KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF GRADUATE STUDIES

COLLEGE OF HEALTH SCIENCES

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DEPARTMENT OF CLINICAL MICROBIOLOGY

ACCURACY OF DIAGNOSIS OF INTESTINAL HELMINTH PARASITES, AND RELATIVE PREVALENCE OF *NECATOR AMERICANUS* AND *ANCYLOSTOMA DUODENALE* INFECTIONS AT THE KOMFO ANOKYE TEACHING HOSPITAL, KUMASI.

A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF PHILOSOPHY DEGREE (MPHIL) IN CLINICAL MICROBIOLOGY.

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 nor 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

There is a growing concern that stool reports from the Komfo Anokye Teaching Hospital (KATH) parasitology laboratory are frequently negative for intestinal helminth parasites. Over the years the wet mount technique has been the only method used at the KATH laboratory for diagnosis of intestinal parasites. The study evaluated the diagnostic sensitivity of the traditional direct wet mount technique using the formol-ether concentration as the gold standard method. After approval from the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital, Kumasi, and with the permission of the hospital management, 2000 stool samples were collected from out-patients who visited the KATH parasitology laboratory between May and October, 2008 to do stool routine examination. Each stool sample was processed within 2 hours after collection using the direct saline wet mount, Kato-Katz and formol-ether concentration methods in accordance with standard protocols.

All hookworm positive stool samples were cultured using the modified Harada-Mori test-tube technique and the isolated hookworm filariform larvae were identified by their morphological features in accordance with established criteria.

Formol-ether concentration, gave the highest overall prevalence of 11.1% of helminth parasites made up of hookworm (2.9%), *Dicrocoelium dendriticum* (2.1%), *Strongyloides stercoralis* (2.1%), *Schistosoma mansoni* (1.8%), *Hymenolepis nana* (1.4%), *Taenia species* (0.6%) and *Trichuris trichiura* (0.1%). The direct wet mount and Kato-Katz detected total prevalence of 3.2% and 5.1% respectively. Direct wet mount was found to be 29.3% sensitive.

Kato-Katz showed good agreement with the ether concentration for detection of hookworms, *T. trichiura* and *S. mansoni* infections (99.1% sensitivity; 95.0% CI, 97.1%-100%, with positive and negative predictive values of 100% (95% CI, 100%-100%) and 99.5% (95% CI, 99.0%-100%), respectively.

Interestingly, no *Ascaris lumbricoides* eggs were detected using any of the three methods employed. EPG (eggs per gram) of feces counted by Kato-Katz method showed that most patients (89.9%) harboured low intensity infections of the parasites found.

All 58 (100%) hookworm positive cases were identified as *Necator americanus.*

This study reconfirmed reports that the direct wet mount has low sensitivity.

It is recommended that stool samples that are negative for parasites by the wet mount method should be re-examined using the formol-ether concentration technique as the confirmatory test. This approach will improve the detection of helminths from stool specimens for accurate diagnosis of intestinal helminth infections, for effective management of patients and ultimately improve the quality of life of individuals in the communities.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Intestinal helminth infections are among the most prevalent and widespread of chronic human infections worldwide (Stoll, 1999). Worldwide, an estimated 1.4 billion humans have ascariasis, 1.2 billion have hookworm infections, 1 billion have trichuriasis, and 200–300 million have schistosomiasis (de Silva *et al.*, 2003; Brooker *et al.*, 2004), with the greatest public health burden occurring in developing countries, particularly in sub-Saharan Africa (Bethony et al., 2006; Van der Werf et al., 2003). Majority of these helminthic infections result from low standard of living, poor socioeconomic status, poor personal hygiene, and poor environmental sanitation (Oduntan, 1974; Bundy and Gutatt, 1996; Hasegawa et al, 1992; Feachem et al., 1983).

Prevalences of helminthic infections exceeding 70% have been reported in some equatorial and tropical communities of West Africa (Brooker et al., 2000). In Ghana, Annan et al. (1986) have reported up to 63% infections among school age children.

Over the years, very low prevalences of 2-3% of helminth infections have been reported at the KATH parasitology laboratory (KATH parasitology records from 1998-2007). The situation could be attributed to low parasite burden resulting from antihelminthic usage, or lack of sensitivity of the wet mount method (Estevez and Levine, 1985) which still remains the main diagnostic tool employed by most hospital laboratories in the developing world (Wirkom et al., 2007).

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 professionalism or technical skill involved (NCCLS, 1997; Melvin and Brooke, 1985; WHO, 2000).

Stool microscopy using direct wet mounts, formol-ether concentration and the Kato-Katz technique offers many relative advantages over other diagnostic methods for detecting intestinal parasites (Parija and Srinivasa, 1999; Watson et al., 1988; Bogoch et al., 2006). 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).
The Kato-Katz technique developed by Kato and Miura (1954) and Katz et al. (1972) allows enumeration of the number of eggs per gram (epg) of faeces thereby providing an indirect evaluation of the intensity of infection and assessment of worm burden (Beaver, 1950; Martin and Beaver, 1968; WHO, 1998).

It is well established that the direct wet mount technique lacks sensitivity but most hospital laboratories in developing countries rely on this method for routine stool examinations as a result of its affordability and simplicity (Smith and Bartlett, 1991; Ahmadi et al., 2007; Ebrahim et al., 1997; Ukaga et al., 2002; Wirkom et al., 2007).

1.2 Routine stool examination at the KATH

Like most hospital laboratories in Ghana, the KATH parasitology laboratory perform routine stool examinations by the direct wet mount technique only (KATH parasitology, SOPs- Stool R/E, 2007). Direct wet mount technique is simple, rapid and inexpensive, but it can miss low intensity infections or if too much debris or fat is present in the preparation (Akujobi et al., 2005). The consequences of misdiagnosis can be grave (Barnabas and Aboi, 2005). Therefore, there is the need for more accurate diagnosis of intestinal helminth infections at the KATH parasitology laboratory which functions as a teaching hospital laboratory.

Over the past decade, hookworm has been the most prevalent helminth parasite reported at the KATH (KATH parasitology data from 1998 to 2007). Recognizing the serious clinical and public health significance of hookworm infections (Chan et al., 1994; Crompton, 2000; Hotez et al., 2005), further focus on it is desirable.

1.3 Hookworm infections and methods of differentiating human hookworm species

The prevalence of the human hookworms, *Ancylostoma duodenale* and *Necator americanus*, alone approaches 740 million, with the foci predominantly within Asia, sub-Saharan Africa, and Latin America (de Silva et al., 2003). Hookworm infection causes considerable medical and public health problems (Hotez and Pritchard 1995; Crompton 2000). The two hookworm species, *N. americanus* and *A. duodenale* produce morphologically identical eggs which are practically indistinguishable (Garcia, 1999, 2001). Traditionally they are reported as hookworm and have been considered identical for treatment purposes (Pillai and Kain, 2003).

There are established differences in the life cycles (Chan et al., 1997; Yu *et al*., 1995), clinical presentations (Stoltzfus et al., 1997), pathogenicity and egg-laying capacities (Beaver et al., 1984; Pawlowski et al, 1991) of *Necator americanus* and *Ancylostoma duodenale*. It is important both for epidemiological and clinical purposes to establish the predominant hookworm species in endemic communities (Brooker et al., 2004).

The two hookworm species can be differentiated through coprocultural techniques (Jozefzoon and Oostburg, 1994; Arcari et al., 2000), of which the modified Harada-Mori test-tube method (Harada and Mori, 1955; Kitvatanachai and Pipitgool, 1999) is most highly recommended. The reared larvae can then be differentiated using distinct morphological features as described by Little (1966).

1.4 Justification for the study

In hospital settings, prompt and accurate diagnosis of infection is critical for guiding clinical management of patients (Isenberg, 1998). The low prevalences of parasites detected at the KATH parasitology laboratory call for better diagnostic method. It is therefore necessary to evaluate the diagnostic sensitivities of the direct wet mount, formol-ether concentration and the Kato-Katz methods. The study will allow true prevalence of helminth parasites reporting at the KATH to be determined.

In the last ten years hookworm has been the most prevalent helminth parasite recovered from stools submitted to the KATH parasitology laboratory (KATH parasitology records from 1998-2007), but there are no published reports on the relative prevalence of

A. duodenale and *N. americanus* reporting at the KATH. It is well reported that *A. duodenale* is associated with greater intestinal blood loss; ingesting 0.15 ml per worm per day compared with about 0.03 ml of blood ingested by *N. americanus*, thus causing severer iron-deficiency anaemia (Albonico et al., 1998; Stoltzfus et al., 1997), hence will require radical treatment and blood analysis for haemoglobin and other red cell indices. It is therefore important to culture all hookworm positive stool samples found in this study to allow identification of the hookworm species.

The study will provide useful data that will inform health policy makers, particularly KATH management, to modify the algorithm for routine stool examinations in hospital laboratories for accurate diagnosis of intestinal parasitic infections.

1.5 Research questions

1. By using the direct wet mount alone, is the KATH parasitology laboratory accurately diagnosing intestinal helminthic infections; compared with other alternative diagnostic methods?
2. What is the 'true' prevalence of the common helminth parasites reporting at the KATH?
3. What proportion of patients reporting at KATH have dewormed in the last three months, and whether dewormer usage affect the wet mount recovery of intestinal helminth parasites in stool samples?
4. What is the parasite burden/load (severity of infection) in infected individuals?
5. What is the relative prevalence of *N. americanus* and *A. duodenale* at the KATH?

6. Will public health be improved if more sensitive diagnostic technique(s) is/are used for stool routine examination at the KATH laboratory?

1.6 Aim and Objectives of the study

Main Aim:

To assess the accuracy of direct wet mount method for routine diagnosis of intestinal helminth parasites, and also determine the relative prevalence of the human hookworm species, *Necator americanus* and *Ancylostoma duodenale*, reporting at the KATH.

Specific objectives:

1. To determine the overall prevalences of intestinal helminth parasites at the KATH, by three different diagnostic methods: the direct saline wet mount, Kato–Katz and formol-ether concentration techniques.

2. To determine the accuracy/sensitivity of wet mount method for diagnosing intestinal helminth parasites, using the formol-ether concentration technique as the diagnostic "gold standard" test.

3. To determine the frequency of antihelminthic usage by patients through a questionnaire survey.

4. To determine the parasite burden (intensity of infection) in affected patients, using the Kato Katz technique.

5. To determine the proportion of hookworm infections represented by *N. americanus* and *A. duodenale* reporting at KATH, using the Harada-Mori test tube culture technique.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Burden of intestinal helminth parasites

Helminth infections are important causes of morbidity and mortality throughout the world, particularly in undeveloped countries and in persons with comorbidities (Chan et al, 1994; De Silva et al., 1997; Guyatt and Bundy, 1991; Anderson and May, 1991). There are about 20 major helminth infections of humans, affecting more than one third of the world's population (Awasthi et al., 2003). The major soil-transmitted helminth (STH) infections, namely ascariasis or roundworm infection (*A. lumbricoides*), trichuriasis or whipworm infection (*T. trichiura*), and hookworms (*N. americanus* and *A. duodenale*) and infection caused by schistosomes account for most of the global helminth disease burden (Bethony et al., 2006; Hotez et al., 2006; De Silva et al., 2003).

Table 1. Major Soil-Transmitted Helminths (De Silva et al., 2003)

Parasite	**Disease**	**Prevalence**
Ascaris lumbricoides	Common roundworm infection, (ascariasis)	800 million to 1.4 billion
Trichuris trichiura	Whipworm infection, (trichuriasis)	600 million to 1 billion
Necator americanus and *Ancylostoma duodenale*	Hookworm infection	580 million to 1.2 billion
Strongyloides stercoralis	Threadworm infection, (strongyloidiasis)	30-300 million
Enterobius vermicularis	Pinworm infection	4 -28% of children

The global picture (Figure 1) of the STHs shows widespread prevalences throughout sub-Saharan Africa, the Americas, China and east Asia (De Silva et al., 2003). An estimated 4.5 billion individuals are at risk of the STHs, 2 billion are infected and more than 135,000 deaths occur worldwide every year (De Silva et al., 2003).

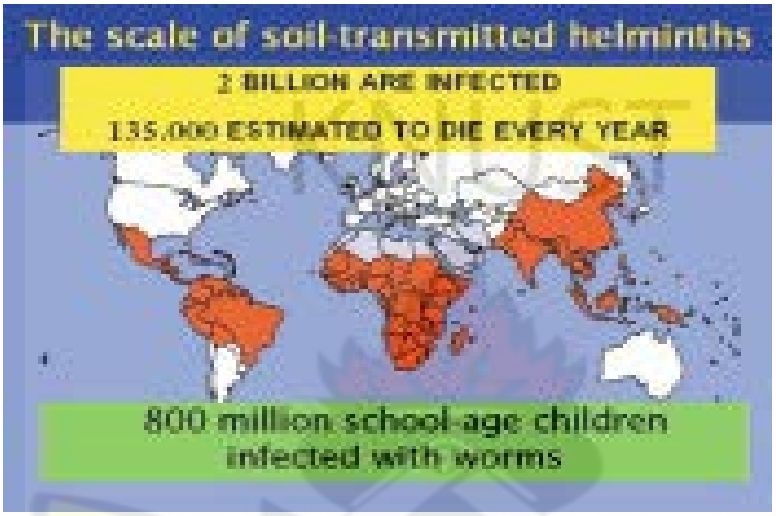

Figure 1: Global picture of intestinal helminth parasites (De Silva et al., 2003)

2.1.1 Epidemiological patterns of helminthic infections

The soil-transmitted helminth (STH) infections are common throughout communities of poor and socioeconomically deprived people of the tropics and subtropics (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 helminth infections (Crompton, and Stephenson, 1990).

Children as young as 6 months can be infected with helminths (Goodman et al,. 2007) which influence their nutritional status to cause growth retardation (Adams et al., 1994) and reduce learning ability with increased absenteeism from school (Beasley et al., 2000; Nokes et al., 1992).

For *A. lumbricoides* and *T. trichiura* infections, convex age-intensity profiles, in which the incidence peaks in childhood, are observed (Woolhouse, 1988). Infection prevalences and intensities rise with increasing age to a peak around 5-10 years old and decline in adults (Bachta et al., 1990; Bundy, 1990).

Unlike other STHs, hookworm infection appears to increases throughout childhood until it reaches a plateau in adulthood with highest prevalence occurring among the middle-aged or even individuals over the age of 60 years (Hotez, 2001; Behnke et al., 2006; Brooker et al., 2004).

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, helminth 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 prevalences of *A. lumbricoides* and *T. trichiura* show no significant difference between males and females (Guyatt and Bundy, 1991).

2.1.2 Clinical indications of intestinal helminth infections

Majority of intestinal helminth infections are chronic and mild, and are usually asymptomatic or 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 1-2 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 Löeffler or Löeffler-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 (Coombs and Crompton, 1991).

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

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 (Bahu et al., 2001; Khuroo, 2001).

In heavy trichuriasis, infected people may show mild anemia, eosinophilia, bloody diarrhea (classic Trichuris dysentery syndrome, or chronic Trichuris colitis), 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 exertional dyspnea (Stoltzfus et al., 1997; Albonico et al., 1998).

Strongyloides stercoralis infection produces burning or colicky abdominal pain, often epigastric, and is associated with diarrhea and the passage of mucus (Oduntan, 1974; Udonsi, 1984; Adams et al., 1994; Drake et al., 2000; Stephenson et al., 2000).

