concentrate or memorize information (Chan et al., 1997). They score less well in school tests and therefore their only chance to gain a few years of schooling in their lives is compromised (Chan et al., 1997).

Soil-transmitted helminthes cause malnutrition, cognitive impairment as well as lowering of resistance to other infections (Lewis et al., 2004). *Ascaris lumbricoides* can cause intestinal

obstruction in children and other complications when adult worms migrate from the small intestine to other parts of the body (Lewis et al., 2004).

They also affect the supply of nutrients and cause anorexia, vomiting and diarrhoea (Chan et al., 1997). Pregnancy complicated by maternal hookworm infection poses a serious threat to the health of mothers and their babies, especially in developing countries (Chan et al., 1997). Women who are anemic during pregnancy are more likely to have ill health, give birth prematurely, and have low birth weight babies with low iron reserves (Chan et al., 1997).

2.4 LABORATORY PROCEDURES FOR THE DIAGNOSIS OF INTESTINAL HELMINTHES

Definitive diagnosis of helminthes infection depends on demonstration of a stage of the parasites life cycle in the human host (Neva and Brown, 1994). The adult worms, that inhabit the intestine, discharge the eggs or larvae they produce in faeces (Neva and Brown, 1994).

Therefore, laboratory diagnosis of intestinal helminthiasis is based on detection and identification of characteristic eggs or larvae in stool specimens (Parija and Srinivasa 1999; CLSI, 1997, 2000b). A wide variety of laboratory methods, including parasitological, molecular, serologic and culture approaches, have been developed over the years for diagnosis of intestinal parasites (Markell et al., 1999).

2.4.1 Visual observation of nature of stool sample (**macrocospic examination**)

Helminthic infections can induce digestive abnormalities and influence the nature of consistency of stool produced (Garcia, 2001). The macroscopic appearance of stool specimen can give a clue to the type of organisms present (Ash and Orihel, 1991; Goodman et al., 2007; Parija and Srinivasa, 1999).

Adult worms of *Ascaris*, *Enterobius* and tapeworm proglottids may be seen when fresh specimen is visually examined (Garcia, 2001). Faecal specimens are described as formed, semi formed, loose or watery, bloody or bloody mucoid (Beaver et al., 1984).

2.4.2 Parasitologic methods (stool microscopy)

Microscopic or parasitological diagnosis is generally sensitive, simple, and economical (Parija and Srinivasa, 1999). If performed correctly, stool microscopy offers some advantages over other diagnostic methods for detecting intestinal parasites (Watson et al., 1988; Bogoch et al., 2006). Diagnostic tests involving microscopy include direct wet mount preparations, concentration methods and the Kato-Katz technique (Markell and Voge, 1976; Watson et al., 1988).

2.4.3 Direct wet mount method

Direct wet mount involves microscopic examination of fresh faecal specimens by wet preparations with physiological saline (saline wet mount) or iodine solution (iodine wet mount) or 1% aqueous solution of eosin (eosin wet mount) (Garcia 2001; Isenberg 1998).

The procedure provides rapid diagnosis for intestinal parasites when they are present in sufficient density in the faecal sample (Engels et al., 1996; Ukaja et al., 2002).

The method is useful for detecting organism motility, including motile larval forms of *Strongyloides stercoralis* and trophozoites of intestinal protozoa (Watson et al., 1988).

The technique is also useful for diagnosis of parasites that may be lost in concentration technique (Melvin and Brooke, 1985). It is particularly useful for the observation of motile protozoan trophozoites and the examination of certain diagnostically important objects such as *Charcot-Leyden* crystals and cellular exudates (Parija and Srinivasa, 1999).

The major disadvantage of direct wet mount method is the lack of sensitivity (Estevez and Levine, 1985; Melvin and Brooke, 1985; Engels et al., 1996; Pearson, 2002).

Bogoch et al (2006) and Akujobi et al (2005) have pointed out that infections of low parasite intensities can be missed even by the most experienced microscopist (Ahmadi et al., 2007) indicated that even when parasites are detected, other species may be present in a density below the diagnostic threshold of the test. Slide preparations from wet mounts dry up easily and motile organisms may not detect if the preparations are not examined quickly after preparation (WHO, 1991).

2.4.4 Concentration methods

Concentration techniques increase sensitivity of stool microscopy to allow the detection of small numbers of organisms that may be missed by using only a direct wet smear (Allen and Ridley, 1970). Basically, concentration techniques operate in two ways, either by sedimentation (Ritchie, 1948) in which the parasite sink to the bottom of the liquid suspension, or by floatation (Truant et al., 1981) in which the parasite forms are suspended in a liquid of high specific density to make them buoyant and float to the surface where they are collected for examination (WHO, 1991).

Some parasite stages have been described as ‘’sinkers’’ and others are ‘’floaters ’’, some do both, and do either (Cox, 1998). Therefore, no ideal method of concentration is capable of detecting all

forms of parasites that may be present in stool specimens. In general, floatation gives a 'cleaner' preparation than sedimentation yet each has a preference over another in certain aspects (Ukaja et al., 2002; Truant et al., 1981; Cheesbrough, 2005).

Concentration by floatation utilizes a liquid suspending medium heavier than the parasite objects so that they float and can be recovered from the film (Cheesbrough, 2005).The floating medium generally employed include brine (that is., saturated aqueous solution of sodium chloride), and zinc sulfate solution having a specific gravity of approximately 1:20 and 1:18 respectively (Garcia, 2001).

The procedure is simple and known to be a more sensitive method if protozoan cysts, nematode and tapeworm eggs (with the exception of *Diphyllobotrium* eggs) are sought (Cheesbrough, 2005). However, eggs of common intestinal helminthes, *Strongyloides* larvae, and protozoan cysts become badly shrunken; sufficient to render the object undiagnosable (Ukaja et al., 2002).

Studies have shown that a sedimentation method recovers the broadest spectrum of parasite species (Truant et al., 1981). The formalin-ether concentration procedure as described by (Allen and Ridley, 1970), and (Ritchie, 1948) provide the best diagnostic outcome in epidemiological studies (Akujobi et al., 2005). The technique requires the use of formalin as a fixative ether (Allen and Ridley, 1970) or ethyl acetate (Young et al., 1979) or gasoline (WHO, 1991; Wirkom et al., 2007).

It uses formalin to fix and preserve the faecal specimen and ether or ethyl acetate to extract debris and fat from the faeces (Truant et al., 1981), leaving the parasites at the bottom of the suspension (Akujobi et al., 2005; Allen and Ridley, 1970). Authors consider the formol ether concentration as the most effective technique that recovers the broadest range of organisms and

hence, the ‘’gold standard’’ method (Wiebe et al., 1999) of all parasitological techniques (Melvin and Brooke, 1985; Garcia, 2001; Cheesbrough, 2005; Markell and Voge, 1976).

The advantages of this method are that it will recover most ova, cysts and larvae and retain their morphology, thereby facilitating identification (Neimeister et al., 1987). There is less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved (Akujobi et al., 2005).

The concentration technique has additional advantage by allowing for transportation and storage after faeces are preserved in formalin (Oguama and Ekwunife, 2007)

Conversely, it has additional disadvantage of destroying trophozoites stages and distorting cellular exudates and liquid stools do not concentrate well (Ash and Orihel, 1991).

Because concentration procedure require a laboratory with trained personnel, centrifuge to separate parasites, electricity to run centrifuges, a well-ventilated work space, adequate water supply, a standard light microscope, and consistent availability of regular supply of reagents, it tends to be expensive running the test (Allen and Ridley, 1970).

2.4.5 Kato-Katz technique

The Kato-Katz technique (Kato and Miura, 1954; Katz et al., 1972) is useful for the quantitative estimation of worm burdens (Markell et al., 1999). It is especially useful for field surveys for helminthes infections since it provides estimates of the intensity of infection (Martin and Beaver, 1968).

