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HIGH PREVALENCE OF HOOKWORM INFECTION AND APPARENT ABSENCE OF *ASCARIS LUMBRICOIDES*: A CASE STUDY AT THE KOMFO ANOKYE TEACHING HOSPITAL IN GHANA

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ABSTRACT

Background: Parasitic helminth infection is one of the major risk factors underlying the high rates of anaemia and malnutrition in many third world countries due to the poor socio-economic and environmental conditions of the people and these play a very significant role in their transmission.

Method: 2000 stool specimens collected between May and October 2008 at the Komfo Anokye Teaching Hospital in Ghana were screened using the Kato-Katz and formol-ether concentration methods for the presence of intestinal helminthes. Prior to sample collection, histories of any antihelmentic drug usage in the last three months preceding this study were collected. Hookworm positive stool specimens were further cultured by the modified Harada-Mori test-tube technique for the identification of the hookworm filariform larvae.

Results: Hookworm (*Necator americanus*) was the most prevalent helminth parasite (2.9%) found in the study. Other parasitic helminthes detected were *Dicrocoelium dendriticum* (2.1%), *Strongyloides stercoralis* (2.1%), *Schistosoma mansoni* (1.8 %), *Hymenolepis nana*, (1.4%) Taenia species (0.6%) and *Trichuris trichuria* (0.1%). *S. mansoni* infection was however high among patients aged between 1 to 15 years. *Ascaris lumbricoides* was not detected in any of the specimens examined.

Conclusion: In this study therefore, gender and dewormer usage did not play any significant role ($p > 0.05$) in the rate and level of helminth infection. Further studies to assess the quality and efficacy of the various types of antihelmentic drugs on the Ghanaian market and the factors contributing to absence of *A. lumbricoides* must be considered.

INTRODUCTION: Helminthes are complex eukaryotic organisms with large genomes, endowing some species with the ability to live for decades in human host [1]. There are about 20 major helminth infections of humans, affecting more than one third of the world's population [2] and the most common ones that account for most of the global helminth disease burden in many sub-Saharan African countries including Ghana are ascariasis, trichuriasis, strongyloidiasis, taeniasis, schistosomiasis and hookworm infections [3-8].

It is common for the host-parasite interplay to seem harmonious, and clinically asymptomatic helminth carriers act as long term reservoirs for transmission. About 7 % of the helminth-infected patients suffer severe and permanent impairments of immunopathological complications such as granulomatous disease and organ failure [9, 10]. Helminth infections have also been shown to play a significant role in anaemia and childhood malnutrition, which often leads to growth retardation, poor intellectual development and impaired cognitive function, resulting in a poorer quality of life and less ability to contribute to society [11-13].

Additionally, immune suppression by helminth infections has well been documented: Schistosome-infected children have been shown to exhibit lower levels of skin reactivity to allergens than uninfected classmates, indicating a helminth-mediated down modulation of the immune system [14].

In Ghana, there are not enough available data on the prevalence of these infections in most of the poor communities which are heavily burdened with these infections. This study was therefore designed to evaluate and provide data on the prevalence of some of the major intestinal helminth infections reported at The Komfo Anokye Teaching Hospital in Kumasi.

MATERIALS AND METHODS:

Study area and population: The study was carried out at the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. The geographical location of this hospital and commercial nature of Kumasi make the hospital accessible, and a referral hospital for the middle and northern parts of Ghana. A non-selective sample of 2000 consented in- and out-patients aged between 1-90 years were recruited for the study between May 2008 to October 2008.

Ethical Issues: The study protocol was approved by the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, in collaboration with the KATH management which also granted the permission to undertake the study.

Specimen Collection: Specimen collection and handling were carried out in accordance with standard protocols reported by Booth *et al*., [15], NCCLS [16], Melvin and Brooke [17] and WHO [18]. Demographic data and history of antihelmentic drug usage in the last three months preceding this study were collected prior to the sample collection.

Parasitological Examination: Parasitological screening methods such as Kato-Katz and Formol-ether concentration were employed. Each specimen was first examined macroscopically and its consistency or nature was recorded in accordance with the description by Ash and Orihel [19], Estevez and Levine [20] and Smith and Schad [21]. The test procedures were carried out in accordance with standard protocols as described by Garcia [22, 23] and WHO [19, 24].