Non-specific clinical manifestations include restlessness, irritability, anorexia, chronic protein energy malnutrition, malabsorption, anasarca and weight loss (Spurchler, 1987).

2.2 Overview of common helminth infections

Intestinal helminth infections that remain prevalent in sub-Saharan Africa, Ghana in particular, include ascariasis, trichuriasis, strongyloidiasis, taeniasis, intestinal schistosomiasis; infection caused by *Schistosoma mansoni* and hookworm infections (Brooker et al., 2000; Feacham et al., 1983; PCD, 1998; Annan et al., 1986).

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

Available data at the KATH parasitology laboratory have noted significant increases in the prevalence of *Dicrocoelium* infections in recent years (KATH parasitology records from 1998-2007).

2.2.1 *Ascaris lumbricoides*

Ascaris lumbricoides is the most common and important soil transmitted helminth (Cheesbrough, 2005). This parasite is cosmopolitan and its distribution is largely determined by local habits in the disposal of faeces, because its eggs reach the soil in human faeces and so contaminate the human environment (Kightlinger et al., 1998). The parasite is one of the major public health problems (O'Lorcain and Holland 2000; Crompton and Savioli, 2007) in communities where the prevailing social environment is characterized by poverty, poor housing, inadequate sanitary practices and overcrowding (Chan, 1997; Chan et al., 1994; Pearson, 2000).

Prevalence of ascariasis worldwide is estimated to be more than 1.3 billion, approximately one - quarter of the world population (Crompton, 1999), and over 250 million suffer from associated morbidity (Crompton and Savioli, 2007).
It is estimated that 204 million cases of ascariasis occur elsewhere in East Asia and the Pacific, 140 million in India, 97 million elsewhere in South Asia, 84 million in Latin America and the Caribbean, 23 million in the Middle East and North Africa and 173 million in sub-Saharan Africa (Pearson, 2002; De Silva et al, 1997, 2003; Peters and Pasvol, 2005).

A. lumbricoides have been shown to play a significant role in childhood 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 (Adams et al., 1994; Drake et al., 2000; O'Lorcain and Holland, 2000).

Study by Oduntan (1974) on the health of Nigerian school children indicated that Ascariasis is most common and intensive in school children aged 6-15 years (Kightlinger et al., 1998).

Surprisingly, no *A. lumbricoides* infection has been reported at the KATH parasitology laboratory since 2006 (KATH parasitology records from 1998-2007).

A recent survey of prevalence of helminthic infections among pupils in Ayigya primary school in Kumasi, Ghana showed prevalence rate of 0% for ascariasis (Tay and Gyampomah, unpublished data).

2.2.2 *Trichuris trichiura*

Infection with *T. trichiura* (trichuriasis), is the third most common helminth infections of humans (Peters and Pasvol, 2005). Adult *Trichuris trichiura* are approximately 40-50 mm in length, the posterior end is thick and the anterior 2/3 of the body is slender, giving a "whiplike" shape to the worm; hence the name whipworm (Neva and Brown, 1994).

Trichuriasis afflicts about 1 billion people throughout the world (Pearson, 2002). It is spread via fecal-oral transmission and high prevalences occur in areas with tropical weather and poor sanitation practices (Bethony et al., 2006). Children are especially vulnerable because of their high exposure risk (Matsubayashi et al., 1965).

Infection rates of up to 75% were found in young schoolchildren in Nigeria (Oduntan, 1974). Tay and Gyampomah (unpublished data) found only about 1% prevalence of trichuriasis among schoolchildren at a suburb in Kumasi, Ghana.

Like *A. lumbricoides*, *T. trichiura* has not been detected in the KATH laboratory in recent years (KATH parasitology records from 1998-2007).

2.2.3 *Strongyloides stercoralis*

Worldwide, an estimated 30–100 million people are infected with *Strongyloides stercoralis.* Strongyloidiasis is endemic in tropical and subtropical countries and more frequently found in rural areas, institutional settings, and lower socioeconomic groups (Pearson, 2002).

Strongyloides is important for its ability to autoinfect and disseminate throughout the organ systems to cause life-threatening infection (hyperinfection syndrome, disseminated strongyloidiasis) in immunosuppressed host (Vadlamudi and Krishnaswamy, 2006).

Human infection is acquired via penetration of intact skin by filariform larvae during contact with contaminated soil or other material contaminated with human feces (Cheesbrough, 2005).

Severe strongyloidiasis carries a high mortality rate (up to 80%) because the diagnosis is often delayed (Vadlamudi and Krishnaswamy, 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.

Strongyloidiasis was the second most prevalent helminthic infection found in schoolchildren in Ayigya, in Kumasi (Tay and Gyampomah, unpublished data). Significantly high rates of *Strongyloides stercoralis* infections have been found among patients who reported at the KATH (KATH parasitology records from 1998-2007).

2.2.4 *Schistosoma mansoni*

The global burden of disease due to *Schistosoma mansoni* is mostly in Africa, though some infections occur in parts of South America, the Caribbean, and the Middle East as well (Michaud et al., 2003; Cheesbough, 2005, Sleil et al., 1985).

Schistosomes have a snail intermediate host, and human contact with water is necessary for infection (WHO, 2002; Hotez et al., 2006).

The adult worms of *S. mansoni* live in the small blood vessels associated with the liver and intestine, and cause serious pathology, morbidity and even death in individuals with heavy, chronic infections (Van der Werf et al., 2003; Chan et al., 1994). They produce eggs that typically have a lateral spine, non-operculated and contain a larva called miracidium, which are discharged in the faeces (Cheesbrough, 2005; WHO, 1994).

De Vlas and Gryseels (1992) indicated that prevalences of *Schistosoma mansoni* are underestimated, as a result of missed diagnosis (Barnabas and Aboi, 2005).

Compared *to S. haematobium* infections, *S. mansoni* infections are relatively low in most communities of West Africa (Tahir, 2002; Booth et al., 2003; Hotez et al., 2006).

Data at the KATH have indicated prevalences of <1% (KATH parasitology records from 1998-2007).

2.2.5 *Taenia species (T. saginata* and *T. solium)*

Taenia saginata (beef tapeworm) and *Taenia solium* (pork tapeworm) cause taeniasis (Cheesbrough, 2005). Humans are the only definitive hosts for *Taenia species* and become infected by ingesting raw or undercooked infected beef or pork (Ukaga et al., 2002). Both species are worldwide in distribution with *T. solium,* more prevalent in poorer communities where humans live in close contact with pigs and eat undercooked pork (Cox, 1998). The adult tapeworms attach to the small intestine by their scolex and reside in the small intestine. The proglottids which mature become gravid, and detach from the tapeworm, and migrate to the anus (Spurchler, 1987). Gravid proglottids and eggs appear in faeces.

The eggs of the two species are morphologically identical; they are round to oval in shape with a thick, radially-striated shell, and contain a six-hooked embryo called oncosphere (Smith and Bartlett, 1991; Ash and Orihel, 1991).

Studies on intestinal helminthic infections in Ghana have indicated relatively low prevalence of Taenia species infection (Annan et al., 1986; PCD, 1998). Data at the KATH also show very low prevalence of taeniasis among patients (KATH parasitology records from 1998-2007).

2.2.6 *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 lifecycle of *H. 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 *Taenia* infections, prevalence of *H. nana* infection in most communities in Ghana have been relatively low (Annan et al., 1986; PCD, 1998).

KATH parasitology records from 1998-2007 have indicated significantly low prevalence of hymenolepiasis among patients.

2.2.7 *Dicrocoelium species*

Dicrocoeliasis, also known as "small liver fluke" disease is caused by *Dicrocoelium dendriticum* and *D. 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 dendriticum* is found throughout Europe, the Middle East, and Asia (Manga-Gonzalez et al., 2001), *Dicrocoelium hospes* is distributed in Ghana, Nigeria, Chad, Togo, Central African republic, Cameroon, Niger, Sierra Leone, Senegal and several sub-saharan savanna 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 oxen and small ruminants in Niger harbour *D. hospes* infection (Manga-Gonzalez et al., 2001). Human infections are generally rare and

are attributed to accidental ingestion of either infected ants or raw or undercooked animal liver (Crompton, 1999).

The presence of *Dicrocoelium* sp. 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).

At the KATH, available data have shown increasing rates of incidence of dicrocoeliasis among patients, particularly in the last two years (KATH parasitology records from 1998-2007).

2.2.8 Hookworm Infections

Despite considerable advances in chemotherapy and control, hookworms rank amongst the most widespread of soil-transmitted intestinal helminth parasites (Chan et al. 1994). An estimated 1.2 billion people worldwide have been infected (Chan 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 prevalences of human hookworm infections in Kenya (Adams et al., 1994), Nigeria (Adenusi, 1997), Mali (Behnke et al., 2000), Côte d'Ivoire (Booth et al., 2003), Zambia (Hira and Patel, 1984) and Ghana (Annan et al., 1986; de Gruijter et al., 2005).

Tay and Gyampomah (unpublished data) noted significantly high prevalence of hookworm infection among schoolchildren in Ayigya community in Kumasi, Ghana.

Available data at the KATH have indicated hookworm infection is the most prevalent helminth among patients that reported at the KATH over the past decade (KATH parasitology records from 1998-2007).

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).

It is reported that *Ancylostoma duodenale* (ancylostomiasis) inhabit cooler, drier climates, while *Necator americanus* (necatoriasis) tends to thrive in more tropical climates (Schad and Banwell, 1984).

Whilst *A. duodenale* is more geographically restricted, *Necator americanus* has global distribution and is the dominant species in most parts of Nigeria (Adenusi, 1997; Overinde, 1978), Togo (Blotkamp et al., 1993; de Gruijter, 2005), Mali (Behnke et al., 2000) and Ghana (Yelifari et al., 2005).

2.2.8.1 Public health and Clinical significance of *A. duodenale* and *N. americanus*

Significant differences in the life histories (Chan et al., 1997; Hoagland and Schad, 1978; Yu *et al*., 1995), clinical presentations (Stoltzfus et al., 1997; Hotez and Pritchard, 1995; Udonsi, 1984), pathogenicity and egg-laying capacities (Beaver et al., 1984; Pawlowski et al, 1991; Albonico et al., 1998) of *Necator americanus* and *Ancylostoma duodenale*, have been described.

Knowledge of the predominant hookworm species in an endemic area is important in both epidemiological and clinical studies (Brooker et al., 2004). The adult worms of *Ancylostoma* and *Necator* are easily identified on the basis of presence of teeth or cutting plates, respectively, around the buccal capsule (Hotez, 1995).

Whilst *Necator* can live in the human intestine from 3-10 years with a maximum life expectancy of 18 years (Beaver, 1988), *Ancylostoma* survives only 1-3 years (Hoagland and Schad, 1978).

The adult male worm of *N. americanus* measures 7-9 mm; the female worm measures 9-11 mm and lays 3000-6000 eggs per day (Hoagland and Schad, 1978). *A. duodenale* is the larger of the 2 species, with male worms measuring 8-11 mm and adult female worms measuring 10-13 mm and lays 10,000-30,000 eggs per day (Hoagland and Schad, 1978).

It is reported that a single *A. duodenale* ingests about 150µl (0.15 ml) of human blood daily while one *N. americanus* ingest about 30µl (0.03 ml) of blood within the same period (Stoltzfus et al., 1997).

The two species have been known to show differences in susceptibility to the same anthelminthic drug and dosage regimen (Rim et al., 1971; Reynoldson et al., 1997; Keiser and Utzinger, 2006).

N. americanus infection is acquired almost exclusively by the percutaneous route (i.e. active penetration of the skin), but *A. duodenale* is able to infect both percutaneously and by the oral route (Looss, 1911; Markell et al., 1999).

A. duodenale has the unique ability to undergo arrested development in humans (Schad *et al*., 1973). After infecting a host, the L3s of *A. duodenale* can temporarily abort maturation and enter an arrested state (hypobiosis) within the host's somatic tissues, and

become reactivated in response to host physiological changes such as pregnancy (Schad, 1990). This phenomenon accounts for infantile ancylostomiasis; with transmission occurring either transplacentally or through maternal milk in lactogenic transmission (Yu et al., 1995, Nwosu, 1981, Chan et al, 1997).

2.2.8.2 Methods of differentiating human hookworm species

Though eggs of the two human hookworm species are morphologically similar and practically indistinguishable (Garcia, 1999, 2001; Ukaga et al., 2002), particular species can be identified by immunological methods such as the ELISA (Zhang et al., 1990), genetic marker (DNA) and ribosomal (RNA) methods based on PCR (Hawdon, 1996; Romstad et al., 1997; Monti et al., 1998; Gasser et al., 2006). But molecular diagnostic approach is costly and not affordable in developing countries (Gasser, 2001).

Traditionally the two hookworm species have been differentiated through coprocultural techniques (Arcari et al., 2000), of which the Harada-Mori test-tube method is most highly recommended.

Egg development and hatching hookworm larvae depend on such factors as temperature, adequate shade and moisture (Smith and Schad, 1990). The larvae of *N. americanus* are susceptible to cold and may fail to develop after refrigeration (Smith and Schad, 1990). Therefore, faecal specimens to be cultured should not be refrigerated (Beaver et al., 1984).

The optimal temperature for development is 20-30°C, with the highest cumulative hatching rates obtained at 30 °C (Udonsi and Atata, 1987). Above temperatures of 35-40°C, development of eggs is arrested and death occurs (Smith and Schad, 1990).

The filariform (L3) larvae of *A. duodenale* and *N. americanus* are known to display contrasting morphology, especially at the head, gut and tail regions, when viewed under the microscope (Wu and Peng, 1965; Yoshida, 1966; Hira and Patel, 1984).

Detailed morphological characteristics of filariform (L3) larvae of hookworm and hookworm-like nematodes have been described by Little (1966) and Yoshida (1966).

Table 2: Differentiation of infective larvae found in faecal cultures

Feature	***Strongyloides***	***Trichostrongylus***	***Ancylostoma***	***Necator***
Length	500 microns	750 microns	660 microns	590 microns
Sheath	No Sheath	Sheath	Sheath 720 microns; striations not clear	Sheath 660 microns; striations clear at tail end
Oesophagus	½ body length	¼ body length	¼ body length	¼ body length
Intestine	Straight	Intestinal lumen zig zagged	Anterior end is narrower in diameter than oesophageal bulb; no gap between the oesophagus and the intestine	Anterior end is as wide as the oesophageal bulb; gap between the oesophagus and the intestine
Tail	Divided into three at the tip	End of tail knob-like	Blunt	Sharply pointed
Head			Blunt	Rounded
Mouth			Mouth spears not very clear and divergent	Mouth spears clear and divergent

2.3 Laboratory Procedures for the Diagnosis of Intestinal Helminths

Definitive diagnosis of helminth infections depends on demonstration of a stage of the parasite's life cycle in the human host (Garcia, 1999, 200). 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 samples (Parija and Srinivasa, 1999; NCCLS, 1997, 2002b). A wide variety of laboratory methods, including parasitologic, molecular, serologic and cultural approaches, have been developed over the years for diagnosis of intestinal parasites (Markell et al., 1999).

2.3.1 Visual observation of nature of stool sample (Macroscopic examination)

Helminthic infections can induce digestive abnormalities and influence the nature or consistency of stool produced (Garcia, 1999, 2001).

The macroscopic appearance of stool specimen can give a clue to the type of organisms present (Ash and Orihel, 1991; Goodman et al., 1999; Parija and Srinivasa, 1999). Adult worms of *Ascaris*, *Enterobius* and tapeworm proglottids may be seen when fresh specimen is visually examined (Garcia, 1999, 2001). Fecal specimens are described as formed, semiformed, soft, loose, or watery (Beaver et al., 1984).