According to Martin and Beaver (1968), the technique entails the examination of a standard sample (determined by the size of the template) of fresh faeces pressed between a microscope

slide and a strip of cellophane that has been soaked in glycerin (Markell and Voge, 1976; Ukaja et al., 2002).

The Kato template may be made of stainless steel, plastic, or cardboard and different sizes have been produced in different countries: a 50mg template with a hole of 9mm on a 1mm thick template; a 41.7mg template has a hole of 6mm on a 1.5 mm thick template; and a 20mg template will have a hole of 6.5 mm on a 0.5 mm thick template (Ebrahim et al., 1997).

The cellophane cover slip, 22 x 30 mm, are pre-soaked for at least 24 hours in a glycerin-malachite green solution of 100 ml pure glycerin, 100 ml distilled water and 1ml of 3% malachite green (Markell and Voge, 1976; Garcia, 2001).

After the faecal film has cleared, eggs in the entire film are counted, and, the number of eggs of each species reported is multiplied by the appropriate multiplication factor to give the number of eggs per gram (epg) of faeces (Martin and Beaver, 1968).

When using a 50 mg template, the multiplication factors is 20; and for a 20 mg template, the factor is 50 (Katz et al., 1972).

The World Health Organization, (WHO, 1998) had recommended the use of template holding 41.7 mg of faeces, and with a multiplication factor of 24. Siegel et al (1990) pointed out that Kato-Katz technique has limited usefulness in detecting infections in diarrhea specimens.

Limitations of this method include difficulty in processing diarrhoea stools (Siegel et al., 1990), lack of sensitivity for diarrhoea stools. If only a single stool sample is examined (Booth et al., 2003). Counting of eggs in Kato-Katz smears can be tedious and time consuming process, and can lead to technical errors (Ebrahim et al., 1997). Other setbacks of the method include high risk of infection for the technicians handling fresh stools (HHS, 1993).

Hookworm eggs clear rapidly, and if slides are not examined within 30-60 minutes, the eggs may no longer be visible. The technique is also known to be unsuitable for detection of cysts, larvae, small fluke eggs or thin-shelled eggs such as *Hymenolepis* species because eggs disappear during the clearing process in a short time of 30-120 minutes (Knopp et al., 2006).

2.4.6 Serological (Immuno-diagnostic) methods

There are increasing availability of non-microscopic methods, such as DNA probes, direct fluorescent antibody methods and enzyme-linked immunosorbent assay-ELISA (Genta, 1988; Char and Farthing 1991; Gasser, 2001). An enzyme linked immunosorbent assay (ELISA) using larval antigen, is employed for the diagnosis of helminthic infections when larvae cannot be found through microscopy (Garcia, 2001).

Serological tests are of value in the diagnosis of acute *trichinosis* and *Strongyloidiasis* (Smith and Bartlett, 1991; Genta, 1988). They have proved to be most useful for distinction between acute and chronic *Schistosoma mansoni* infection (Valli et al., 1997). Serological methods are sensitive but are expensive for use in the developing world and may show cross reactivity with other helminthic infections (Valli et al., 1997). Another disadvantage of sero diagnositic approach is that tests might remain positive even after cure by chemotherapy (Knopp et al., 2006).

2.4.7 Molecular diagnosis

Molecular techniques such as polymerase chain reaction (PCR) using primers derived from different genetic markers are useful diagnostic tools (Michaud et al., 2003).

The technique is desirable in differentiating two morphologically identical species such as *Ascaris duodenale* and *Necator americanus* (de Gruijter et al., 2005).

Polymerase chain reaction amplified fragments can be analyzed by using restriction fragment length polymorphisms (PCR-RFLP) analysis has been used to distinguish the two human hookworms *Ancylostoma duodenale and Necator americanus* (Hawdon, 1996) and from infection with *Oesophagostomumbiurcum*, whose eggs are morphologically indistinguishable from hookworm (Verweij et al., 2001). These ‘’high-technology’’ methods are sensitive and specific, and allow distinction between morphologically identical parasite species. However, they are often too expensive for use in the developing countries (Valli et al., 1997; Hotez et al., 2006).

2.4.8 Culture techniques (Coprocultures)

Faecal culture or coproculture involves in-vitro breeding of hookworm and other intestinal nematode larvae (Jozefzoon and Oostburg, 1994; Arcari et al., 2000). It is useful for detecting latent infections and for the diagnosis of *Ancylostoma duodenale, Necator americanus* and *Strongyloides stercoralis* infections (Hsieh, 1961; de Kaminsky, 1993).

Coproculture is essentially a concentration method as the procedure is used for recovery of larvae when they are too scanty to be detected by other parasitological methods (Markell and Voge, 1976).

Standard methods of culturing hookworm and *Strongyloides* larvae include the Baermann, Harada-Mori test tube (Harada and Mori, 1955), agar-plate (Koga et al., 1990) and charcoal cultures (Markell and Voge, 1976; Smith and Bartlett, 1991), each of which has been modified in various ways to enhance yield or ease of maintenance (Hsieh, 1961; Koga et al., 1991; Watson and Al-Hafidh, 1957).

The specimen to be cultured must be fresh stool that has not been refrigerated, because some parasites (especially *Necator americanus*) are susceptible to cold and may fail to develop after refrigeration (Smith and Schad, 1990).

In general, cultural methods are not suitable for routine diagnostic practices or for screening asymptomatic patients because they require too much time to be clinically useful (Smith and Bartlett, 1991; Beaver et al., 1984; Isenberg, 1998). Therefore faecal culture largely remains a research technique, where rapid results are not that important (Jozefzoon and Oostburg, 1994; WHO, 1998, 2000).

2.4.9 The Baermann technique

The Baermann technique relies on the principle that active larvae will migrate out of a faecal specimen that has been placed on a wire mesh covered with several layers of gauze (Garcia, 2001).

It is documented as the most efficient technique available for the laboratory diagnosis of *Strongyloides stercoralis* infection, and very useful for monitoring following the results of antihelminthic therapy (Markell and Voge, 1976).

Various modifications of the technique are also used for the recovery of other intestinal nematode larvae (Watson and Al-Hafidh, 1957).

Limitations of Baermann method include the fact that the technique requires the use of a special apparatus, which is generally used for repeated cultures, but each individual sample requiring a separate apparatus, which makes it difficult to adopt for use in large-scale laboratory or epidemiologic studies (Markell and Voge, 1976). It is also too cumbersome a method for routine use (Watson and Al-Hafidh, 1957).

2.4.10 The agar plate culture

The agar plate culture has been found to be most useful for the diagnosis of *Strongyloidesstercoralis* infection (Koga et al., 1990, 1991, 1992). In a study that looked at the prevalence of *Strongyloides stercoralis*, the diagnostic efficacy of the agar plate culture method was found to be high as 93.9%, compared to only 28.5 for the Harada-Mori filter paper culture (Koga et al., 1990).

Limitations of this method include the requirement for preparation of agar plates, daily search for furrows on agar plates and the use of dissenting microscope to examine the plate for the presence of tracks (Garcia, 2001). The procedure is relatively expensive and difficult to carry out (Garcia, 2001).

2.4.11 Charcoal cultures

Charcoal cultures are made by thoroughly mixing one part of soft or softened (with water) faeces with five to ten parts of fine, granulated (not powdered), hardwood charcoal (BDH), or bone charcoal (Ebonex, Melvindale), which is made damp throughout and packed into a covered but not air-tight container (Garcia, 2001).

Charcoal culture continues to be documented as the most suitable method for obtaining large quantities of *Strongyloides* larvae. Disadvantages of this method are that it requires additional organic material (that is., charcoal), as well as daily observation for adequate moisture (Garcia, 2001).