Helminth Infection Intensity Determination by Kato Katz Technique: The faecal sample was pressed through a Kato nylon screen mesh (HelmR test kits, Belo Horizonte, Brazil) of size 200µm and the sieved stool was transferred into the 6 mm diameter hole of the cardboard template on a microscope slide. The template was then removed and the remaining sample (approximately 42 mg) was covered with the glycerol soaked cellophane strip of size 25×35 mm and 50µm thick. The prepared slide was microscopically

examined and the eggs of each of the helminth species were recorded. The eggs per gram (epg) of faeces was calculated and used to estimate the infection intensity of the parasites based on the classification reported by WHO [25] for major soil-transmitted helminths and *S. mansoni* infections. Loose and watery stool specimens were not analyzed by the Kato-Katz method because of the technical difficulties that limit analysis of such specimens by this method [15, 26].

Cultivation and Detection of Hookworm Larvae from Stool samples: Hookworm ova positive stool samples in the Formol-ether concentration method were cultured by a modified Harada-Mori technique [27-29]. The culture tubes were incubated upright in darkness for 10 days at 25°C and then heated in a water bath at 50°C for 15 minutes to kill the infective larvae. This was followed by centrifugation at 1000 rpm for 5 minutes and after aspirating the supernatant, the sediment was stained with a drop of Lugol's iodine. A slide of the sediment was then prepared and examined microscopically for the presence of larvae as described by Wu and Peng [30] (1965), Yoshida [31] and Little [32]. The length of each larva and sheath were measured and recorded. All the laboratory procedures were carried out in accordance with standard protocols [16-18].

RESULTS AND ANALYSIS:

Demographic Characteristics: A total of 2000 patients comprising of 1,185 (59.25%) females and 815 (40.75%) males, with ages ranging between 1 to 90 years were studied. About 71% of the patients were aged between 16 and 40 years while the 41 years and above patients constituted 13.5% (**Fig. 1**). Antenatal care attendants were the highest, followed by patients whose conditions were not clearly diagnosed (**Table 1**).

Antihelmentic Drug Usage: A total of 620 patients of the studied population reported of having taken some form of antihelmentic drug in the 3 months preceding the study. The highest antihelmentic drug usage was found among the 26-30 years age group, followed by those 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 (**Fig. 2**).

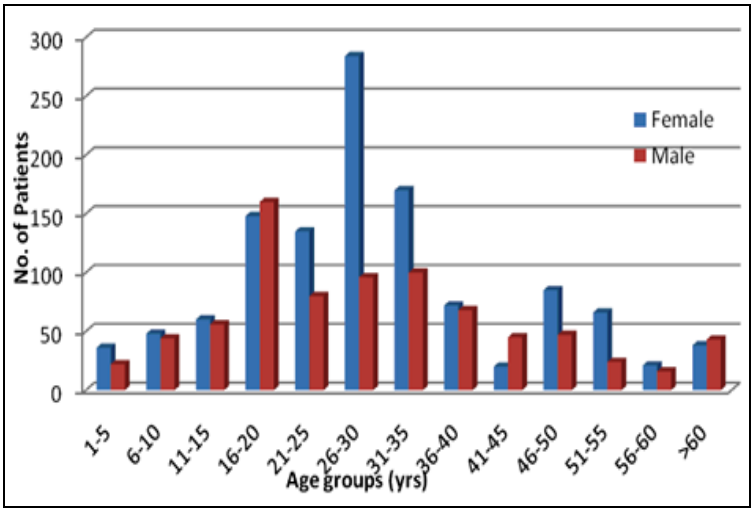

FIG. 1: DEMOGRAPHIC DATA OF PATIENTS STUDIED FOR HELMINTH INFECTIONS AT KATH

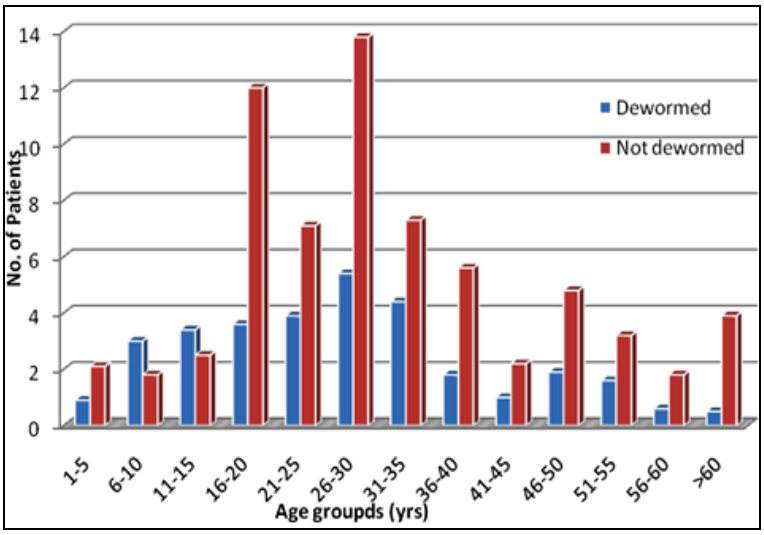

FIG. 2: REPORTED ANTIHELMENTIC DRUG USAGE AMONG THE STUDIED POPULATION

Organoleptic Characters of Stool Specimens: Most of the stool specimens (75.7%) were normal in nature, while 120 were diarrhoeal specimens and 39 contained blood and/or mucus (**Table 1**).