2.3.2 Parasitological methods (Stool microscopy)

Microscopic or parasitologic diagnosis is generally sensitive, simple, and economical (Parija and Srinivasa, 1999). If performed correctly, stool microscopy offers many

advantages over other diagnostic methods for detecting intestinal parasites (Watson et al., 1988; Bogoch et al., 2006). Diagnostic tests involving microscopy include direct wet preparations, concentration methods and the Kato-Katz technique (Markell and Voge, 1976; Watson et al., 1988).

2.3.2.1 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, 1999, 2001; Isenberg, 1998).

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

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 techniques (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; Garcia, 1999).

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

Akujobi et al (2005) and Bogoch et al (2006) 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 be detected if the preparations are not examined quickly after preparation (WHO, 1991).

2.3.2.2 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 flotation (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 some 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, flotation gives a “cleaner” preparation than sedimentation yet each has a preference over another in certain aspects (Ukaga et al., 2002; Truant et al., 1981; Cheesbrough, 2005).

Concentration by flotation utilizes a liquid suspending medium heavier than the parasite objects so that they float and can be recovered from the surface film.

The floating medium generally employed include brine (i.e., saturated aqueous solution of sodium chloride), and zinc sulfate solution having a specific gravity of approximately 1.20 and 1.18 respectively (Garcia, 1999, 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 helminths, *Strongyloides* larvae, and protozoan cysts become badly shrunken; sufficient to render the object undiagnosable (Ukaga 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 Ritchie (1948), and Allen and Ridley (1970) provide the best diagnostic outcome in epidemiological studies (Akujobi, 2005). The technique requires the use of formalin as a fixative and ether (Allen and Ridley, 1970) or ethyl acetate (Young et al., 1979) or gasoline (WHO, 1991; Wirkom et al., 2007) or hemo-de (Knight et al., 1976) as a lipid removing agent.

It uses formalin to fix and preserve the faecal specimen and ether or ethyl acetate to extract debris and fat from the faeces, 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, 1999, 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, 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 the disadvantage of destroying trophozoites stages and distorting cellular exudates and liquid stools do not concentrate well (Ash and Orihel, 1991).

Because concentration procedures 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).

2.3.2.3 Kato-Katz technique

The Kato-Katz technique (Kato & 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 helminth 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; Ukaga 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 50 mg template has a hole of 9 mm on a 1 mm thick template; a 41.7 mg template has a hole of 6 mm on a 1.5 mm thick template; and a 20 mg template will have a hole of 6.5 mm on a 0.5 mm thick template (Ebrahim et al., 1997).

The cellophane coverslip, 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 1 ml of 3% malachite green (Markell and Voge, 1976; Garcia, 1999, 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 WHO (1998) had recommended the use of a 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 diarrhoeal specimens.

Limitations of this method include difficulty in processing diarrhoeal stools (Siegel et al., 1990), lack of sensitivity, if only a single stool sample is examined (Booth et al., 2003). Counting of eggs in Kato-Katz smears can be a tedious and time consuming process, and can lead to technical errors (Kato and Miura, 1954; Ebrahim et al., 1997).

Other drawbacks 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 (Garcia, 1999). 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).

2.3.2.4 Serological (Immuno-diagnostic) methods

There are increasing availabilities 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 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 serodiagnositic approach is that tests might remain positive even after cure by chemotherapy (Knopp et al., 2006).

2.3.2.5 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 *A. duodenale* and *N. americanus* (de Gruijter et al., 2005).

PCR 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 *Oesophagostomum biurcum*, 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 world (Valli et al., 1997; Hotez et al., 2006).

2.3.2.6 Cultural 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 (Garcia, 1999), 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).

2.3.2.6.1 The Baermann technique

The Baermann technique relies on the principle that active larvae will migrate out of a fecal 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 following the results of anthelminthic 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 this 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 adapt 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).

2.3.2.6.2 The agar plate culture

The agar plate culture has been found to be most useful for the diagnosis of *Strongyloides stercoralis* infection (Koga et al., 1990, 1991, 1992). In a study that looked at the prevalence of *S. stercoralis*, the diagnostic efficacy of the agar plate culture method was found to be as 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 dissecting microscope to examine the plate for the presence of tracks (Garcia, 2001). The procedure is relatively expensive and difficult to carry out (Garcia, 2001).

2.3.2.6.3 Charcoal cultures

Charcoal cultures are made by thoroughly mixing 1 part of soft or softened (with water) faeces with 5 to 10 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 (i.e., charcoal), as well as daily observations for adequate moisture (Garcia, 2001).

2.3.2.6.4 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* from *Ancylostoma* infections. Sasa et al. (1958) and Hsieh (1961) have noted that the modified Harada-Mori culture technique has several advantages over other methods for cultivating hookworm larvae.

2.3.2.6.4.1 Principles of the modified Harada-Mori culture technique

The technique employs a filter paper to which fecal material is smeared and inserted into a test tube containing about 3 ml distilled water (Sasa et al., 1958; Hsieh, 1961).

The capillary flow of water upward through the paper provides sufficient moisture by continuous soaking of the filter paper and the faecal film (Sasa et al., 1958; Hsieh, 1961).

By the capillary flow, soluble elements of the faeces are carried to the top of the paper, where they are either volatilized or accumulate in a dark deposit (Garcia, 2001). Incubation under suitable conditions favors hatching of ova and further development of larvae (De Kaminsky, 1993).

Advantages of this method are that it does not require additional organic material like charcoal or agar, individual sample tubes can be incubated together in large numbers, require readily available inexpensive materials, has high detection rate for hookworm species, simple to perform, and safe discard of used filter papers (Harada and Mori, 1955; Beaver et al., 1964; Sasa et al., 1958; Hsieh, 1961).

More so, the experiment can be set up in any room, provided adequate precaution is taken to prevent human contact with faeces containing the ova of hookworm (Garcia, 2001; HHS, 1993).

A disadvantage of the Harada-Mori technique is that the fecal cultures must be monitored closely to prevent desiccation caused by evaporation (Garcia, 2001).

2.3.2.7 Choice of diagnostic method in routine parasitology laboratory

In hospital settings, prompt and accurate diagnosis of infection is critical for guiding clinical management of patients (Isenberg, 1998). The choice of a particular technique for routine use is influenced by its affordability (low cost), simplicity (ease of performance), sensitivity (effectiveness in detecting parasites in scanty numbers) and level of professionalism (technical skill) involved (NCCLS, 1997; Melvin and Brooke, 1985; WHO, 2000).

In developing countries, routine methods are chosen largely on account of their affordability and rapidity (i.e. cheap, easy to carry out, and non-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). For instance, many authors have indicated that the direct wet mount technique 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 laboratories in developing countries rely on it as the main diagnostic tool for routine stool examinations (Cheesbrough, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 THE STUDY AREA

The study was carried out at the parasitology laboratory of Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. KATH is the second largest tertiary and teaching hospital in Ghana. The institution in collaboration with the College of Health Sciences and the Kwame Nkrumah University of Science and Technology offers clinical training for undergraduate and post-graduate medical professionals (KATH Annual report, 2007). The geographical location of the hospital and commercial nature of Kumasi make the hospital accessible, and the preferred referral hospital for all areas that share boundaries with the Ashanti Region and beyond. The vision of the hospital as outlined by the management is to become a medical centre of excellence offering clinical and non-clinical services of the highest quality standards comparable to any international standards (KATH Annual report, 2007).

The clinical microbiology department of the diagnostics directorate has a separate parasitology laboratory, located at the polyclinic section of the hospital. The unit provides a broad range of diagnostic parasitology services (KATH parasitology, SOPs, 2008) for the hospital and serve as a reference laboratory for other primary health care facilities in the Ashanti region of Ghana. The KATH parasitology laboratory receives between 30 and 50 stool samples daily for routine examinations (KATH parasitology, Stool R/E record). The conventional algorithm for diagnosis of intestinal parasites at the KATH is based only on the direct wet mount technique (KATH parasitology, SOPs, 2008).

3.2 Materials for the study

Materials including equipment, reagents and other laboratory supplies used for the study have been outlined in Appendix 1.

3.3 Study population

A non-selective sample of 2000 consenting subjects were recruited over a period of six months, commencing from May to October, 2008, for this study. Any out-patient who reported at the KATH parasitology laboratory during the study period, with a request form from a clinician to do stool routine examination, was eligible for the study. However, only those who gave informed consent were selected. Stool samples that were delivered to the laboratory from the wards or in-patients were excluded from the study largely on account of difficulties in obtaining information that was critical for the study, and for hardly meeting the sample collection and inclusion criteria (Siegel et al., 1990; Morris et al., 1992).

3.4 Ethical issues

The study protocol were reviewed and approved by the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital, in Kumasi. Permission to undertake the study at the KATH parasitology laboratory was sought and granted by the hospital management and the heads of diagnostics directorate and department of clinical microbiology.

Adult participants and parents/guardians of children, less than 15 years of age, who were enrolled for the study gave informed consent after full explanation about the purpose and the techniques of the study was given.

3.5 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 10 grams of stool in the containers provided and to deliver them to the laboratory within 2 hours after collection (Booth et al., 2003). Clients who were unable to produce specimens same day in the hospital were allowed to send the specimen containers home and asked to bring freshly passed stool sample the following day. Each specimen was labelled with a study number, date and time specimen was collected, and time specimen was received. Clients who delivered inadequate stool specimens (about 10 grams 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).
A short questionnaire (Appendix 5) was administered to gather information on place of residence (community) and anthelminthic drug usage in the last three months preceding this study. Data on age, sex, and clinical indications/diagnosis for the stool examination were obtained from the laboratory request forms that had been filled out by the requesting clinician.

3.6 Laboratory procedures

3.6.1 Parasitologic examinations

Three parasitological methods were used for this study, namely the direct wet mount, Kato-Katz and formol-ether concentration techniques. Each specimen was first examined macroscopically and its consistency or nature was recorded as either formed (F), semi-formed (SF), semi-formed with blood (SB), 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 were received; none was preserved in the refrigerator or any preservative added prior to processing, as this would kill ova and hinder successful cultivation of hookworm larvae (Estevez and Levine, 1985; Smith and Schad, 1990). The test procedures were carried out in accordance with standard protocols as described by Garcia (1999, 2001) and the WHO (1991, 1994). The steps have been outlined in appendices 2, 3 and 4.

3.6.1.1 Choice of a "gold standard" method for the study

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

The technique has been mentioned as the "gold standard" method (Wiebe et al., 1999) for several clinical and epidemiological studies on intestinal parasites (Allen and Ridley, 1970; Akujobi et al., 2005; Ahmadi et al., 2007; NCCLS, 1997; Watson et al., 1988; Oguama and Ekwunife, 2007).

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

3.6.1.2 Using the Kato-Katz technique to determine infection intensity.

A commercial disposable Kato kit (HelmR test kits: from Brazil AK Industriae Commercio Ltd., Belo Horizonte, Brazil; containing a cardboard template- with a hole of 6 mm diameter and 1.0mm thick, nylon screen of mesh size 200 ųm, and hydrophilic cellophane strips of size 25x35 mm and 50 ųm thick) was used for this study.

Using an electronic weighing balance of 0.0001g sensitivity, the amount of sieved faecal sample delivered by the template (as descrbed in Appendix 3) was measured. Measurements of 20 sieved faecal samples were made, and the average amount was found to be 42 mg.

Figure 2: Photograph of Kato Katz materials

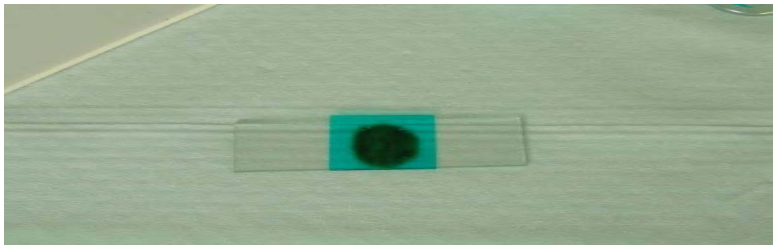

Figure 3: Photograph of Kato Katz thick smear

The number of eggs for each helminth species found in the Kato smear were counted and recorded separately. The eggs per gram (epg) of faeces was calculated as follows: if "n" number of eggs of parasite species are found in 42 mg of stool specimen, then 1000 mg (i.e. 1g) of the faecal specimen contains "n" X (1000/42) or ("n" X 24) epg.

Because the same batch of Kato kit was used for this study, the multiplication factor of 24 was used for all parasite ova detected by the Kato-Katz method. The "epg" values obtained were used to estimate the infection intensity of the parasites based on the classification developed by the WHO (2002) for the major soil-transmitted helminths and *S. mansoni* infections as represented in table 3.

Table 3: Classification of infection intensities by the Kato Katz (WHO, 2002)

Parasite	Light intensity	moderate intensity	Heavy intensity
A. lumbricoides	1-4,999 epg	5,000-49,999 epg	≥50,000 epg
T. trichiura	1- 999 epg	1,000- 9,999 epg	10,000 epg
Hookworms	1-1,999 epg	2,000- 3,999 epg	4,000 epg
S. mansoni	1-99 epg	100-399 epg	≥400 epg

3.6.1.3 Limitations of the Kato-Katz technique

Stool samples that were either loose or watery in nature could not be processed by the Kato-Katz method because of the limited usefulness and technical difficulties associated with analyzing diarrhoeal specimens by this technique (Siegel et al., 1990; Booth et al., 2003). As have been pointed out by Ebrahim et al. (1997), counting of eggs in the Kato smears was found to be a tedious and time consuming process.

3.6.2 Stool culture for diagnosis of Hookworm larvae

Stool samples which were found to contain hookworm ova by the ether concentration method, were cultured immediately by the Harada-Mori technique (Harada and Mori, 1955), using the modifications of Sasa et al. (1958) and Hsieh (1961) as follows:

3.6.2.1 Test procedure- modified Harada-Mori test-tube filter paper cultivation

A narrow strip (13 by 120 mm) of filter paper (Whatmann No. 3) was cut, and one end was trimmed to become slightly tapered. About 4 g of hookworm positive stool sample was placed on the middle portion of the filter paper and spread to make a thick smear of faeces (1 to 2 mm thick) on the middle third (one side only) of the strip of filter paper and inserted into a 15-ml conical-tip centrifuge; ensuring that the tapered end of the paper strip was near the bottom of the tube.

About 3 to 4 ml distilled water was added to the bottom of each tube so that the meniscus was about 5 mm below the fecal spot. The tubes were loosely covered with caps that had been punched creating holes in them to allow air into the culture system.

Each tube was labeled with the study number and date of culture (which was always the same day sample was collected).

The tubes were kept upright in a rack and incubated at room temperature (24^{o}- 28^{o}C) in the dark for a maximum period of 10 days (Figure 4). The volume of liquid in each tube was monitored daily and adjusted as needed, to maintain the original water level, for optimum humidity.

At the end of the incubation, the tubes were placed in a water bath at 50^{0}C for 15 minutes to kill the infective larvae. The tubes were uncovered and by means of forceps, the filter paper strips were discarded into a disinfectant, and the tubes centrifuged at 1000 rpm for 5 minutes to concentrate the larvae.

The supernatant was aspirated and a drop of Lugol's iodine was added to the sediment to allow easy identification of larval features.