2.4.12 The Harada-Mori test tube culture

A clean and simple test-tube method for the culturing of hookworm and other intestinal nematode larvae from eggs was devised by Harada and Mori in 1955.The original Harada-Mori test-tube method (Harada and Mori, 1955) has been variously modified by others (Sasa et al., 1958; Hsieh, 1961) for diagnosis and differentiation of *Necator americanus* from *Ancylostoma duodenale* infections. Sasa et al. (1958) and Hsieh (1961) have noted that the modified Harada-Mori culture technique has several advantages over other methods for culturing hookworm larvae.A disadvantage of the Harada-Mori is that the faecal cultures must be monitored closely to prevent desiccation caused by evaporation (Garcia, 2001).

4.13 Choice of diagnostic method in routine parasitological laboratory

In hospital and health centers, quick and accurate diagnosis of infection is critical for guiding clinical management of patients (Isenberg, 1998). The choice of a particular technique is influenced by its affordability, simplicity, sensitivity and level of professionalism involved (Melvin and Brooke, 1985; WHO, 2000; CLSI, 1997).

In developing countries, routine methods are chosen largely on account of their affordability and rapidity (that is, cheap, easy to carry out, and time consuming procedure), and often disregarding the sensitivity and consequences of misdiagnosis that may ensue from employing a method of low sensitivity (Barnabas and Aboi, 2005; Wirkom et al., 2007; Oguama and Ekwunife, 2007).

Many authors have indicated that the direct wet mount lacks sensitivity (Smith and Bartlett, 1991; Ukaga et al., 2002; Markell and Voge, 1976), yet because the method is inexpensive, simple and rapid to perform, hospital and health centers in developing countries laboratories rely on it as the main diagnostic tool for routine stool examinations (Cheesbrough, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDY AREAS

3.1.1 PROFILE OF THE STUDY AREAS

The study areas are Gia and Kajelo communities in the Kassena-Nankana East and West Districts (KND) of the Upper East Region of Ghana.

The Kassena-Nankana District is in the North-Eastern part of Ghana. It shares boundaries with Burkina Faso to the North, the Bolgatanga Municipality to the South and to the west with Builsa and Sisala Districts. The districts has a total land masses of 1,675km^2, eighty percent, (80%) of which is arable, while the remaining twenty percent, (20%) is covered by forest, rivers, hills, and eroded areas (Kassena- Nankana District, 1996).

The Gia community is one of the several rural communities participating in the Tono irrigation project (TIP).The Gia community has a Sub-Chiefdom within the Kassena-Nankana Traditional Area. The Kajelo community is also one of the several rural communities in the Kassena-Nankana District. The community however, does not partake in the Tono irrigation project. The Kajelo community has a Sub-Chiefdom within the Paga Traditional Area.