TABLE 1: RELATIVE FREQUENCIES OF THE TYPES OF CONSISTENCY OF THE STOOL SAMPLES

Consistency type	Frequency	% Frequency
Semi-formed (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**

Helminth Parasites Prevalence: The prevalence of hookworm was 2.9 % and the overall prevalence rate of helminth parasites in the population studied was 11.1% (**Table 2**).

TABLE 2: PREVALENCE OF INTESTINAL PARASITES AMONG THE 2000 PATIENTS EXAMINED

Helminth parasite	Prevalence (%)
Hookworm	58 (2.9)
Dicrocoelium dendriticum	43 (2.1)
Strongyloides stercoralis	42 (2.1)
Schistosoma mansoni	37 (1.8)
Hymenolepis nana	28 (1.4)
Taenia species	13 (0.6)
Trichuris trichuria	2 (0.1)
Ascaris lunbricoides	0 (0.0)
Total (overall prevalence)	**226** (11.1)

Helminth Infection Intensities: The intensity of helminth infection was measured on the basis of egg counts per gram (epg) of stool [33], determined by the Kato-Katz method. Infection intensities for the soil transmitted helminths (STHs) and *S. mansoni* cases detected in the study were classified in accordance with the criteria prescribed by WHO [25]. All cases of STHs found in the study were of low intensity (**Table 3**). 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).

TABLE 3: INFECTION INTENSITY OF STHS AND *S. MANSONI* USING THE KATO-KATZ

Helminth	Infection Intensity (%)		
	Light	Moderate	Heavy
Hookworm	60.0	0.0	0.0
Ascaris lumbricoides	0.0	0.0	0.0
Trchuris trichura	2.2	0.0	0.0
Schistosoma mansoni	15.6	17.8	4.4
Total Results	77.8	17.8	4.4

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 described by Wu and Peng [30], Yoshida [31] and Little [32]. All the hookworm larvae species recovered morphological features of *Necator americanus* (**Fig. 3**).

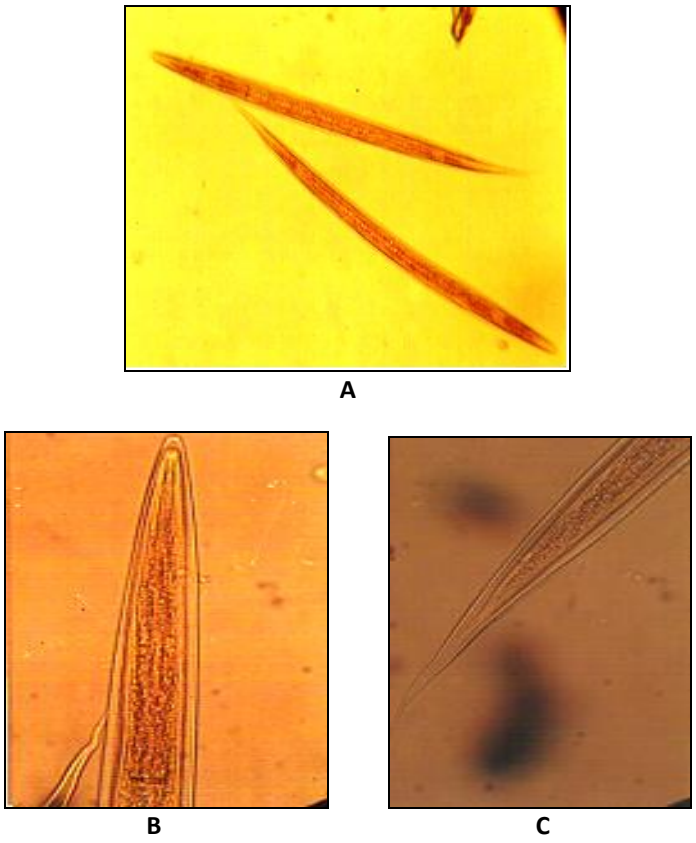

FIG. 3: PICTURES OF HOOKWORM LARVAE RECOVERED FROM THE STOOL SPECIMENS (A: WHOLE LARVAE, B: ROUNDED HEAD OF LARVAE AND C: SHARPLY POINTED END OF LARVAE)

DISCUSSION: Poor nutrition in general and anaemia in particular are the main underlying causes of poor pregnancy outcomes in developing world [34]. Hence stool examination for parasitic intestinal infections is one of the routine medical examinations for pregnant women who attend antenatal clinics in health institutions in Ghana and this accounted for the high numbers of the female participants (59.3%) in the study. Antihelmentic drug usage within the three preceding months of the study period was reported in only 31 % of the patients. This did not significantly ($p<0.05$) support the speculation that many people regularly take antihelmentic drugs.