Figure 4. Photograph of the modified Harada-Mori culture set up (source: the study)

3.6.2.2 Microscopic examination of larval features

Using a Pasteur pipette, a drop of the sediment was placed on a clean glass slide. A coverslip was placed and microscopically examined using the 10X objective (low power) for the presence of larvae. All larvae recovered were found to be sheathed. The length of each larva and sheath were measured by means of an ocular micrometer on a calibrated microscope and recorded. Typical morphological characteristics, especially in the head and tail regions were identified and confirmed under 40X objective (high dry power) lens, with the condenser iris sufficiently closed to give good contrast.

3.6.2.3 Identification of hookworm larvae

The details of larval features were compared with the morphologic characteristics of larval nematodes described by Wu and Peng (1965), Yoshida (1966) and Little (1966). The criteria presented in Table 2 were used for species identification of the cultured larvae. This allowed the hookworm larvae recovered in the study to be identified as *Necator americanus.*

3.7 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 (NCCLS, 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 biosafety precautions (NCCLS, 2002a, 2002b; HHS, 1993) were followed at all times. Waste was disposed off in accordance with infection control practices at the KATH.
4. For QC of the concentration method, preserved stool specimens known to contain parasite ova and larvae was included in each batch of samples to be concentrated, and for the faecal culture, known hookworm-positive and negative fresh stool samples were included in each batch of stool samples cultured. 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 this research was calibrated, and the objectives and oculars used for the calibration procedure was used for all measurements done with the microscope. The calibration factors for the 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 parasite ova and larvae from parasitological text were reviewed.

3.8 STATISTICAL ANALYSIS

The data obtained from 2000 samples were entered onto the statistical package of EPI-INFO (Dean et al., 1995). Analysis of the data was done using the chi-square (χ^2) test for categorical variables. Using the statistical software, SPSS version 13, the sensitivity, specificity, negative and positive predictive values (Hoehler, 2000) were determined to evaluate the performance of the three parasitological methods employed in the 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 categorical variable, when appropriate. Data on infection intensity of the main soil-transmitted helminths and *S. mansoni* was analyzed as a continuous variable, as well as a categorical variable, where groups consisted of light, moderate, and heavy infection as defined in the methods section.

Analytical outcomes were presented in Damy tables, histograms and pie charts.

3.8.1 Sensitivity

Sensitivity is the ability of a test to correctly identify 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 (Hoehler, 2000). High sensitivity allows accurate detection of disease, and therefore reduced incidence of misdiagnosis.

3.8.2 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 number of samples identified by the reference test as negative, expressed as a percentage. High specificity suggests that the test is good for "ruling out" disease (Hoehler, 2000).

3.8.3 Predictive values

Positive predictive value (PPV) expresses the proportion of persons with positive test who 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 negative test who do not have the disease condition. Both PPV and NPV can be calculated using the table (Hoehler, 2000) below:

Disease condition	**Test Outcome**	
	Positive	Negative
Present	**a**	**b**
Absent	**c**	**d**

They are calculated using the formula: PPV= a/(a+b) or true positive/all negative, and NPV=d/(d+c) or true negative/all negative.

3.8.4 Kappa value *(K)*

The kappa, *k* value is used to estimate the overall performance of a test (in this study, the direct wet mount) relative to the gold standard test, which in this study is the formol-ether concentration method. It can also be used to determine the combined correlation of sensitivity and specificity of a test against another test. Kappa values range between 0-1. If Kappa = 1, then there is perfect agreement. If Kappa = 0, then there is no agreement. The higher the *k* value, the stronger the agreement, and '*k*' values greater than 0.9 are considered excellent.

3.8.5 P-value

P-values provide 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 significance level is set at 0.05, any test resulting in a p-value under 0.05 would be significant. Therefore, the null hypothesis will be rejected in favour of the alternative hypothesis.

CHAPTER FOUR

4.0 RESULTS AND ANALYSIS

4.1 Demographic characteristics of studied patients

Out of the 2000 studied subjects 1,185 were females and 815 were males, representing 59.25% and 40.75%, respectively. Figure 5 shows the sex distribution of the studied patients.

Figure 5: Sex distribution of patients

Their ages ranged from 1 to 90 years, and the median age group was 27 years.

A total of 270 patients representing 13.5% of the studied population were children aged 1-15 years. The proportion of females to males among the children was approximately 1:1. The number of female patients was higher in all age groups except for age groups 16-20, 41-45 and over 60 years where males slightly outnumbered females. 1420 (71%) of the studied patients were aged between 16 and 40 years while the over 41 years constituted 13.5% of studied patients.

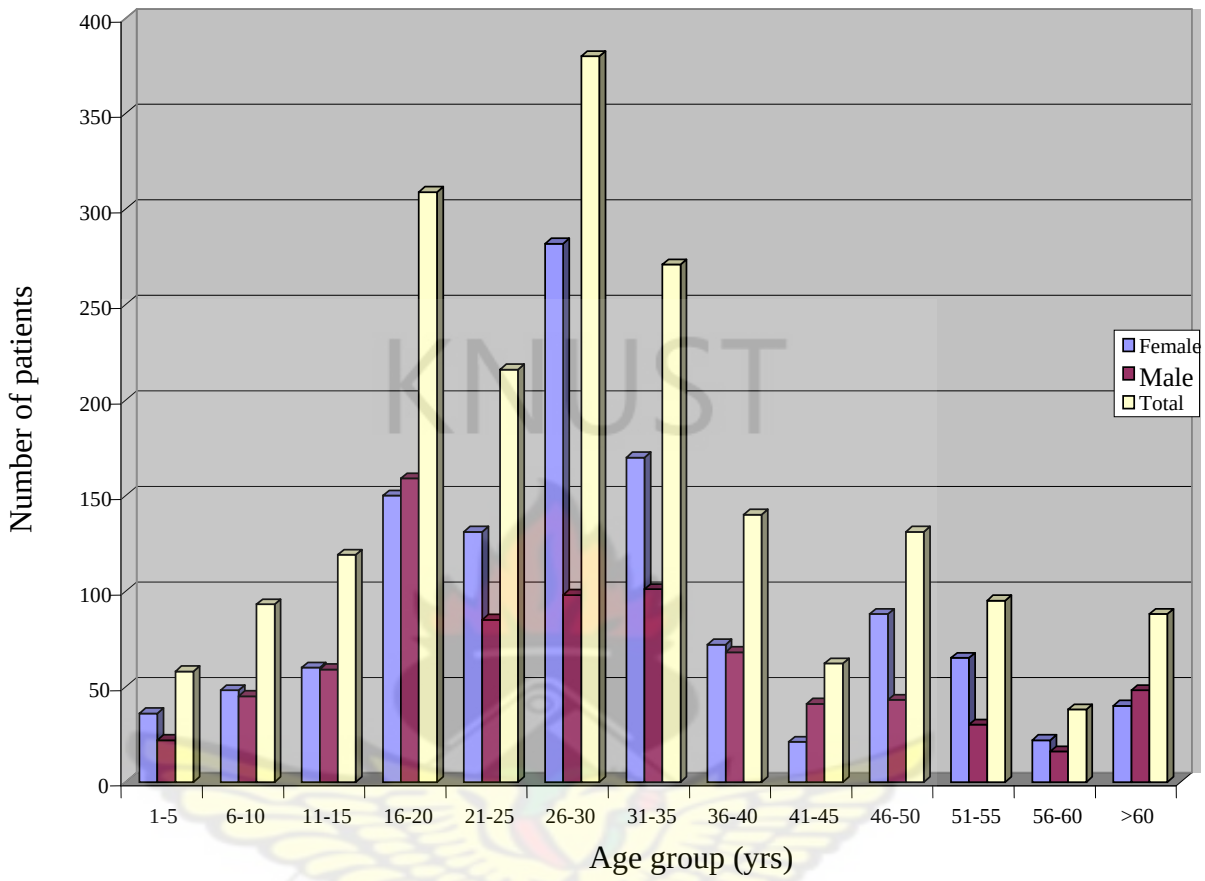

Figure 6. Distribution of patients by age and sex

4.2 Clinical diagnosis of patients

Table 4 represents the frequencies of clinical diagnoses for the studied patients. Most pregnant women, who performed stool examination as part of routine antenatal care services, had their laboratory request forms filled out as “cyesis” or “ANC” for clinical diagnosis.

In this study, the two clinical indications were put together as “ANC” (Table 4).

Table 4: Clinical diagnosis of patients as indicated on laboratory request form

Clinical diagnosis	Frequency	% frequency
ANC*	775	38.75
No diagnosis indicated	536	26.8
Anaemia	187	9.35
Med. Exams	112	5.6
Abdominal pain	83	4.15
Diarrhoea	77	3.85
Others†	71	3.55
Helminthiasis	56	2.8
Gastroenteritis	45	2.25
Dysentery	38	1.9
Nausea	20	1.0
Total cases	2000	100

ANC*- Antenatal care Others† -included urinary tract infection (UTI), malaria, chronic kidney disease (CKD), chronic liver disease (CLD), and enteric fever.

As shown in Table 4, "ANC" cases were the highest, accounting for 775 (38.75%) of the studied population. No clinical diagnosis was indicated for 536 (26.8%) patients. Whilst the clinical diagnosis for 506 (25.3%) patients correlated with known indications for intestinal helminth diseases, 71 (3.55%) 'Others' including urinary tract infections (UTI), malaria, chronic kidney disease (CKD), and enteric fever were not related to published manifestations of helminthiasis.

4.3 Nature of stool specimens (macroscopic examination)

Table 5 represents the relative frequencies of the types of consistency of 2000 stool samples collected from the studied patients.

Table 5: Relative frequencies of the types of consistency of the stool samples

Consistency type	Frequency	Frequency (%)
Semiformed (soft)	1514	75.7
Formed (hard)	327	16.35
Loose	101	5.05
Watery	19	0.95
Bloody-mucoid	18	0.9
Soft with blood	12	0.6
Soft with mucus	9	0.45
Total	**2000**	**100**

As shown in Table 5, most of the stool specimens (75.7%) were normal semiformed or soft in nature, 120 (6.0%) were diarrhoeal specimens (including 101 loose and 19 watery stools) and 39 (1.95%) samples contained blood and/or mucus.

4.3.1 Analysis of diarrhoeal stools

In this study, 120 diarrhoeal stools (Table 4) could not be processed by the Kato-Katz method due to technical challenges as explained under section 3.6.1.3. Results of microscopical examination of the samples by the formol-ether concentration and direct wet mount methods are given in Table 6.

Table 6. Results of analysis of diarrhoeal stools by the methods used

Helminth parasite	Formol-ether conc.	Direct Wet Mount	*Kato Katz
Present	2	0	-
Absent	118	200	-
TOTAL	**120**	**200**	**200**

* Specimen inappropriate for Kato-Katz method.

The two parasites identified by the ether concentration technique were *Taenia sp*. and *S. stercoralis*. The positive parasitologic findings involved only 0.1% (2/2000) of the studied population and 0.88% (2/226) of the total helminth parasites detected by the reference method (Table 7). As shown in Table 6, no helminth parasite was found in 200 diarrhoeal stools examined by the direct wet mount method.

4.4 Dewormer usage by patients

Overall, 620 patients representing 31.0% of the studied population were found to have taken some form of antihelminthic drug (dewormer) in the 3 months preceding the study. The highest dewormer usage was found in clients aged 26-30 years followed by persons in the 31-35 age group.The age groups 1-5 and those over 55 years had the least number of persons who had dewormed in the 3 months preceding the study.

Chi square test of association between dewormer usage and the presence of intestinal parasites indicated no significant association ($p=0.95$, $\chi^2=0.003$).

The relative percentages of dewormer usage for the age groups of the studied patients are given in Figure 7.

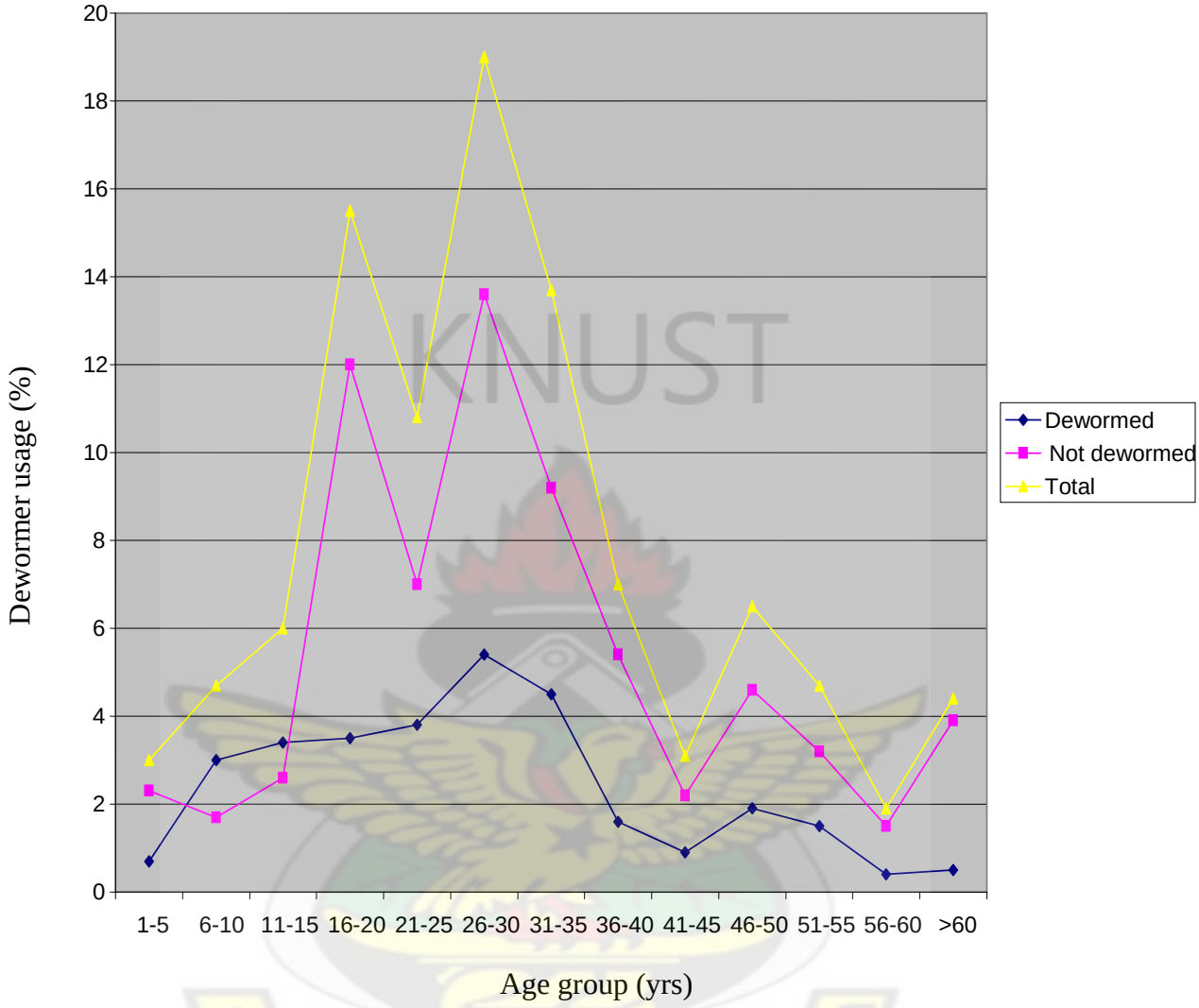

Figure 7: Relative percentages of dewormer usage by studied patients

4.5 Prevalence rates of helminth parasites by the parasitological methods used

The overall prevalence rate of helminth parasites detected by the formol-ether concentration (gold standard) method was 11.1%. Kato-Katz and direct wet mount methods gave overall prevalences of 5.2% and 3.25% respectively. The prevalences of the seven intestinal helminths detected by the three methods are represented in Table 7.