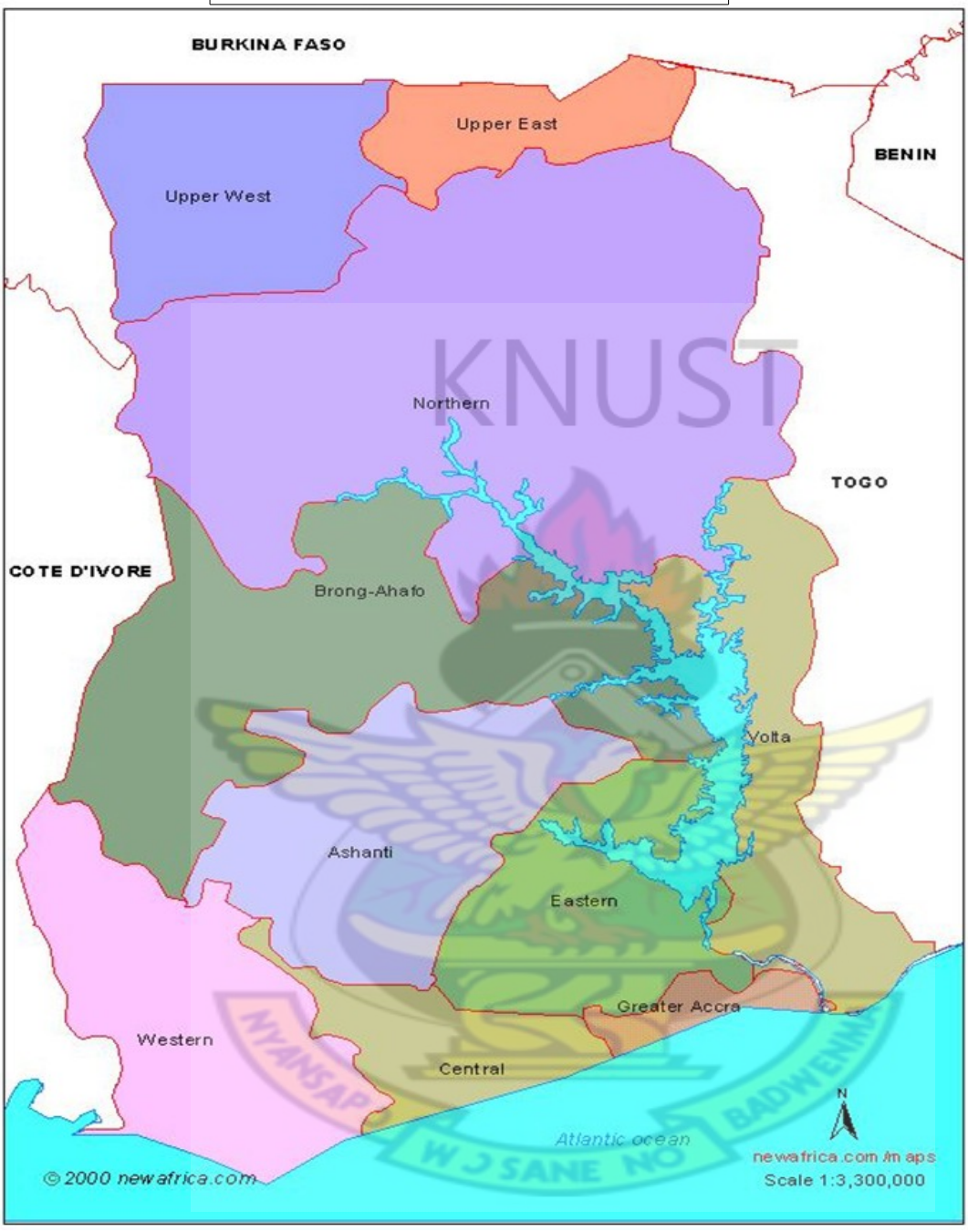

Figure 1: Administrative Map of Ghana

Source: Ghana Geographical Department

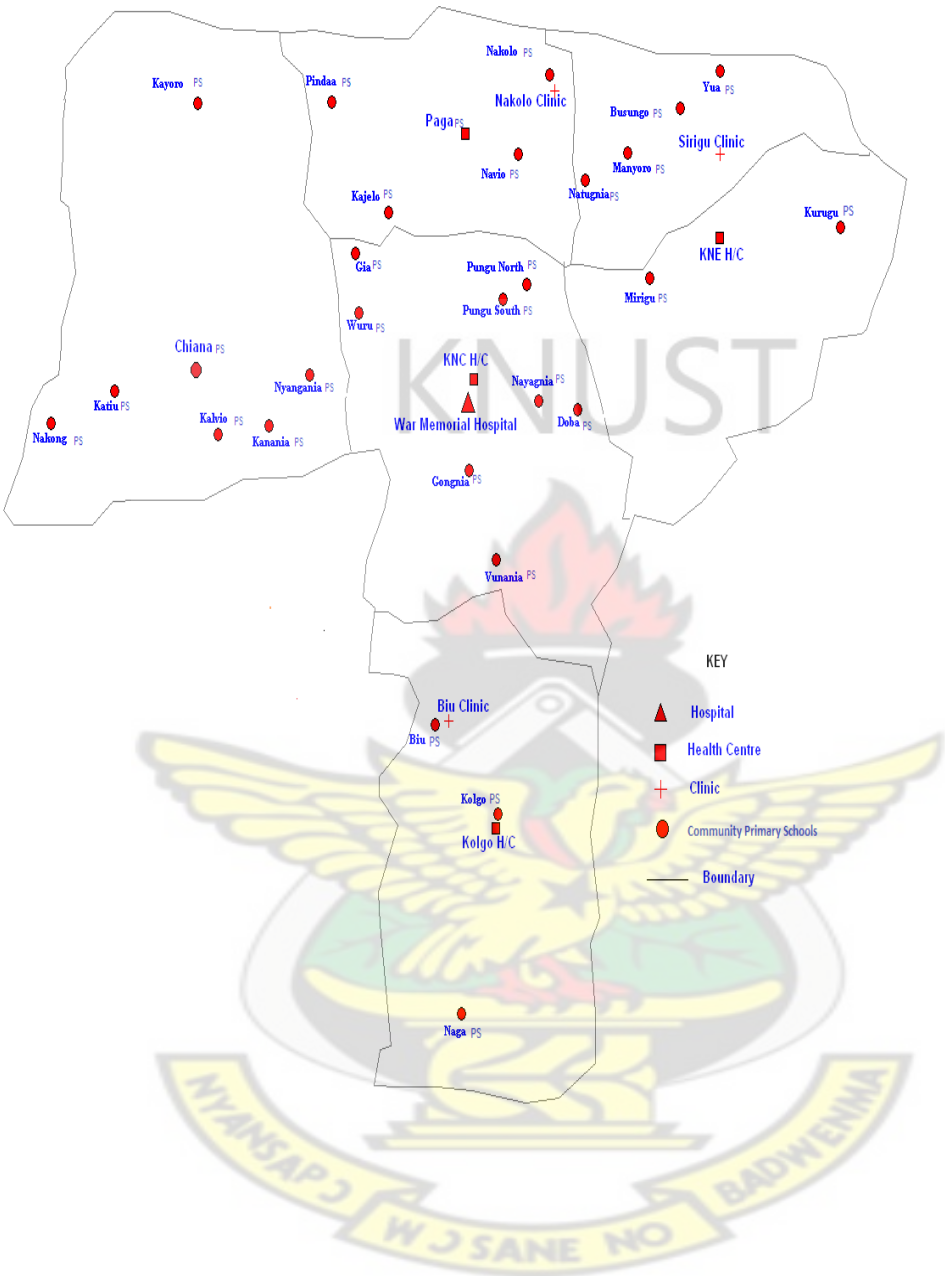

Figure 2: Map of primary schools, hospitals and health centers in the Kassena-Nankana District in the Upper East Region of Ghana.

Source: Ghana Survey Department

3.1.2 DEMOGRAPHIC FEATURES

Gia is located about 10km east of the District capital, Navrongo, and lies in one of the less densely populated areas in the District. The community has 5 zones; Bagnia, Kwosongo, Nangwao, Naa and Kansila. The people live in dispersed settlements of compounds, connected by outer walls and surrounded by their farmlands for subsistence farming.

The general extended family system is practiced with most of the households headed by men. The total estimated population of Gia is about 2110, (interview, Kassena-Nankana East District Health Management Administration, 2010) and it is served by one community health center.

Kajelo is located about 12km west of the Paga District, and lies in one of the less densely populated areas in the District. The community has 6 zones; Batiu, Nabua, Kansila, Kachuno and Yunania. The people also live in dispersed settlements of compounds, connected by outer walls and surrounded by their farmlands for subsistence farming.

The people practice extended family system with most of the households also headed by men. The total estimated population of Kajelo is about 2860, (interview Kassena-Nankana West District Health Administration, 2010) and is served by a community health center.

3.2 STUDY POPULATION

Gia primary school has a pupil population of 358 (School enrolment for 2010/2011 academic year). In all 198 pupils out of the 358 between the age group 5 to 15 years were selected. All the 198 were involved in the study. Pupils above and below the age group mentioned were excluded from this study (Morris et al., 1992; Chan et al., 1997).

Kajelo primary school has a pupil population of 276 (school enrolment for 2010/2011 academic year). In all 196 pupils out of the 276 between the age group 5 to 15 years were selected. All the

196 pupils were involved in this study. Pupils above and below the age group mentioned were excluded from this study (Morris et al., 1992; Chan et al., 1997).Consented subjects (pupils) were recruited over a period of six months, commencing from October 2010 to March 2011 for this study.

3.3 ETHICAL CONSIDERATION

Ethical clearance was approved by the Kassena-Nankana District Health Management Team (DHMT). Permission to undertake the study at the War Memorial Hospital and Tongo Health Centre Laboratories was sought and granted by the hospital management and the head of the District Laboratory.

The heads of both schools and parents/guardians of the pupils, who were enrolled for the study, gave informed consent after full explanation about the purpose and the techniques of the study was given.

3.4 SAMPLE COLLECTION

Consented subjects were provided with clean, dry, leak-proof and wide-mouthed plastic specimen containers. They were given instructions on how to avoid contamination of stool sample with urine, and further instructed to collect about half of stool in the containers provided and to deliver them to school within 2 hours after collection (Booth et al., 2003).

Pupils who were unable to produce specimens on the same day in the school were allowed to send the specimen containers home and asked to bring freshly passed stool samples the following day. Each specimen was labeled with a study number, date and time specimen was collected, and time specimen was received. Pupils who delivered inadequate stool specimens (half of the

container was considered adequate for all the tests adopted for the study with enough remaining for preservation) and/or delivered them later than 2 hours after collection were not included in the study (Morris et al., 1992). The samples collected were then transferred within one hour to the laboratory for analysis.

A semi-structured questionnaire (Appendix) was administered to gather information on age, sex, parental level of education, type of water usage and anthelminthic drug usage in the past one year.

3.5 LABORATORY PROCEDURES

3.5.1 PARASITOLOGICAL EXAMINATIONS

Two parasitological methods were used for this study, namely the direct wet mount and formol-ether concentration techniques. Each specimen was first examined macroscopically and its consistency was recorded as formed (F), semi-formed (SF), bloody-mucoid (BM), loose (L) or Watery (W), in accordance with the description by Ash and Orihel (1991). Samples were analyzed fresh, in batches, as soon as they are received; none was preserved in the refrigerator or any preservative added prior to processing, as this will kill ova and larvae of the organism (Smith and Schad, 1990).The test procedure were carried out in accordance with standard protocols as described by Garcia (1999, 2001) and WHO (1991, 1994). The steps have been outlined below;

3.5.2 DIRECT WET MOUNT TECHNIQUE, SALINE PREPARATION

With a marker the study identification number was written at one end of the slide and a drop of physiological saline was placed in the center of the slide. With a wooden applicator stick, a small portion of stool specimen was picked and thoroughly emulsified to make a thin uniform saline

suspension- not too thick that faecal debris may obscure organism, and not too thin that blank spaces may be present.

The suspension was carefully covered with a cover slip in a way as to avoid air bubbles. The slide is then placed on the stage, and the preparation was examined systematically under the low power (10 xs) objective so that the entire cove slip area is scanned for parasite ova, cysts, larvae and trophozoites.

When organisms or suspicious objects were seen, the high dry (40x) objective was used to see more the detailed morphology of the object for confirmation.

3.5.3 FORMOL-ETHER CONCENTATION TECHNIQUE

With a wooden applicator stick, 1 gram of stool specimen was added to 10ml of 10% formalin in a small beaker and thoroughly emulsified, and brought into suspension.

The suspension was strained through a double layer of wet gauze directly into a 15ml centrifuge tube. The gauze was then discarded, and more 10% formalin was added to the suspension in the tube to bring the total volume to 10ml.

3ml of ether was added to the suspension in the tube, rubber stoppered and shaked vigorously for 10 seconds.