However, children aged between 6 to 15 years who had dewormed were more than those who had not dewormed. This observation is probably a reflection of the national deworming programme for school children in Ghana under the School Health Education Programme [35-37] but there was no significant association between dewormer usage and the prevalence of helminth parasites realized from the study. This appears to provide strong indications for further studies to assess the efficacies of the various types of medicines commonly used to treat helminth infections in Ghana. The study in addition, did not show any significant association ($p<0.05$) between gender and helminth infection reported.

Out of the overall prevalence (11.1%) of parasitic helminthes in the studied population, hookworm was the highest (2.9%) and these supported our preliminary survey (unpublished data) which identified hookworm as the predominant parasite among school children in the Ayigya community of Kumasi, Ghana. Earlier epidemiological studies have also reported the predominance of hookworm infections in some communities in Ghana [38, 39], Mali [40], Kenya [41] and Nigeria [42]. As reported by Hotez *et al*., [4], hookworm infection is still a significant public health problem in Sub-Saharan Africa. Hookworm infection is associated with high intestinal blood loss; ingestion of 0.15 ml per worm per day can occur and these can results in severe iron- and or protein- deficiency anaemia [43, 44]. Species identification is important in a community as its transmission influences the burden of iron deficiency anaemia in that community and the efficacy of antihelmentic therapy also depends on the infecting species [45, 46]. In susceptible children, hookworms cause intellectual, cognitive and growth retardation, intrauterine growth retardation, prematurity and low birth weight among neonates born to infected mothers.

Out of a total of 226 specimens that showed the presence of helminthes, 25.7% were positive for hookworm (table 2). *Necator americanus* was the only prevalent species detected (Fig 3) and it was of light intensity (table 3). Other studies conducted by Tay *et al*., [38] at Kintampo in the Brong Ahafo Region of Ghana also revealed high prevalence of hookworm in the country. These findings supported the reports that *N. americanus* predominates in developing countries of the tropics and subtropics [47- 49]. However, because only 58 stool samples were cultured for larvae, the data available cannot be considered sufficient enough to conclude the absence of *Ancylostoma duodenale* infection in Ghana. *N. americanus* infection is acquired almost exclusively by active penetration of the skin [50-53].

Other parasitic helminthes such as *Dicrocoelium dendriticum* (2.1 %), *Strongyloides stercoralis* (2.1%), *Schistosoma mansoni* (1.8%), *Hymenolepis nana* (1.4%), Taenia *species* (0.6%) and *Trichuris trichuria* (0.1%) which were earlier reported in Ghana by Odei [54] and Wolfe [55] are still prevalent in the country (Table 2). These parasitic helminthes were also recently reported in other parts of the country [38, 39]. Our findings therefore, seem to agree with reports by Odei [54], King [56], Manga-Gonzalez *et al*., [57] and Pillai and Kain [58] that dicrocoeliasis is a zoonotic infection. *Schistosoma mansoni* infection was predominant among the 1 to 15 years age groups, with those in the 10-15 years age group accounting for most of the moderate and heavy infections.

Ascaris lumbricoides infection was not detected in any of the specimens examined and this disagreed with the previous reports of 2.1% prevalence in the Brong Ahafo Region of Ghana [38]. Ascariasis occurs through ingestion of food and or water contaminated with faeces containing *Ascaris* eggs. Its transmission and distribution is largely determined by inadequate sanitary practices and improper faeces disposal [8, 25, 59]. The absence could be due to the fact that adult worms of *Ascaris* are regularly found in stool of infected individuals who then self-medicate with antihelmentics [12] after seeing these worms in their stool, in addition to improvement in prevailing social environment and behaviours of people in the communities [60, 61].

CONCLUSION: In this study, a true association between dewormer usage and not being infected could not be statistically established ($p>0.05$). Also, hookworm infections are still prevalent in Ghana. Hence studies to assess the quality and efficacy of the various types of antihelmentic drugs on the Ghanaian market are necessary. Further, studies are also needed to determine the contributing factors for the absence of *A. lumbricoides* and confirm its absence.

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