Table 7: Prevalence of intestinal helminth parasites

Helminth parasite	Formol-ether conc. (%), n=2000	Kato Katz (%) n= 1880	Direct Wet Mount (%), n=2000
Hookworm	58(2.9)	54(2.8)	22(1.1)
Dicrocoelium dendriticum	43(2.1)	0(0.0)	12(0.6)
Strongyloides stercoralis	42(2.1)	6(0.3)	17(0.8)
Schistosoma mansoni	37(1.8)	34(1.8)	8(0.4)
Hymenolepis nana	28(1.4)	2(0.1)	4(0.2)
Taenia species	13(0.6)	0(0.0)	2(0.1)
Trichuris trichiura	2(0.1)	2(0.1)	1(0.05)
Ascaris lumbricoides	0(0.0)	0(0.0)	0(0.0)
TOTAL (overall prevalence)	**226(11.1)**	**98 (5.2)**	**66(3.25)**

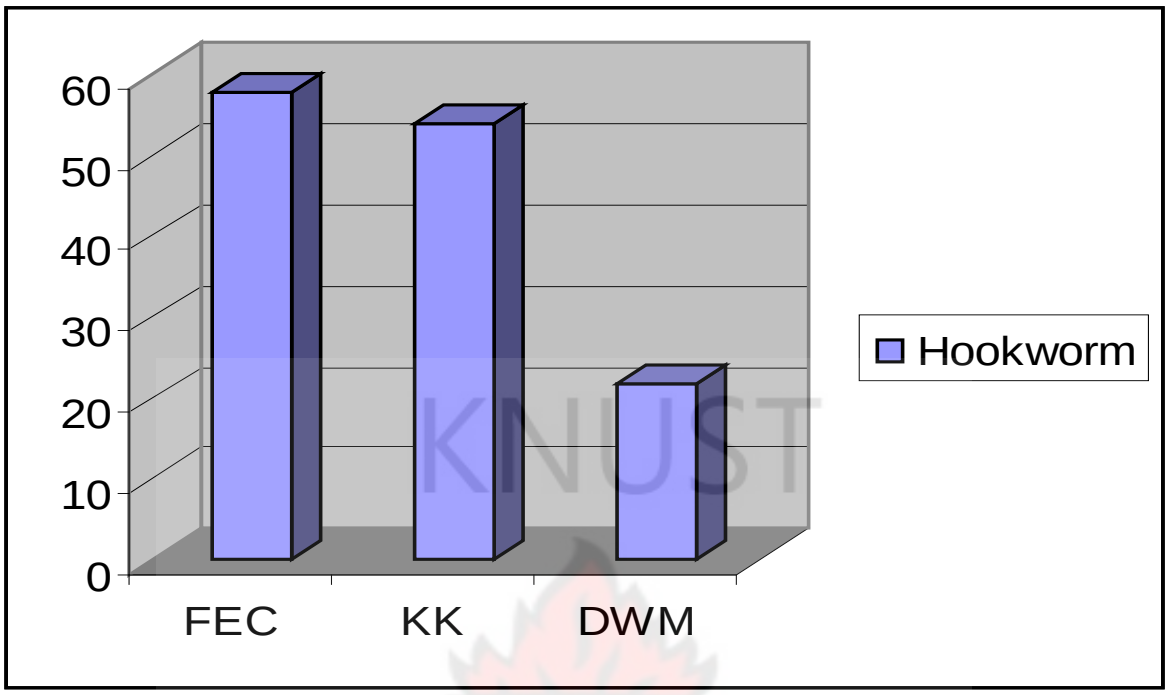

Figure 8: Prevalence rates of hookworm as detected by the methods
(FEC- Formol-ether concentration; KK- Kato-Katz; DWM- Direct wet mount)

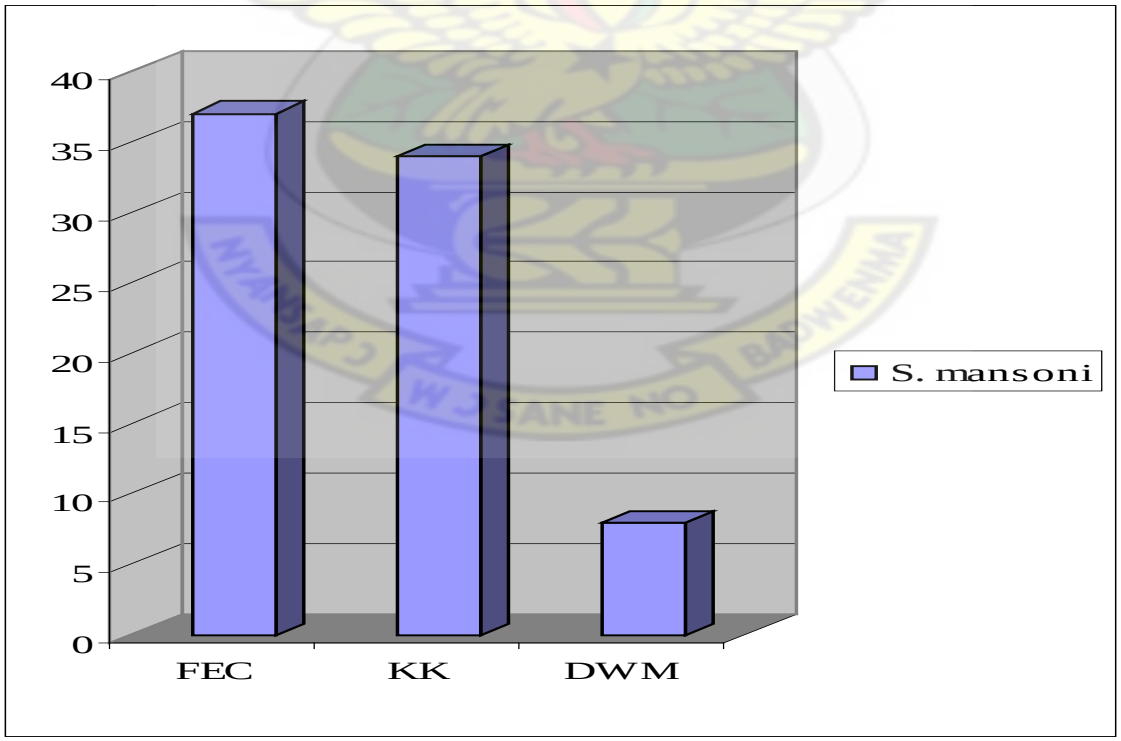

Figure 9: Prevalence rates of *S. mansoni* infection as detected by the methods
(FEC- Formol-ether concentration; KK- Kato-Katz; DWM- Direct wet mount)

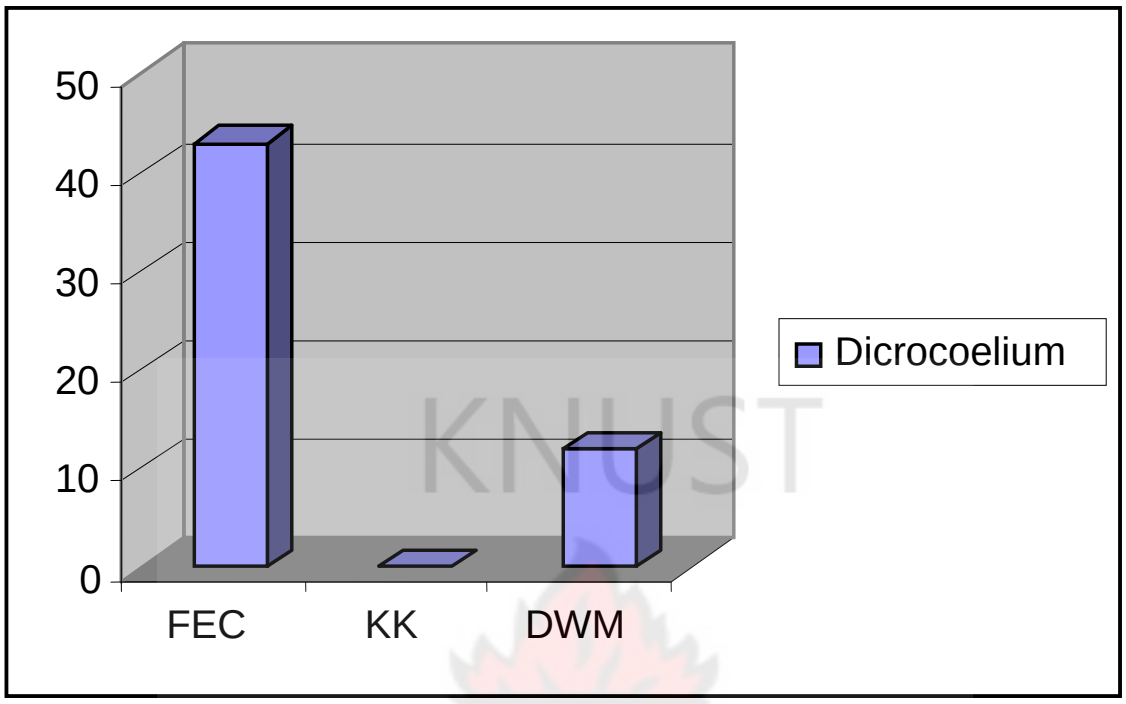

Figure 10: Prevalence rates of *Dicrocoelium* infection as detected by the methods (FEC- Formol-ether concentration; KK- Kato-Katz; DWM- Direct wet mount)

4.5.1 Test performance of direct wet mount

The performance of direct wet mount was evaluated in relation to formol-ether concentration which is the gold standard test, and the evaluation results are shown in Table 8.

Table 8. Performance of direct wet mount against the gold standard in detection of intestinal helminth parasites

Gold standard/Reference		Direct wet mount test		Total Results
Method	Result	†Positive	*Negative	
Formol-ether concentration technique	†Positive	66	160	226
	*Negative	0	1774	1774
Total Results		66	1934	2000

Positive predictive value, PPV=29.2% (66/226)

As shown in Table 8, the wet mount method detected a total of 66 intestinal helminth parasites as against 226 by the gold standard. The evaluation results gave sensitivity and specificity of the wet mount method as 29.2% (66/226) and 100% (1774/1774), respectively.

Table 9. Comparison of Kato-Katz versus the gold standard for detection of STHs and *S. mansoni* infections.

Parasite	Formol-ether conc. (%) n=2000	Kato Katz (%) n=1880
Hookworm	58(2.9)	54(2.8)
T trichiura	2(0.1)	2(0.1)
A lumbricoides	0(0.0)	0(0.0)
S mansoni	37(1.8)	34(1.8)
TOTAL RESULTS	**97(4.8)**	**90 (4.7)**

4.5.2 Performance of Kato-Katz for detection of STHs and *S. mansoni* infections

As shown in Table 10, both the Kato-Katz and formol-ether concentration techniques showed good agreement for detection of the three major STHs (hookworms, *T. trichiura* and *A. lumbricoides*), and *S. mansoni* infections. A comparison of the performance of the Kato-Katz versus the gold standard results gave sensitivity of 92.8% (90/97), and a total agreement of 92.8% (95% CI; 90.8-94.8). Significantly, no *A. lumbricoides* eggs were found using both methods.

4.5.3 Infection intensities

The intensity of infection was measured on the basis of egg counts, expressed as eggs per gram (epg) of stool (Beaver, 1950), counted by the Kato-Katz method. Infection intensities for the STHs and *S. mansoni* cases detected in the study were classified as light, moderate and heavy in accordance with the criteria by the WHO (2002).

Table 10 summarizes infection intensity of STHs and *S. mansoni* as detected in the study.

Table 10. Infection Intensity of STHs and *S. mansoni* using the Kato-Katz

Organism	Infection Intensity (%)			Total positive by Kato-Katz (%)
	Light	Moderate	Heavy	
Hookworm	54 (60.0%)	0 (0.0%)	0 (0.0%)	54 (60.0%)
Ascaris lumbricoides	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Trichuris trichiura	2 (2.2%)	0 (0.0%)	0 (0.0%)	2 (2.2%)
Schistosoma mansoni	14 (15.6%)	16 (17.8%)	4 (4.4%)	34(37.8%)
Total Results	70 (77.8%)	16 (17.8%)	4 (4.4%)	90 (100%)

As shown in Table 10, all cases of STHs found in the study were of low intensity. *S. mansoni* infections gave 16 (17.8%) moderate intensity and 4 (4.4%) high intensity infections.

Differences in the intensity of *S. mansoni* infection were tested for all age groups. It was evidenced that a significantly high number of individuals infected with medium and high *S. mansoni* burden belonged to 10-15 age group ($P < 0.05$).

4.5.4 Operational characteristics of parasitological methods used in this study

The parasitological methods employed in this study were the direct wet mount, the Kato-Katz thick smear and the formol-ether concentration techniques. Assessment of their operational characteristics is crucial for decision regarding the suitability of a method for a specific laboratory or area.

Characteristics usually sought include the technical simplicity or ease of use, rapidity or time taken to perform the test, the stability of the test under user conditions, accuracy or reliability (sensitivity and specificity) of test results, the predictive value of the test and affordability or cost of test, which is largely determined by the cost of equipment and materials required to perform test. However, some of these characteristics may be qualitative and subjective. The operational characteristics of the methods used in this study are provided in Table 11.

Table 11: Operational characteristics of the methods

Characteristics	Direct wet mount	Kato-Katz	Formol-ether conc.
Procedural steps	Very few	Few	Several
Ease of performance	Very easy	Easy	Less easy
Rapidity (Total time required)	Results in less than 1 hr.	Results ready in 1-2 hrs	Results ready next day.
Essential facilities and/or materials required for test	Physiological saline	Kato kit, Glycerol-malachite green solution	Formalin, Diethyl-ether, Centrifuge, Electricity supply, Centrifuge tubes, Gauze pads, Test tube racks, Fume chamber
Sensitivity	29.2%	92.8% (detection of STHs & *S. mansoni* infections)	Reference method: 100% sensitive for broad range of parasite ova, larvae & cysts.
Specificity	100%	100%	Reference method (100%)
Potential barriers to routine use of method	Nil of note	Inadequate trained personnel, Kit not readily available on the local market.	Few trained personnel, Requirement for centrifuge, reliable electricity, and fume chamber, Reagents are expensive and stock-outs.
Affordability (cost of test)	*Cheap	?Less expensive	?Expensive

*Cost of test as per the National health insurance tariff is GH¢1.34 for stool routine examination, based on the direct wet mount technique.

? Rates are not available, as they are not routinely done in any hospital laboratory in Ghana.

4.6 Species identification of hookworm filariform larvae

The criteria for species identification of hookworm larvae cultivated by the Harada-Mori technique were based on distinct morphological characteristics of larval nematodes that have long been described by Wu and Peng (1965), Yoshida, (1966), and Little (1966). Figures 12 and 13 show some details of filariform (infective) larvae reared from hookworm-positive stool samples in this study.

The recovered larvae were identified on the basis of distinct morphological characteristics as presented in Table 12.