With an applicator stick the plug of debris was loosened by a spiral movement and the supernatant (comprising the top three layers) was decanted, in a single movement, into a bowl containing disinfectant; allowing the last few drops of residual fluid to flow back onto the sediment.

The deposit was resuspended with a disposable Pasteur pipette. It was necessary to add a drop of physiological saline to have sufficient fluid to resuspend the deposit/sediment. A few drops of the suspension was transferred onto a microscope slide and covered with a cover slip.

The preparation was scanned using the low power (10 xs) objective, and in a systematic manner as to observe the entire cover slip area. If an organism or suspicious objects are seen, the higher magnification (40 xs) objective was used to observe its detailed morphology.

3.5.4 CHOICE OF A ‘’GOLD STANDARD” METHOD FOR THE STUDY.

Enough evidence in the literature support the fact that the formol-ether concentration or various modifications of it is the most sensitive and specific of all parasitological tests for intestinal helminthes (Wirkom et al; 2007, Matsubayashi et al., 1965; Neimeister et al., 1987).

The technique has been mentioned as the ‘’gold standard’’ method (Wiebe et al., 1999) for several medical and epidemiological studies on the intestinal parasites (Akujobi et al., 2005; Ahmadi et al., 2007; CLSI, 1997; Oguama and Ekwunife, 2007).

Because it continues to be documented as the most sensitive and specific for the detection of parasite ova and larvae in faecal specimens, it was chosen as the ‘’gold standard’’ method for this study (Wiebe et al., 1999).

3.6 QUALITY CONTROL (QC)

1. To ensure quality control, all the laboratory procedures including collection and handling of specimens, were carried out in accordance with standard protocols (CLSI, 1997; Melvin and Brooke, 1985; WHO, 1991).

2. All the reagents were checked for contamination each time they were used.

3. To ensure general safety, disposable gloves were worn and universal bio safety precautions (CLSI, 2002a, 2002b; HHS, 1993) were followed at all times. Waste was disposed of in accordance with infection control practices at the War Memorial Hospital and Tongo Health Centre respectively.

4. For quality control of the concentration method, preserved stool specimens known to contain parasite ova and larvae was included in each batch of samples to be concentrated. This approach ensured that the procedures were precise.

5. Ten percent of slide preparations were randomly selected for confirmation by the supervising clinical parasitologist.

6. The microscope used for the research was calibrated, and the objectives and oculars used for the calibration procedure were used for all the measurements done with the microscope. The calibration factors for 10x and 40x objectives was posted on the microscope for easy access.

7. To ensure accurate identification of parasite species, bench aids for the diagnosis of intestinal parasites WHO (1994), and diagrams of various parasites ova and larvae from parasitological text were reviewed.

3.7 STATISTICAL ANALYSIS

The data obtained from the 394 samples were entered into the computer using Microsoft excel 2007 version. Analysis of the data was done using the chi-square (x^2) test for categorical variables. Using the statistical software SPSS version 11, the sensitivity, specifity values were determined to evaluate the performance of the two main parasitological methods employed in this study. Significant associations were identified based on a P-value of < 0.05 and 95% confidence interval.

Age was analyzed as both a continuous variable and a categorical variable, when appropriate. Data on the infection intensity of the soil-transmitted helminthes was analyzed as a continuous variable, as well as categorical variable, were groups consisted of loose, watery, semi- formed and formed as defined in the method used.

Analytical outcomes were presented as bar graphs, pie charts and histograms

3.7.1 Sensitivity

Sensitivity is the ability of a test to identify correctly all positive samples. It is calculated as the number of true positive samples detected by that test being evaluated, divided by the number of samples identified by the reference method as positive, expressed as a percentage. High sensitivity enables accurate detection of diseases, and therefore reduced incidence of misdiagnosis (Hoehler, 2000).

3.7.2 Specificity

Specificity is the ability of a test to detect correctly all negative samples. It is calculated as the number of true negative samples recognized by the test being evaluated, divided by the reference test as negative, and expressed as a percentage. High specificity suggests that the test is good for ‘’ruling out’’ disease (Hoehler 2000).

3.7.3 Predictive value

Positive predictive value (PPV) expresses the proportion of persons with positive test that have the disease condition, that is, the probability that a positive test is a true positive. Negative

predictive value (NPV) is the probability that a negative test is a true negative, or the proportion of persons with the negative test who do not have the disease condition (Hoehler, 2000).

3.7.4 P-value

P-value provides a sense of the strength of the evidence against the null hypothesis. The smaller the P-value, the more strongly the test rejects the null hypothesis, that is, the hypothesis being tested. The most commonly used level of significance is 0.05. When the significant level is set at 0.05, any test resulting in a p-value under 0.05 is significant. Therefore, the null hypothesis will be rejected in favor of the alternative hypothesis.

CHAPTER FOUR

4.0 RESULTS

4.1 COLLECTION AND EXAMINATION OF STOOL SAMPLES.

A total of 394 subjects were examined for soil-transmitted helminthes between October 2010 and March 2011.The Gia primary school was where the first samples were collected. A total of 198 subjects were recruited at Gia primary school for this study. The Kajelo primary school was where the second samples were collected. A total of 196 subjects were recruited for this study. Overall, there were more females than males in the 394 subjects examined with female's population of 202 representing 51.3% and males 192 representing 48.7%.

There was an overall prevalence of 9.40% in the subjects with the formol-ether concentration technique. The direct wet mount method gave an overall prevalence of 2.79%.

4.2NATURE OF STOOL SPECIMENS (Macroscopic examination)

The table below represents the relative frequencies of the types of consistency of 394 stool samples corrected from the studied patients.

Table 1: Relative frequencies of consistency of stool samples.

Consistency type		Frequency	Percentage (%)
Semiformed		228	57.87
Formed		127	32.23
Loose		10	2.54
Mucoid		5	1.27
Watery	24	6.09	
Total		394	100

As shown in Table 1 above, most of the stool specimens 228 (57.87%) were semiformed or soft in nature, 127 (32.23%) of the stool specimen were formed or hard in nature. Loose stool was 10 (2.54%), mucoid stool was 5 (1.27%) and whiles watery stool was 24 (6.09%).

4.3.1COMPARISON OF THE CURRENT PREVALENCE OF SOIL-TRANSMITTED HELMINTHES INTHE GIA COMMUNITY WITH THE PREVIOUS PREVALENCE.

According to the unpublished data from the Neglected Tropical Disease Control Unit of the Ghana Health Service on the prevalence of soil-transmitted helminthes in the Gia community in 2007 was 10%. The study used the direct wet mount as the sole diagnostic method.

The current study in 2010-2011 has revealed that, the prevalence of soil-transmitted helminthes in Gia is 3.54% with the direct wet mount method and a prevalence of 10.61% with the formol-ether concentration technique.

The formol-ether concentration technique shows that, there is relatively high prevalence of soil-transmitted helminthes in the Gia community. The direct wet mount method gave a low prevalence as against the 2007 indicating that with the direct wet mount method there is a decrease in the soil-transmitted helminthes in the Gia community.

4.3.2PREVALENCE OF SOIL- TRANSMITTED HELMINTHES IN THE GIA COMMUNITY

Table 2: Showing the prevalence of soil-transmitted helminthes in the Gia community.

Helminthes	Direct wet mount		Formol-ether concentration	
	n=198		n=198	
	Positive	Percentage (%)	Positive	Percentage (%)
Hookworm	1	0.51	10	5.05
Strongyloides stercoralis	5	3.03	7	3.54
Ascaris lumbricoides	0	0.00	4	2.02
Trichuris trichuira	0	0.00	0	0.00
Total	6	3.54	21	10.61

4.3.3 PREVALENCE OF SOIL-TRANSMITTED HELMINTHES IN THE KAJELO COMMUNITY.

There is no available data from the unpublished data on Kajelo community from the Neglected Tropical Disease Control Unit of the Ghana Health Service. Simple random sampling was done to select the Kajelo community from the various communities in the Kassena-Nankana District. The Kajelo community was then selected in addition to the Gia community to know the current prevalenceof soil-transmitted helminthes in those communities.