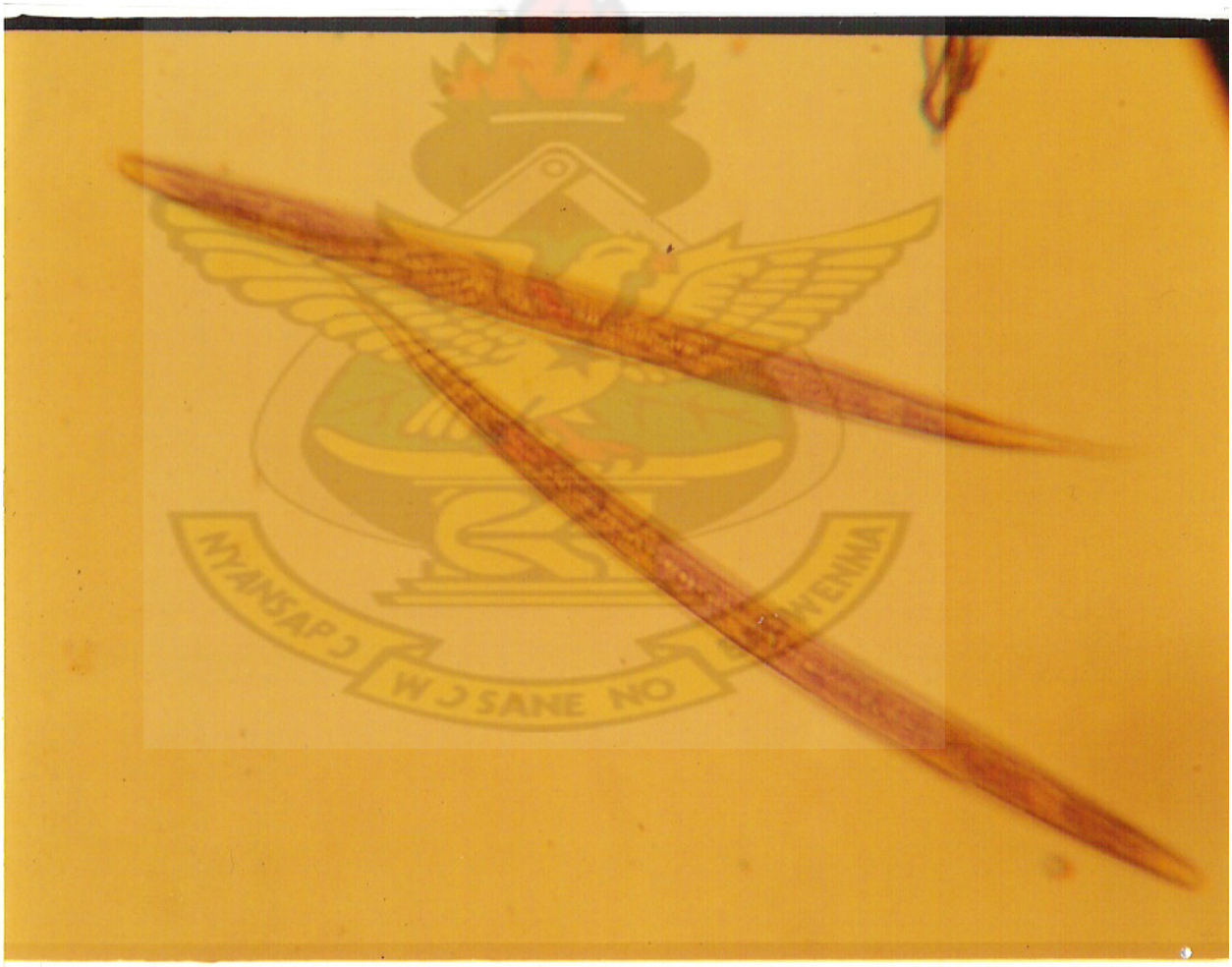

Figure 11. Hookworm L3 larvae recovered from hookworm positive faecal sample

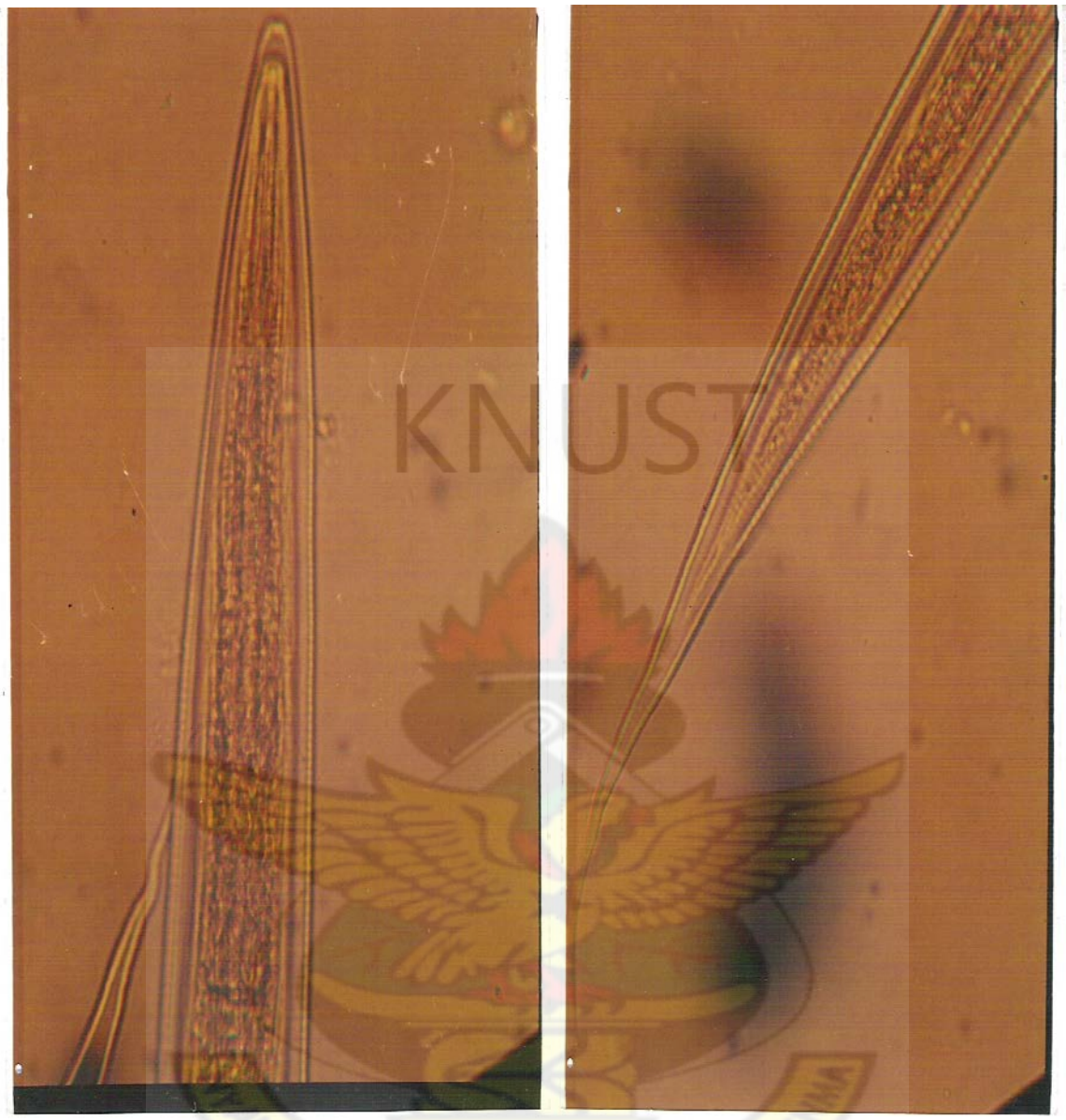

Figure 12: Rounded head (left) and sharply pointed tail (right) of hookworm larvae with striations clearly visible at the tail end (source: the study)

4.6.1 Identification of hookworm species recovered from the study

Hookworm larvae recovered from all the 58 hookworm-positive faecal samples showed the same characteristics as presented in Table 12.

Table 12. Comparative morphological characteristics of hookworm larvae recovered from the study.

Morphological characteristic	**Larvae reared from hookworm-positive stool samples in the study (n=58)**	***Ancylostoma***	***Necator***
Length	Ranged from 580 -600 microns (ave. 590 microns)	660 microns	590 microns
Sheath	Present (Figures 11 & 12); measured 655-665 microns (ave. 660 microns)	Sheath 720 microns	Sheath 660 microns
Intestine	Anterior end is as wide as the oesophageal bulb with gap between the oesophagus and the intestine (Figure 12 left)	Anterior end is narrower in diameter than oesophageal bulb; no gap between the oesophagus and the intestine	Anterior end is as wide as the oesophageal bulb; gap between the oesophagus and the intestine
Tail	Sharply pointed; with striations clearly visible at the tail end (Figures 11 & 12)	Blunt; striations not clear	Sharply pointed; striations clear at tail end
Head	Rounded (Figures 11 & 12)	Blunt	Rounded

On the basis of established morphological features for differentiating species of hookworm larvae (as presented in Table 2), all larvae recovered in this study were identified as *Necator americanus*. No *A. duodenale* was found.

CHAPTER FIVE

5.0 DISCUSSION

Routine diagnosis of intestinal helminth infections is based on the recovery of helminth eggs and/or larvae in stool samples examined through a variety of parasitologic methods (Goodman et al., 2007). Prior to the study, information from several hospital laboratories across the country revealed that the direct wet mount procedure was the sole diagnostic tool used for stool routine examination at all levels of health care delivery in Ghana, that is, the primary/district level, secondary/regional level and tertiary/teaching hospital laboratories. It was observed that teaching hospital laboratories, KATH in particular, could be adequately resourced to perform stool concentration technique as a confirmatory test when results obtained with direct wet smears were negative for intestinal parasites.

In the last three years, new microscopes have been delivered to the KATH parasitology laboratory and stool microscopy have been performed by very experienced laboratory technologists, yet the overall number of parasites recovered from stools have remained low. The study was therefore, intended to find out if there was some critical diagnostic factor responsible for the low recovery of parasites, and hence, the need for a diagnostic evaluation of the wet mount method using the formol-ether concentration technique as the gold standard method for the study.

5.1 Demographic characteristics

Of the 2000 studied patients, 1,185 (59.3%) were females and 815 (40.7%) were males. The higher female ratio can clearly be linked to the fact that pregnant women who

reported to the KATH antenatal clinic (ANC) were made to do stool routine examination as part of the routine antenatal care, so that requests for stool examination that were received from the antenatal clinic (ANC) were far more than those from the polyclinic out-patient departments (OPD) and other specialist clinics within the hospital. This finding contrast patient demographic data from the Korle-Bu Teaching Hospital (KBTH) parasitology laboratory, which showed no significant difference in sex distribution of patients who performed stool routine examinations (KBTH parasitology laboratory records from 2000-2007). It was observed that at Korle-Bu Teaching Hospital, a satellite laboratory at the maternity block performs investigations for the ANC clients. The differences in the sex distribution of patients reporting at the two different parasitology laboratories can thus be understood.

In this study, children aged between 1 to 15 years constituted only 13.5% of the total clients studied. This low proportion of children is significant because most studies on helminthiasis have been targeted to children as researchers have identified them as most vulnerable to helminthic infections (Annan et al., 1986; Kightlinger et al., 1998; Perason, 2002). Therefore, constituting just about one eighth of the total clients studied could contribute to the overall low prevalence of helminth infections found in the study. Patients aged between 16 and 40 years accounted for 72% whilst 14.5% comprised clients aged 41 years and above, of the study population. The large number of pregnant subjects may have significantly influenced the age distribution of the studied patients because most pregnant women were aged between 16 and 40 years, as shown by the records at the ANC unit of KATH (KATH, annual report, 2007).

There is evidence in the literature of increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria (Nacher et al., 2002; Spiegel et al., 2003). In this study however, association between malaria attack and co-infection with intestinal helminth was not tested for, due to inadequate clinical data.

It was observed that the proportion of children who submit stool for routine examination at the KATH parasitology laboratory could have been higher if there was a policy at KATH to screen children with malaria for intestinal helminth infections. It is suggested that if stool examination were performed for all children with malaria, the overall prevalence rates of helminth infections found in the study could have been higher.

5.1.1 Clinical diagnosis of patients

Clinicians have a responsibility to ensure that laboratory investigations ordered for their patients are appropriate, and contribute to patient care (Grimshaw and Russel, 1993). The study showed that only 506 clients, representing 25.3% of the studied patients had provisional diagnoses or clinical summaries that could be related to intestinal parasitic diseases. As expected, most of the intestinal helminth parasites were found among patients whose clinical summaries were somehow associated with intestinal parasitic infections.

The study noted that 30.4% patients either had no clinical diagnosis indicated on their laboratory request forms or clinical summaries were unrelated to the investigation ordered. Stool specimens from such patients were found negative for helminth parasites, which implied that the request for stool examination were probably inappropriate.

From the results, the proportion of inappropriate tests is significant enough to cause an overall low prevalence of positive parasitological findings.

In the light of this observation, it is perhaps necessary to consider the appropriateness of some of clinicians' request for laboratory tests, particularly stool routine examinations.

5.2 Dewormer usage by studied patients

The study showed that 31.0% of the studied patients had taken some form of anthelminthic drug (dewormer) in the 3 months preceding the study. The study findings do not sufficiently support the speculation that many people regularly take anthelminthic drugs. In deed, on account of the many different shots and deworming products available on the market today, together with various campaigns and programmes aimed at encouraging de-worming treatments, the level of dewormer usage observed in the study can be considered as much lower than expected.

Significantly, the number of children aged 6 to 15 years who had dewormed were more than those who had not dewormed (Figure 7). This observation was probably a reflection of the national deworming programme for school children in Ghana under the School Health Education Programme (http://www.unicef.org/media/media_38248.html).

Strikingly, the study established no significant association between dewormer usage and the presence of helminth parasites. 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 extreme caution. Nevertheless, the observation provides strong indication for the other studies to assess the efficacies of the various types of medicines commonly used to treat helminthic infections in Ghana.

5.3 Prevalences of intestinal helminth parasites

Hookworm was the most prevalent helminth parasite found among the studied patients. The study result is consistent with the data obtained from the KATH parasitology laboratory over the past decade (KATH parasitology records, 1998-2007), which had found hookworm to be the commonest helminth parasite among patients reporting at the KATH. The findings support a recent survey by Tay and Gyampomah (unpublished data) which identified hookworm as the predominant helminth parasite among the schoolchildren in the Ayigya community of Kumasi in Ghana. Other epidemiological studies have reported the predominance of hookworm infections in some communities in Mali (Behnke et al., 2006), Kenyan (Brooker et al., 2004), and Nigeria (Adenusi and Ogunyomi, 2003).

This observation reconfirms report by Hotez et al. (2006) that hookworm infection still remains a significant public health problem in Africa.

The prevalence rate detected through the wet mount method compared well with available data at the KATH (Parasitology records, 1998 - 2007), and as expected, the prevalence of hookworm detected through the concentration method was significantly higher than rates that have been reported over the years at the KATH parasitology (KATH parasitology records, 1998 - 2007).

With these observations, it became obvious that the persistence of hookworm infection is established in routine practice (KATH parasitology records, 1998 - 2007). However, the true proportion of individuals infected had been underestimated (Ahmadi et al., 2007) because diagnosis by the wet mount is barely 30% sensitive, as was demonstrated in this study and others (Akujobi et al., 2005; Engels et al., 1996; Oguama and Ekwunife, 2007).