The direct wet mount method gave a prevalence of 2.55% in the Kajelo community. The formol-ether concentration technique gave prevalence of 8.16% also in the Kajelo community

Table 3: Prevalence of soil-transmitted helminthes in the Kajelo community.

Helminthes	Direct wet mount		Formol-ether concentration	
	n=196		n=196	
	Positive	Percentage (%)	Positive	Percentage (%)
Hookworm	0	0.00	3	1.53
Strongyloides stercoralis	5	2.25	13	6.63
Ascaris lumbricoides	0	0.00	0	0.00
Trichuris trichuira	0	0.00	0	0.00
Total	5	2.55	16	8.16

4.3.4OVERALL PREVALENCE OF SOIL-TRANSMITTED HELMINTHES IN THE GIA AND KAJELO COMMUNITIES.

Out of the 394 stool samples examined from the two communities gave an overall prevalence of 2.79% with the direct wet mount method and 9.40% with the formol-ether concentration technique

Table 4: Showing overall prevalence of soil-transmitted helminthes in Gia and Kajelo communities.

Helminthes	Direct wet mount	Formol-ether concentration

	n=394		n=394	
	Positive	Percentage (%)	Positive	Percentage (%)
Hookworm	1	0.25	13	3.30
Strongyloides stercoralis	10	2.54	20	5.08
Ascaris lumbricoides	0	0.00	4	1.02
Trichuris trichuira	0	0.00	0	0.00
Total overall prevalence	11	2.79	37	9.40

Table 5: Performance of direct wet mount method against the formol- ether concentration technique or the gold standard in detection of the soil-transmitted helminthes parasites.

Gold standard		Direct wet mount method		Total results
Method	Result	Positive	Negative	
Formol-ether Concentration Technique	Positive	20	40	60
	Negative	0	334	334
Total results		20	374	394

Positive predictive value, PPV= 33.3% (20/60)

As shown in the table 7, the direct wet mount method detected a total of 20 soil-transmitted helminthes parasites as against 60 by the formol- ether concentration technique or the gold standard. The evaluation results gave sensitivity of 33.3% (20/60) and specificity of 100% (334/334), respectively.

4.4 SURVEY BY QUESTIONNAIRE ADMINISTRATION

A total of 394 questionnaires were administered to the pupils in Gia and Kajelo communities in the Kassena-Nankana district. However, not all the pupils answered all the questionnaires posed to them. The questionnaires were analyzed below:

4.4.1 SOME BIODATA OF RESPONDENTS

4.4.1.1 AGE DISTRIBUTION OF RESPONDENTS

Table 6: Age Distribution of Respondent

Age	Frequency	Percent
5	1	0.3
6	8	2.0
7	22	5.6
8	30	7.6
9	59	15.0
10	85	21.6
11	77	19.5
12	45	11.4
13	28	7.1
14	37	9.4
15	2	0.5
Total	394	100.0

Table 6 above provides information on the age distribution of the respondents. They were within the age group 5 to 15 years and the mean age was 10.5 years. A total of 85 subjects representing 21.6% of the studied population were pupils of 10 years of age. In addition, a total of 77 subjects representing 19.5% of the studied population were pupils of 11 years of age. The least age group was 5 years representing 0.3% of the studied population.

4.4.1.2 SEX DISTRIBUTION OF RESPONDENTS

Figure 3: Sex distribution of respondents.

Figure 3 above shows the sex distribution of respondents. Out of the 394 studied subjects 192 were males and 202 were females representing 48.7% and 51.3% respectively.

4.4.1.3 DISTRIBUTION OF THE EDUCATIONAL LEVEL OF RESPONDENTS RELATIVES.

Figure 4: Distribution of educational level of respondents' relatives

Figure 4gives an illustration of the educational level of the respondents' parents or guardians. Majority of respondents' parents or guardian have had no formal education 180 (45.7%). This was followed by respondents' parents or guardian with primary level of education 123 (31.2%). Those with senior high school education were the third highest with 67 (17%). Those with tertiary level of education were the least with 24 (6.1%).

Most of the respondents 319 (81%) stay with their parents in the communities. This was followed by respondents who stay with their guardian in the communities 75 (19%).

4.4.1.4 SOURCE OF DRINKING WATER OF RESPONDENTS

Figure 5: Source of drinking water of respondents

Figure 5 illustrates the source of drinking water among respondents in their homes. Majority of respondents 383 (97.2) uses boreholes as their source of drinking water. Those who use water from streams are10 (2.3%). However, a respondent said they use pipe borne water in their house 1 (0.3%).

4.4.2.1 KNOWLEDGE ABOUT HOW RESPONDENTS GOT TO KNOW ABOUT SOIL-TRANSMITTED HELMINTHES (WORMS).

Figure 6: Knowledge of respondents about soil-transmitted helminthes

Figure 6 illustrate knowledge of respondents about worms. Majority of respondents 244 (61.9%) got to know about worms through their teachers. This was followed by 77 (20.0%) of respondents who got to know about worms through the community nurse. In addition 35 (8.9%) of respondents got to know about worms through their parents or guardian. Respondents who got to know about worms through their friends were 19 (4.8%). Furthermore, 17 (4.3%) of respondents got to know about worms through a doctor.Lastly, 2 (0.5%) of respondents got to know about worms through a deworming exercise in their school.

4.4.2.2 KNOWLEDGE OF RESPONDENTS ABOUT DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHES

Figure 7: Response on the diagnosis of soil-transmitted helminthes among studied subjects

Figure 7 above gives an illustration of the responses of respondents on the diagnosis of soil-transmitted helminthes. A large number of respondents 142 (36.0%) were diagnosed of worms at the hospital. This was followed by respondents 133 (33.8%) were diagnosed of worms at the health center. In addition, 95 (24.1%) of respondents were diagnosed of worms at the

clinic. The number of respondents who were diagnosed of worm's infestation at the CHPScenterswas 24 (6.1%).

4.4.2.3 RESPONSES ON LABOATORY TEST DONE TO DIAGNOSE SOIL-TRANSMITTED HELMINTHES.

Figure 8: Response on any laboratory test done to diagnose soil-transmitted helminthes.

Figure 8 illustrate responses on whether there was a laboratory test done to diagnose soil-transmitted helminthes. Majority of the respondents 278 (70.6%) indicated that there was test done to diagnose soil-transmitted helminthes. This was followed by 116 (29.4%) of respondents who responded that there was no laboratory test done to diagnose soil-transmitted helminthes.

CHAPTER FIVE

5.0 DISCUSSION

The study demonstrated that all cases of soil-transmitted helminthes were of low density and required a more sensitive method for their detection (Schad and Banwell, 1984). What is less well known is that soil-transmitted helminthes commonly coexist in resource-poor countries, and many individuals are concomitantly infected with enteroparasites (Crompton 1999; Montressor et al., 2002).

Diagnosis of soil-transmitted helminthes infections is based on the recovery of helminthes eggs and or larvae in stool samples examined through a variety of parasitological methods (Goodman et al., 2007). Information from several hospitals and health centers across the country revealed that the direct wet mount method is the sole diagnostic tool used for stool routine examination at all levels of health care delivery in Ghana.

The Centre for Neglected Tropical Diseases did a study at Gia community in 2007 with the direct wet mount method as the sole diagnostic tool (unpublished) and had 10% prevalence of soil-transmitted helminthes. The study in 2010-2011 has revealed a less prevalence of 3.54% with the direct wet mount in the Gia community and 10.16%, a higher prevalence with the formol-ether concentration technique. In addition the overall prevalence in the Gia and Kajelo communities was 2.79% with the direct wet mount and 9.40% with the formol-ether concentration in the two communities. The study was therefore, intended to find out if the prevalence of soil-transmitted helminthes in the two communities have reduced with the direct wet mount or increased with the formol-ether concentration technique. Furthermore, the study was to find out if there was the need to evaluate the direct wet mount method using the formol-ether concentration technique as the diagnostic gold standard test for the study.

5.1 Demographic characteristics

Of the 394 studied subjects, 192 (48.7%) were males and 202 (51.3%) are females. The higher female ratio can be attributed to most parents now embracing the idea of sending their female children to school.

In this study, children aged between 5-10 years constituted 52.1% of the total subjects studied. This proportion of children is significant because most studies on helminthes have targeted children between this age group (Annan et al., 1986; Pearson, 2002). Children between the ages of 11-15 years also constituted 48.0% of the total subjects studied.

5.2 Dewormer usage by studied subjects

The study showed that 302 (76.6%) of the studied subjects had taken some form of anthelminthic drug (dewormer) in the three months preceding the study. Of the studied subjects 61 (15.5%) had taken some form of anthelminthic drug (dewormer) six months preceding the study. This was followed by 19 (4.8%) of the studied subjects who had taken some form of anthelminthic drugs one year preceding the study. In addition, 12 (3.1%) of the studied subjects had taken anthelminthic drugs more than one year preceding the study. The study findings sufficiently support the speculation that the subjects have had some form of deworming before the study. Indeed, on account of the many deworming products available on the market today, together with various campaigns and programmes aimed at encouraging deworming treatments, the level of dewormer usage observed in the study can be considered as much higher than expected.

Significantly, the number of children who had dewormed was more than those who had not dewormed. However, the study established no important association between dewormer usage and the presence of helminthes parasite. The analysis is handicapped by lack of data on the type

of anthelminthic drug taken, dosage regimen, etc. Therefore, interpretation of the results may have to be done with caution.

5.3 Prevalence of Soil-Transmitted Helminthes parasites

The prevalence of 5.08% *Strongyloides stercoralis* detected by the formol-ether concentration technique compared with 2.54% by the direct wet mount method highlighted the lack of sensitivity of the direct wet mount for detection of *Strongyloides stercoralis* infection. This observation had been reported by Estevez and Levine (1985). It was clear from the study that the prevalence of strongyloides stercoralis has been underestimated as a result of missed diagnosis. Indeed, the study finding collaborate other studies that reported spurious and genuine *Strongyloidesstercoralis* infections elsewhere in Ghana (Odei, 1966; Wolfe, 1966).

In the study, 0.25% prevalence of hookworm was detected by the direct wet mount method as compared to the 3.30% by the formol-ether concentration technique. Hookworm infection was found in pupils in almost all the age group examined. This finding is significant because hookworm is not known to be endemic in the Upper East Region probably because of missed diagnosis or solely because the direct wet mount is the sole diagnostic method used in most laboratories in the Upper East Region.

Based on this study and other studies elsewhere (De Vlas, 1992; Hotez et al., 2006; Michaud et al., 2003), hookworm infection is best detected in stool samples by the formol-ether concentration technique.

Ancylostosomiasis is associated with greater intestinal blood loss; ingesting 0.15ml per worm per day and causing severe iron-deficiency anemia (Albinoco et al., 1998), while Necator americanus infection is acquired almost exclusively by active penetration of the skin,

Ancylostosoma duodenale is able to infect both percutaneously and by the oral route (Looss, 1911), and also causes infantile ancylostosomiasis, through transplacental or lactogenic transmission. Ancylostosoma duodenale is also reported to differ in susceptibility to the same anthelminthic and dosage regimen (Reynoldson et al., 1997). Consequently, the efficacy of anthelminthic therapy depended on the infecting species of hookworm (Horton, 2003).

The low prevalence of Ascaris lumbricoides (1.02%) and the absence of Trichuris trichuira can be attributed to the very low average annual rainfall of 850mm and the extreme average annual temperature range of (18-45^{0}C) in the Kassena-Nankana district. The eggs of both species require an optimal temperature of about 31 ^{0}C (Seamster, 1950) for embryonation whilst temperature of 38 ^{0}C is lethal (WHO, 1967). Areas where the average annual rainfall falls below 1400mm, usually demonstrate absence of transmission (Brooker et al., 2000). Several studies in Sahalean countries; Mali (De Clerq et al., 1995), Mauritania (Urbani et al., 1997), Niger (Develoux et al., 1986) and Sudan (Magambo et al., 1986) have demonstrated absence of transmission of these two parasites.

In addition, it cannot be attributed to lack of diagnostic sensitivity because the formol-ether concentration technique (Allen and Ridley, 1970) was used in this study. The method is well noted for its diagnostic capability and is widely used in epidemiological and clinical studies on diagnosis of intestinal helminthes infections (Melvin and Brooke, 1985; Garcia 2001). Unfortunately the study did not assess the risk factors and epidemiological patterns of the various helminthic infections.

The transmission and distribution of Ascaris lumbricoides and Trichuris trichuira are largely determined by inadequate sanitary practices and the local habits in the disposal of feces (WHO, 2002). Current opinions suggest that the absence of Ascaris lumbricoides is due to improvement

in prevailing social environment and behaviors of people in communities (Montressor et al., 1998). However, there is no consistent evidence to support these assertions.

Therefore, there is the need for further studies to assess current trends of ascariasis, trichuriasis and other helminthes in understanding their epidemiological and public health importance within the communities (Crompton and Savioli, 2007).

5.4 Performance of the formol-ether concentration and direct wet mount method

The overall performance of intestinal helminthes infections as detected by formol-ether concentration (which was chosen as the gold standard method for the study) and the direct wet mount method were 9.40% and 2.79% respectively. Analysis of the diagnostic performance of the direct wet mount method gave sensitivity of 33.3%, relative to the performance of the formol-ether concentration technique.

In other words, the direct wet mount method exhibited the lowest performance; being about three times less sensitive than the formol-ether concentration technique for the detection of intestinal helminthes parasites.

Indeed, significant difference ($p<0.05$) observed in the sensitivities of the two methods (formol-ether concentration technique and direct wet mount method) used in this study have been reported in other studies that have compared these methods (Martin and Beaver, 1968; Watson et al., 1988; Goodman et al., 2007).

Lack of sensitivity of the direct wet mount method is highlighted in this study and others elsewhere (Akujobi et al., 2005; Estevez and Levine, 1985), and hence support the argument that most laboratories in the country underestimate the true prevalence rates of helminthes infections among pupils.

The overall prevalence of 2.79% of intestinal helminthes parasite observed in this study for the direct smear is comparable to the rates that have been reported elsewhere (Oguama and Ekwunife, 2007; Akujobi et al., 2005). This support the fact that the direct wet mount is less sensitive in identifying helminthes parasites in stools of pupils. The need for introducing stool concentration technique in routine laboratory practice becomes compelling as reliance on the wet mount method alone may miss about three-quarters of helminthes infections.

5.5 Operational characteristics of the methods used in the study

Of the two Parasitological methods used the direct wet mount was found to be the simplest, more affordable and required minimum labour and skill to perform. It is rapid to perform, involving very few steps to complete. It does not require any special equipment or materials other than physiological saline. Other authors have described similar characteristics (Cheesbrough, 2005; Bogoch et al., 2006; Garcia, 2001).

In 394 stool specimens examined for helminthes parasites, the wet mount method gave a sensitivity of 33.3% relative to the recovery rate of helminthes parasite with the formol-ether concentration. This implies that only a third of helminthes infected pupils would have been analyzed with only the direct wet mount method. The findings of this study is in consistent with others (Akujobi et al., 2005; Goodman et al., 2007; Oguama and Ekwunife, 2007), have shown that the direct wet mount was less sensitive to the formol-ether concentration method.