In this study, whilst hookworm infection was more prevalent among males, the prevalence of Strongyloidiasis was highest among females. The observation on these two helminth parasites is similar to reports by Pearson (2002), Adenusi and Ogunyomi (2003), Brooker et al. (2004), Yelifari et al. (2005) and Bethony et al. (2006).

The prevalence of 2.1% of *Dicrocoelium dendriticum* detected by the concentration method compared with only 0.6% by the wet mount method (Table 7) highlighted the lack of sensitivity of the wet mount technique for detection of *Dicrocoelium* infection. This observation had been reported by Engel et al. (1996), and Estevez and Levine (1985). It was clear from the study results that the prevalence of dicrocoeliasis reported at the KATH parasitology laboratory have been underestimated as a result of missed diagnosis.

The findings on prevalence rate of dicrocoeliasis seem to disagree with reports by King (1971), Manga-Gonzalez et al. (2001) and Pillai and Kain (2003) that dicrocoeliasis is a rare zoonotic infection.

In deed, the study finding collaborate other studies that reported spurious and genuine human *Dicrocoelium* infections elsewhere in Ghana (Odei, 1966; Wolfe, 1966).

This is because stool routine examination performed at the hospital is based on the wet mount method alone, which could not detect most *Dicrocoelium* infection, as shown in this study.

During the microscopy works in the study, it was noted that the small-sized egg of *Dicrocoelium dendriticum* (CDC, 2006) was often overlooked by inexperienced microscopist.

This observation, coupled with the lack of sensitivity of the wet mount technique (Estevez and Levine, 1985) could possibly explain the seemingly rare prevalence of *Dicrocoelium* infection in some diagnostic centres where laboratory staffs may not have been adequately trained (Bogoch et al., 2006).

In the study, 1.8% prevalence of *Schistosoma mansoni* was reported.

It was identified as the only infection that had light (1-99 epg), moderate (100-399 epg), and heavy (≥400 epg) worm burdens according to the criteria used by the WHO (2002). *Schistosoma mansoni* infections were mostly in patients aged 1 to 15 years, with those in the 10-15 years age group accounting for most of the moderate and heavy infections. This finding is significant because *S. mansoni* is not known to be endemic in the Ashanti region probably because the snail intermediate host may not be common, though the prevalence of *S. haematobium* is significantly high in the region (KATH parasitology records, 1998-2007).

Based on the findings of this study and other studies elsewhere (Sleil et al., 1985; De Vlas, 1992; Hotez et al., 2006; Michaud et al., 2003), *S. mansoni* infection is best detected in stool samples by the formol-ether concentration and the Kato-Katz methods.

Another significant finding of this study is the fact that no *Ascaris* infection was detected among the 2000 stool samples that were analyzed by three different parasitological methods.

In the last two years, laboratory data at the KATH have consistently showed 0% prevalence on *A. lumbricoides* infection (KATH parasitology records, 1998 - 2007) as assessed by the presence of eggs in stool samples submitted by patients for routine

examination. Similar results were obtained for the prevalence of *A. lumbricoides* infections in a recent survey on helminthic infections among pupils in Ayigya primary school in Kumasi, Ghana (Tay and Gyampomah, unpublished data).

The reason can be multifactoral but cannot be attributed to lack of diagnostic sensitivity because the formol-ether concentration technique (Allen and Ridley, 1970) was used both in this study and the Ayigya survey. The method is well noted for its diagnostic capability and is widely used in epidemiological and clinical studies on diagnosis of intestinal helminth infections (Melvin and Brooke, 1985; De Kaminsky, 1993; Garcia, 1999, 2001; Knopp et al., 2006).

Unfortunately the study did not assess the risk factors and epidemiological patterns of the various helminthic infections. Therefore it still remains unclear as to why *Ascaris lumbricoides* recorded 0% prevalence.

It could be due to the fact that adult worms of Ascaris are regularly found in stool of infected individuals who go for self medication (Adams et al., 1994) after seeing these worms in their stool. Parents of infected children purchase over-the-counter medicine for the treatment of their children.

The transmission and distribution of *Ascaris lumbricoides* is largely determined by inadequate sanitary practices and the local habits in the disposal of faeces (Anderson, 1980; Feachem et al., 1983; WHO, 2002).

Current opinions suggest that the absence of *A. lumbricoides* is due to improvement in prevailing social environment and behaviours of people in communities (Montresor et al., 1998; Warren, 1990).

However, there is no consistent evidence to support these assertions.

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

5.4 Performance of the formol-ether concentration, direct wet mount, and Kato-Katz techniques

The overall prevalence of intestinal helminth infections as detected by the formol-ether concentration (which was chosen as the gold standard method for the study), Kato-Katz and direct wet mount methods were 11.1%, 5.2% and 3.25%, respectively. Analysis of the diagnostic performance of direct wet mount and the Kato-Katz methods gave sensitivities of 29.2% and 43.4%, respectively, relative to the performance of the formol-ether concentration method.

In other words, the direct wet mount exhibited the lowest performance; being about three times less sensitive than the formol-ether concentration method whilst the Kato-Katz method is about twice as less sensitive as the formol-ether concentration method for the detection of intestinal helminth parasites. This observation supports the 33% sensitivity of the direct smear method reported by Tay and Gyampomah (unpublished data).

In deed, significant differences ($p < 0.05$) observed in the sensitivities of the three methods (formol-ether concentration, Kato-Katz and direct wet mount) used in this study have been reported in other studies that have compared these methods (Martin and Beaver, 1968; Watson et al., 1988; Engels et al., 1996; Akujobi et al., 2005; Goodman et al., 2007; Oguama and Ekwunife, 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; Oguama and Ekwunife, 2007; Watson et al., 1988), and hence support the argument that available data at the KATH parasitology laboratory underestimates the 'true' prevalence rates of helminth infections among patients reporting at the hospital.

The overall prevalence rate of 3.25% of intestinal helminth parasites observed in this study for the direct smear is comparable to the rates that have been reported at the KATH laboratory over the years (KATH parasitology records, 1998-2007). This could be due to the fact that majority of infected patients have low worm burden, as shown in this study. The need for introducing stool concentration technique in routine laboratory practice becomes compelling as reliance on the wet mount technique alone may miss about three–quarters of helminth infections.

Compared with the ether concentration method, which was used as the gold standard test, the Kato-Katz method showed overall sensitivity of only 43.4% in detection of intestinal helminth parasites. However, it gave a good sensitivity of 92.8% (95% CI; 90.8-94.8) for

detection of hookworms, *T trichiura* and *S. mansoni* infections. These observations demonstrate a good agreement between the Kato-Katz and formol-ether concentration methods for the diagnosis of the three major STHs and *S. mansoni* infections, and lack of sensitivity of the Kato-Katz method for the detection of such helminth parasites as *S. stercoralis, Dicrocoelium species, Taenia species* and *H. nana* was highlighted in the study. Several other authors have obtained similar results (Kato and Miura, 1954; Katz et al., 1972; Martin and Beaver, 1968; Ebrahim et al., 1997; Engels et al., 1996).

When there is a good indication of infection with *S. stercoralis, Dicrocoelium species, Taenia species* and *H. nana* the choice of the direct wet mount over the Kato-Katz method is desirable, because the rates of prevalence obtained by the Kato-Katz method for these parasites were significantly lower than those obtained by the direct smear method.

5.5 Operational characteristics of the methods used in the study

The operational characteristics of the methods used in the study have been described in Table 11. Of the three parasitologic methods used, the direct wet mount was found to be the simplest, most 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; Arcari et al., 2000; Bogoch et al., 2006; De Kaminsky, 1993; Garcia, 1999, 2001).

In 2,000 stool specimens examined for helminth eggs, the wet mount method gave a sensitivity of only 29.2% relative to the recovery rate of helminth parasites with the formol-ether concentration. This implies that only about a third of helminth infected individuals would have been identified if stool samples had been analyzed with only the wet mount method. The findings of this study as well as others (Watson et al., 1988; Akujobi et al., 2005; Goodman et al., 2007; Oguama and Ekwunife, 2007) have showed that the wet mount method was the least sensitive method of the three parasitological methods evaluated.

Although some characteristics of the wet mount technique make it the preferred choice for 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 caused missed diagnosis of infections leading to grave clinical and public health consequences, as reported by Barnabas and Aboi (2005).

The Kato-Katz method was found to be relatively rapid and easy to perform, required minimal training and involved few steps to complete. Its high sensitivity and specificity for the diagnosis of the major STHs and *S. mansoni* compared to the formol-ether concentration is well noted in this study and others (Kato and Miura, 1954; Katz et al., 1972; Ebrahim et al., 1997).

The technique is a useful tool for the quantification of egg counts to determine infection intensities. These qualities make the Kato-Katz the most frequently employed method in research works (Katz *et al.*, 1972, WHO, 2000).

However the difficulty in obtaining the Kato kit from the local market, its lack of sensitivity for parasites other than the major STHs and *S. mansoni*, as well as technical challenges in processing diarrhoeal stools (Siegel et al., 1990), offer some limitations to its use in routine practice.

The formol-ether concentration was used as the reference method or gold standard (Wiebe et al., 1999) for the study. Unlike the direct smear and the Kato-Katz methods, the formol-ether concentration was time consuming and required highly trained personnel and skill to perform 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 most expensive of the three parasitological methods evaluated in the study.

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

It is most desirable to perform the formol-ether concentration as a confirmatory test on all stool samples that are negative for helminthic parasites by the wet mount technique. This approach will ensure accurate diagnosis of intestinal helminthic infections to allow

effective and efficient management and control of parasitic diseases which ultimately improves quality of life of individuals in the community.

5.6 Prevalence of Hookworm species

Table 13 presents the prevalence rates of the two hookworm species as were cultivated from among 58 hookworm-infected subjects whose stools were cultured by the modified Harada-Mori test tube faecal culture technique (Harada and Mori, 1955; Sasa et al., 1958; Hsieh, 1961), to yield L3 larvae of hookworms.

In accordance with the criteria for species identification as described by Little (1966) and presented in Table 12, all isolated larvae were found to be pure *N. americanus* variety.

The results showed 100% prevalence of *N. americanus* infections among hookworm-infected patients reporting at the KATH. *A. duodenale* larvae were not found among the isolated hookworm larvae.

The study results imply that when hookworm infection is diagnosed at the KATH parasitology laboratory, it is most likely of *N. americanus* infection.

However, because only 58 stool samples were cultured for larvae, the data available cannot be considered sufficient enough to conclude the absence of *A. duodenale* infection among hookworm-positive patients reporting at the KATH.

It is not fully understood why *A. duodenale* was not found, but because it is reported to be relatively common in northern parts of Ghana (De Gruijter et al., 2005), there is a possibility that *A. duodenale* may be discovered when a larger sample of hookworm-positive cases are cultured for identification.

Ancylostomiasis is associated with greater intestinal blood loss; ingesting 0.15 ml per worm per day and causing severer iron-deficiency anaemia (Albonico et al., 1998). While *N. americanus* infection is acquired almost exclusively by active penetration of the skin (percutaneous route), *A. duodenale* is able to infect both percutaneously and by the oral route (Looss, 1911), and also causes infantile ancylostomiasis, through transplacental or lactogenic transmission (Yu et al., 1995, Nwosu, 1981). *A. duodenale* is also reported to differ in susceptibility to the same anthelminthic and dosage regimen (Rim et al., 1971; Reynoldson et al., 1997). Consequently, the efficacy of anthelminthic therapy depended on the infecting species of hookworm (Horton, 2000).

Against this background therefore, it is important to identify the type of hookworm species being transmitted in a community because it influences the burden of iron deficiency anaemia in the community (Crompton and Savioli, 2005), such that hookworm infected individuals in a community with *Ancylostoma* species will require some radical treatment and efficient management of anaemia.

5.7 CONCLUSIONS AND RECOMMENDATIONS

It is concluded from the study that requesting stool routine examination for patients whose clinical summaries or provisional diagnoses are not related to known clinical manifestations of intestinal parasitic infections appears to provide no diagnostic benefit. Consequently, the health systems should develop evidence-based clinical practice protocols for appropriate laboratory investigations, and ensure that house officers and junior medical staff do not order laboratory tests inappropriately.

It is strongly suggested that a study should be conducted on inappropriate request for some laboratory tests at the KATH, including a conservative estimate of the excess cost of such investigations to the patient and the hospital.

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 on the Ghanaian market and their relative efficacies, including the dynamics of dewormer usage and worm burdens in communities.

The study clearly indicated that the formol-ether concentration method is superior to the Kato-Katz and direct wet mount methods for routine diagnosis of intestinal helminth infections. It is concluded that the low prevalences of intestinal helminth parasites reported at the KATH laboratory is due to lack of sensitivity of the traditional wet mount method. Hence, the data available at the KATH parasitology laboratory underestimates the 'true' prevalences of intestinal helminth infections at the hospital.

As a general conclusion, intensity of infections among patients reporting at the KATH are mostly of low grade intensity which require the formol-ether concentration method to ensure accurate diagnosis of most helminth infections.

It is therefore recommended that stool samples that are found negative for parasites by the traditional wet mount method should be re-examined by the formol-ether concentration technique as a confirmatory test.

It is strongly suggested that all regional and teaching hospital laboratories in Ghana be adequately resourced to perform the concentration method in routine practice.

The use of the formol-ether concentration as a confirmatory test will sufficiently reduce misdiagnosis of intestinal helminthic infections and its attendant public health consequences.

Additionally, data from laboratories that use the formol-ether concentration as a confirmatory test can be assumed to reflect the 'true' prevalences of helminthic infections at the hospitals.

The fact that *Dicrocoelium dendriticum* rated second highest in prevalence suggest increasing genuine human dicrocoeliasis in the community as reported by Odei (1966) and Wolfe (1966). In order to improve the detection and identification of *Dicrocoelium* species, it will be desirable to organize training workshops on stool microscopy for laboratory staffs at all levels of health care to improve their technical and diagnostic skills for diagnosing *Dicrocoelium* species and other parasites that may rarely be found in stool specimen.

Because very few studies have been published on dicrocoeliasis in Ghana, 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 *A. lumbricoides* transmission and its overall prevalence appear to have diminished as was found by Tay and Gyampomah (unpublished).

Further study is necessary to assess current trends of ascariasis and in understanding their epidemiological and public health importance within the community (Crompton and Savioli, 2007).

It is concluded from the study that hookworm infection continues to persist in the community, and that all 58 hookworm-positive faecal samples that were cultured by the Harada-Mori technique were the *Necator americanus* species.

The presence of both *Ancylostoma duodenale* and *Necator americanus* infections have been reported in humans in northern Ghana but in this study, the data on hookworms is still not sufficient enough to conclude the absence of *Ancylostoma duodenale* infection. It is therefore strongly recommended that large scale epidemiological studies on human hookworm species in various communities in Ghana be undertaken to gather adequate data on the relative prevalence of the two human hookworm species in various communities in Ghana as had been reported for Nigeria (Adenusi, 1997; Adenusi and Ogunyomi, 2003; Oyerinde, 1978).

It is important to identify the prevalent hookworm species in various communities to provide useful information in designing effective integrated control of hookworm and anaemia in endemic communities of Ghana.

5.7.1 Limitations and constraints

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

1. Difficulty in obtaining the Kato-Katz kits as they are not readily available on the local market.

2. Initial difficulty in recruiting clients for the study as most patients perceived a potential delay in the release of their laboratory results.

3. In-patient samples could not be included in the study due to technical challenges.

4. Kato-Katz could not be performed on diarrhoeal stools, due to technical difficulties.

5. 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.