Although some characteristics of the direct wet mount method makes it the preferred choice for the diagnosis of intestinal helminthic infections in resource –limited countries (Wirkom et al., 2007), reliance on it as the sole diagnostic tool in routine practice is very likely to cause misdiagnosis of infections.

The formol-ether concentration technique was used as the reference method or gold standard (Wiebe et al., 1999) for the study. Unlike the direct wet mount method, the formol-ether concentration was time consuming and required highly trained personnel and skill to perform the test.

Essential equipment and material requirements include fume chamber, centrifuge, reliable supply of electricity, centrifuge tubes and tube racks, gauze pads, formalin and diethyl-ether or ethyl acetate (Cheesbrough, 2005). These requirements make it the more expensive of the two parasitological methods evaluated in the study.

Nonetheless, based on the findings of this study and others (Akujobi et al., 2005; Oguama and Ekwunife, 2007), the formol-ether concentration remained most cost – effective method for diagnosis of latent helminthic infections, assessment of anthelminthic drug sensitivity, and epidemiological and clinical studies.

It is more desirable to perform the formol-ether concentration technique as a confirmatory test on all stool samples that are negative for helminthic parasites by the direct wet mount method. This approach will ensure accurate diagnosis of intestinal helminthic infections to allow effective management and control of parasitic diseases which ultimately improves quality of life of individuals in the community.

5.6 Some findings in questionnaire survey

The age group analysis indicated that the populations of Gia and Kajelo communities have more female pupils than male pupils in the schools. The population of male and female pupils in the communities, even though there were more females than males, the difference was not much, hence, one cannot say that there is a higher population of females than males in the communities.

Majority of the relatives of pupils in Gia and Kajelo communities had no formal education since the differences in their level of education were known to be higher than those with primary level of education, high school education and tertiary level of education.

Most of the pupils about 81% in the Gia and Kajelo communities stay with their parents in the communities. Those who stay with their guardian were 19% in the communities.

Also, it was revealed that, out of the 394 questionnaires administered 97.2% of the respondents were bore-hole water dependents. Those who use stream in their homes were 2.3%. Pipe-borne water dependent was 0.3%. The communities however, are scarcely dependent on pipe-borne water supply. The high dependency on borehole-water has also contributed immensely to the outcome of the result as compared to stream and pipe water users in general. Other studies have shown that helminthes infections among piped and stream users in underdeveloped countries may arise from the poor quality of the two since efforts to minimize microbial contaminants and to monitor the water quality are important (Chan et al., 1997).

Majority of the pupils knew about soil-transmitted helminthes. About 89.6% of respondents knew something about soil-transmitted helminthes. 9.9% of the respondents did not know about soil-transmitted helminthes. However, 0.5% of the respondents did not answer the questionnaire. This observation of pupils having knowledge about soil-transmitted helminthes is good towards its eradication.

5.7 CONCLUSION AND RECOMMENDATIONS

5.7.1 Conclusion

The study has resulted in a good number of findings in the Gia and Kajelo communities of the Kassena-Nankana district. These include:

A 3.54% prevalence of soil-transmitted helminthes with the direct wet mount method at Gia has reduced from 10% in 2007. However, there is a slightly higher increase with the formol-ether concentration with a prevalence of 10.16% at Gia.

The overall prevalence was 2.79% at both Gia and Kajelo communities with the direct wet mount method and 9.40% with the formol-ether concentration technique.

The study clearly indicated that the formol-ether concentration method is superior to the direct wet mount methods for routine diagnosis of intestinal helminthes infections. It is concluded that the low prevalence of intestinal helminthes parasites is due to lack of sensitivity of the traditional direct wet mount method. Hence, Parasitological laboratories in the country underestimate the 'true' prevalence's of intestinal helminthes infections at the hospitals and health centers.

5.7.2 Recommendations

It is recommended that stool samples that are found negative for parasites by the traditional direct wet mount method should be re-examined by the formol-ether concentration technique as a confirmatory test.The higher sensitivity of the formol-ether concentration technique being used as a confirmatory test increases detection of intestinal helminthic infections, but health safety makes it difficult to adopt it for routine clinical diagnosis.

Also, from the chi-squared analysis, it was found that the direct wet mount and the concentration technique are dependent with (p-0.001) at 0.05% significant level.

In this study, a true association between dewormer usage and not being infected could not be statistically established ($p>0.05$). Therefore, further extensive studies are recommended to assess the various types of anthelminthic drugs in Ghanaian market and their relative efficacies, including the dynamics of dewormer usage and worm burden in communities.

The facts that strongyloides stercoralis rated highest in prevalence suggest increasing genuine strongyloidiasis in the communities as reported by Odei (1966). In order to improve the detection and identification of strongyloidiasis species, it will be prudent to organize training workshops on stool microscopy for laboratory staffs at all levels of health care to improve their technical and diagnostic strongyloidiasis species and other parasites that may rarely be found in stool specimen. Further studies are needed to establish the epidemiological characteristics and factors that expose individuals to a high risk of developing associated disease.

The study results show that *Ascaris lumbricoides* and *Trichuris trichuira* transmission and their overall prevalence appear to have diminished. Further studies are necessary to assess current trends in ascariasis and *trichuriasis* in understanding their epidemiological and public health importance within the communities (Crompton and Savioli, 2007).

It is recommended from the study that *Schistosoma* infection which is not soil-transmitted helminthes parasite continues to persist in the communities and that both the direct wet mount method and the formol-ether concentration technique detected some species of *Schistosoma*. From this study and that of Fuseini et al., (2009), it has been revealed that *Schistosoma mansoni*a non-soil-transmitted parasite is still the leading helminthes parasite in the Upper East Region of Ghana. More study should be undertaken to know the relative prevalence of *Schistosoma mansoni* in the Upper East Region of Ghana.

The presence of hookworm infections have also been reported in the Northern Ghana and also by this study. Hookworm infection also continues to persist in the communities in the Upper East Region of Ghana. It is therefore recommended that large scale epidemiological studies on hookworm in various communities in Ghana be undertaken to gather adequate data on the relative prevalence of hookworm in various communities in Ghana.

The environmental conditions in the communities satisfy the condition for development and successful transmission of these soil-transmitted helminthes as a result of uncontrolled defecation due to the absence of inadequate latrine systems. This observation indicates that parasitolosis could become a problem in the communities if care is not taken.

To forestall this situation, a well-organized health education program on the mode of transmission and the impacts on soil-transmitted helminthes infections coupled with the provision of toilet facilities are desirable in the Gia and Kajelo communities in the Kassena-Nankana district.

5.7.3 Limitations and constraints

Some of the limitations and constraints that confronted this study include:

1. The pupils in Gia and Kajelo speak Kassem. Most of the pupils do not understand the English language so more often an interpreter was sought after to interpret the English language to them.
2. Unwillingness of some parents prevented their wards to be part of this study. This was due to the fact that some research which has already taken place promised the pupils of some incentives which were never fulfilled by the researchers.
3. Ethyl acetate was a preferred substitute for diethyl ether, but it could not be obtained from the local market. The concentration method was, therefore, performed using diethyl ether with its attendant health risks.

4. Because there was no fume chamber, the stool samples were processed in the open with their attendant odour or biohazard issues.

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APPENDIX

RESEARCH QUESTIONNAIRE

Please tick the appropriate box or fill the spaces provide

Number:…….. Age……. Sex:……

1. Who do you stay with?
2. What is your level of education of your parents/ guardian?

   Primary ☐ Secondary ☐ Tertiary ☐

3. What is the source of water for drinking and cooking in your home?

   ---

4. Have you heard about worms? a) yes ☐ b) no ☐
5. How did you got to know about worms? …….
6. Have you been affected with worms before? a) yes ☐ b) no ☐
7. Where were you diagnosed of worm infection? ☐
8. Were you given any treatment?a) yes ☐ b) no ☐
9. Was there any laboratory test done? a) yes ☐ b) no ☐
10. When was the last time you dewormed? ☐