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

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APPENDICES

Appendix 1. Materials used for the study

1.1 Equipment:

i. Binocular microscope (with 10X, 40X, and 100X objectives)

ii. Centrifuge, with head and cups to hold 15ml conical tubes

1.2 Reagents:

i. Diethyl ether

ii. Formalin (10%)

iii. Lugol's iodine solution (1%)

iv. Physiological saline solution, isotonic (0.85% NaCl)

v. Glycerol-malachite green solution (1ml of 3% aqueous malachite green added to 200ml of 50% glycerol; the solution is mixed well and poured onto the cellophane strips and soaked in this solution in a staining jar for at least 24 hr prior to use).

1.3 Other laboratory supplies:

i. Kato-set (HelmR test kits: from Brazil AK Industriae Commercio Ltd., Belo Horizonte, Brazil; containing a 41.7mg cardboard template- with a hole of 7.5mm diameter and 1.0mm thick, number 105-sized nylon mesh screen, hydrophilic cellophane strips of size 25x35 mm and 50 µm thick).

ii. Applicator sticks, wooden.

iii. Beakers (50ml, 100 ml and 500 ml).

iv. Centrifuge tubes 15ml (ether-resistant).

v. Coverslips (22 X 22 mm)

vi. Disinfectant (Izal)

vii. Disposable plastic Pasteur (transfer) pipettes

viii. Dropping/'Squeeze' bottles, 250 ml.

ix. Face shield/Mask

x. Filter paper (Whatmann No.3).

xi. Forceps.

xii. Gauze.

xiii. Glazed tile.

xiv. Gloves.

xv. Markers for indelible labeling.

xvi. Measuring Cylinders: 200ml and 1000ml.

xvii. Microscope slides (75mm X 25mm).

xviii. Pergamon absorbent sheets.

xix. Slide boxes.

xx. Staining jar with lid.

xxi. Test tube racks.

Appendix 2. Direct Wet Mount Technique, Saline Preparation

Step	Procedure
1	With a marker the study identification number is written at one end of the slide and a drop of physiological saline is placed in the centre of the slide.
2	With a wooden applicator stick, a small portion of stool specimen (approximately 2 mg which is about the size of a match head) is picked and added to the drop of saline and thoroughly emulsified to make a thin uniform saline suspension– not too thick that faecal debris may obscure organisms, and not too thin that blank spaces may be present.
3	The suspension is carefully covered with a cover slip in a way as to avoid air bubbles.
4	The slide is then placed on the microscope stage, and the preparation is examined systematically under the low power (X 10) objective so that the entire cover slip area is scanned for parasite ova, cysts, larvae and trophozoites. When organisms or suspicious objects are seen, the high dry (X40) objective is used to see more the detailed morphology of the object for confirmation.

Appendix 3. Kato-Katz Technique

Step	Procedure
1	The Kato template (with hole) is placed in the centre of a microscope slide.
2	With wooden applicator stick, a small mount of faecal material is placed on the "Pergamon" absorbent sheet and a small sized nylon mesh screen is pressed on top of the faecal material so that some of the faeces will sieve through the screen and accumulate on top of the screen.
3	A spatula is used to scrape across the upper surface of the screen so that sieved/filtered faeces accumulate on the spatula.
4	The collected sieved faecal specimen is added in the hole of the template so that it is completely filled.The side of the spatula is used to pass over the template to remove excess faeces from the edge of the hole.
5	The template is carefully removed from the slide so that a cylinder of faeces is left on the slide.
6	The faecal material is covered with pre-soaked hydrophilic cellophane strip (as described in appendix 1, 1.2, v). The microscope slide is then inverted and the faecal sample is firmly pressed against the hydrophilic cellophane strip on a smooth hard surface (a glazed tile), to spread faecal material evenly.
7	The slide is carefully removed by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. The slide is then placed on the bench with the cellophane upwards.
8	After 30 minutes incubation at room temperature (allowing water to evaporate and glycerol to clear the faecal material), the Kato thick smear is microscopically examined, with the X10 objective, in a systematic manner so that the entire coverslip area is observed, and the number of eggs of each species is reported.
9	Having used the WHO recommended 41.7 mg template, the number of eggs per gram (epg) of faeces is obtained by multiplying the number of eggs found by a factor of 24.

Appendix 4. Formol-ether concentration technique

Step	Procedure
1	With a wooden applicator stick, 1 gram of stool specimen is added to 10ml of 10% formalin in a small beaker and thoroughly emulsified, and brought into suspension.
2	The suspension is strained through a double layer of wet gauze directly into a 15 ml centrifuge tube. The gauze is then discarded, and more 10% formalin is added to the suspension in the tube to bring the total volume to 10 ml.
3	3 ml of ether is added to the suspension in the tube, rubber stoppered and shaked vigorously for 10 seconds.
4	With an applicator stick the plug of debris is loosened by a spiral movement and the supernatant (comprising the top 3 layers) is decanted, in a single movement, into a bowl containing disinfectant; allowing the last few drops of residual fluid to flow back onto the sediment.
5	The deposit/sediment is resuspended with a disposable Pasteur pipette. Sometimes it is necessary to add a drop of physiological saline to have sufficient fluid to resuspend the sediment. A few drops of the suspension is transferred onto a microscope slide and covered with a coverslip.
6	The preparation is scanned using the low power (X10) objective, and in a systematic manner as to observe the entire coverslip area. If an organism or suspicious objects are seen, the higher magnification (X 40 objective) is used to see observe its detailed morphology.

Appendix 5 : QUESTIONNAIRE

Accuracy of diagnosis of intestinal helminthic infections, and epidemiology of *N. americanus* and *A. duodenale* infections at the KATH.

1. Study No: …… 2. Date of collection:…..…… 3. Age (yrs):..…..

4. Sex: Male [1] Female [2] 5. Resident (Community/Locality):……

6a. Have you taken any dewormer in the last three months? Yes [1] No [2]

6b. If 'Yes' indicate source of drug: Hospital prescription [1] Per self (Pharmacy) [2]

7. Clinical indications for stool test (diagnosis as indicated on laboratory request form):

Abdominal pain [1] Nausea [2] Diarrhoea [3] Anaemia [4] Helminthiasis [5]

Gastroenteritis [6] Dysentery [7] Medical exam [8] ANC [9]

No diagnosis indicated [10] Other (specify)…….

Appendix 6. Preparation of glycerol-malachite green solution

1. A stock solution of malachite green, 1% solution is prepared as follows:

Malachite green crystals …….……. 1g

Distilled water …….. 100ml

2. A working solution of glycerol-malachite green solution is prepared as follows:

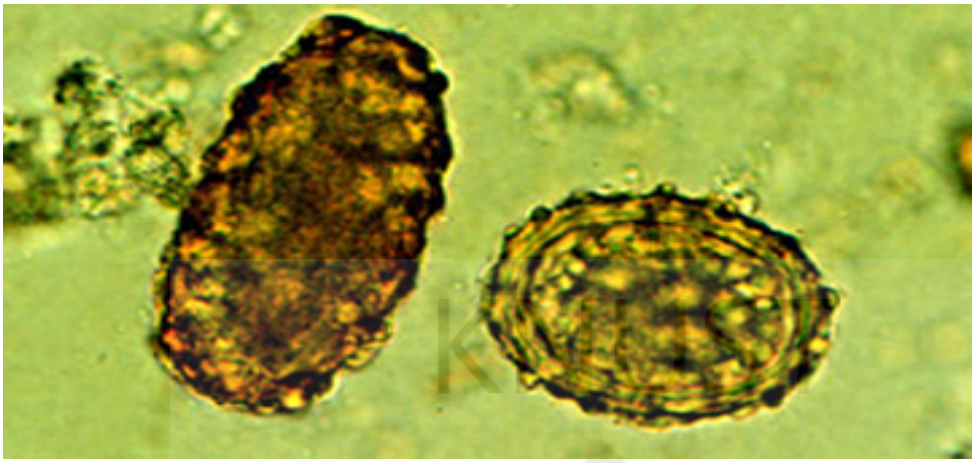
Glycerol ……..100ml

Malachite green, 1% stock solution ……...1ml

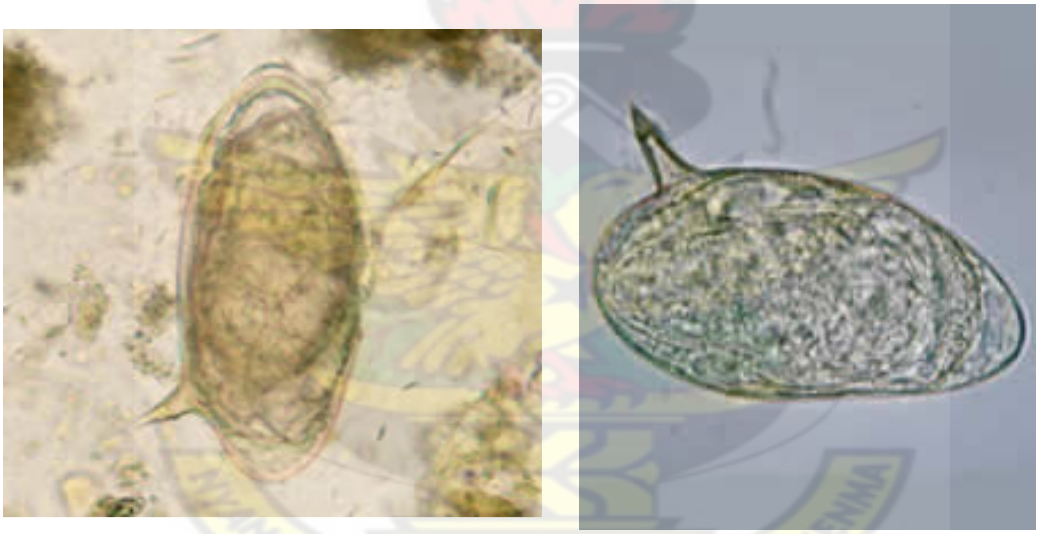
Distilled water ……100ml

The glycerol, malachite green stock solution and distilled water are mixed and poured into a 250ml glass stoppered bottle, and then labeled as "GLYCEROL-MALACHITE GREEN SOLUTION". It is mixed gently before use.

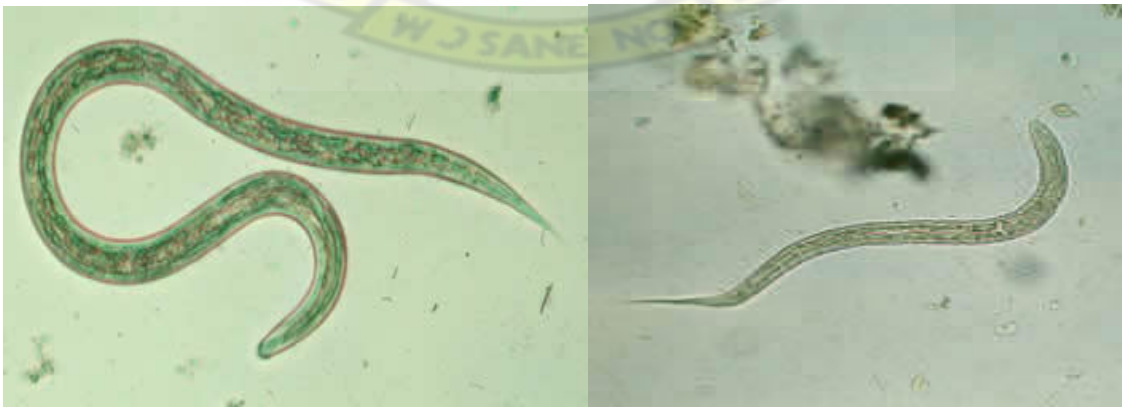
Appendix 7: Diagnostic characteristics of commonly detected parasite eggs and larvae in stool specimens (courtesy CDC, 2006: DPDx).



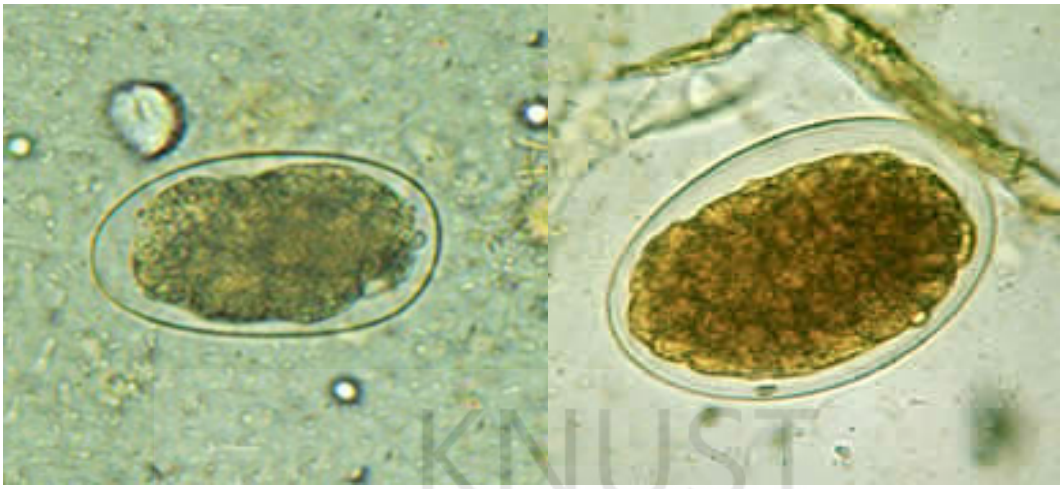
Unfertilized and fertilized *Ascaris lumbricoides* eggs (left and right, respectively



Ova of *Schistosoma mansoni*

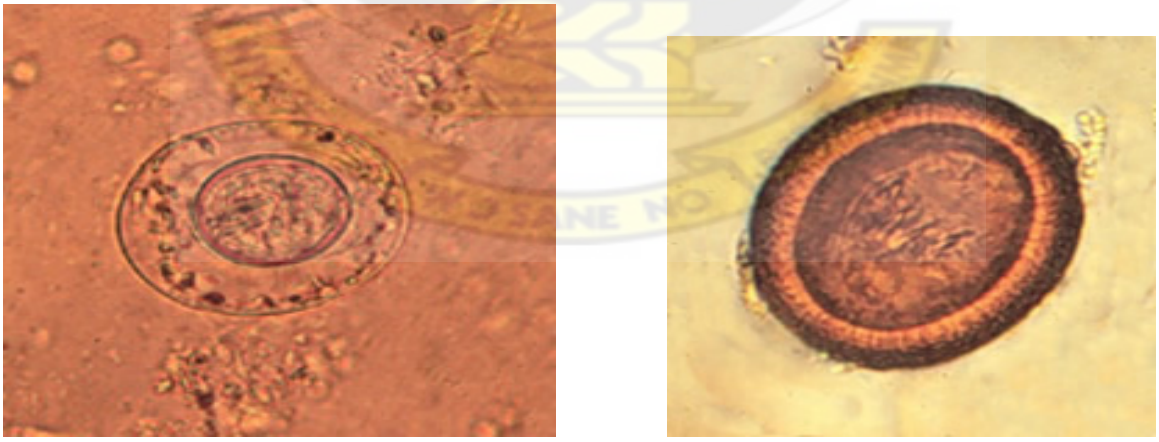


Strongyloides stercoralis larva



Hookworm eggs; 8-cell stage and morula stage eggs (left and right, respectively).

Eggs of *Dicrocoelium dentriticum* and *Trichuris trichiura* (left and right, respectively).



Eggs of *Hymenolepis nana* and *Taenia* species (left and right, respectively).