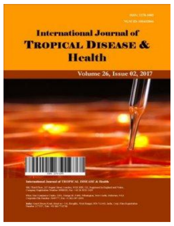

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Prevalence of Soil-Transmitted Helminths among School Pupils in the Upper East Region of Ghana Using Direct Wet Mount Technique and Formol-Ether Concentration Technique

Yaw Sam[1,2], Fred Jonathan Edzeamey[3*], Enock Henebeng Frimpong[1], Ansah Kofi Ako[2] and Kwaku Appiah-Kubi[4]

[1]Department of Clinical Microbiology, School of Medical sciences, Kwame Nkrumah University of Science and Technology, Ghana.
[2]Laboratory Department, Konongo-Odumase Government Hospital, Ghana.
[3]Laboratory Department, Ashanti-Bekwai Municipal Hospital, Ghana.
[4]Department of Applied Biology, School of Applied Sciences, University of Development Studies, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. Author YS, EHF and FJE designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author KA managed the analyses of the study. Author AKA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The prevalence of soil-transmitted helminths has been reported to be high among residents especially school pupils in small communities located in Ghana and Sub-Saharan Africa as a whole. Gia is one of the communities in the Kassena-Nankana district of the Upper East Region of Ghana where the prevalence of soil-transmitted helminths was reported in 2007 to be 10% by the direct wet

**Corresponding author: E-mail: kwesi.mkk@gmail.com;*

mount method. The study sought to determine the current prevalence of soil-transmitted helminths among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the formol-ether concentration techniques and also to compare the performance of the two techniques employed in the study.

Methods: Three hundred and ninety-four (394) pupils were recruited from the primary schools in the Gia and Kajelo communities for the study. The study was carried out from October 2010 to March 2011. Stool samples were collected from subjects and processed within two hours after collection using the direct wet mount and the formol-ether concentration techniques.

Results: Out of the 394 stool samples examined in the study, 2.79% (Hookworm 0.25 %, *Strongyloides stercoralis* 2.54%, *Ascaris lumbricoides* 0.00% and *Trichuris trichuira* 0.00%) prevalence was observed with the direct wet mount method whilst 9.40% (*Strongyloides stercoralis* 5.08%, Hookworm 3.30%, *Ascaris lumbricoides* 1.02% and *Trichuris trichuira* 0.00%) prevalence was observed with the formol-ether concentration technique. The formol-ether concentration technique demonstrated a higher sensitivity as compared to the direct wet mount technique.

Conclusion: There is high soil-transmitted helminthic infection among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district of the Upper East Region of Ghana. However, it has been underdiagnosed due to the use of the direct wet mount technique in clinical laboratories in these communities.

Keywords: Prevalence; soil-transmitted helminths; formol-ether; wet mount; school-age children; Ghana.

1. INTRODUCTION

Soil-transmitted helminths (STHs) are worms that mostly come into contact with the soil through contamination with human faeces from an infected person [1]. Some of the species of soil-transmitted helminths that are important regarding public health are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichuira*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1]. Soil-transmitted helminths are also known in many endemic communities as common intestinal worms [2].

Prevalence of helminthic infections exceeding 70% of the population has been reported in equatorial and tropical countries of West Africa [3]. In Ghana, up to 63% infections among school- age children have been reported [2]. The major soil-transmitted helminths in Ghana are *Ascaris lumbricoides*, *Trichuris trichuira*, hookworms *Ancylostoma duodenale* and *Necator americanus* and *Strongyloides stercoralis* [4].

More than one billion people are infected with intestinal helminths worldwide. They are the most prevalent of chronic human infection [2]. The morbidity associated with these parasites is strongly related to parasite burden [2], these include pathological effects such as stunting growth, anaemia and deficient cognitive functions. The disease burden is particularly high in developing countries [2].

Over 35.4 million school- aged children in Africa are infected with *A. lumbricoides*, 40.1 million with *T. trichuira* and 41.1 million with hookworm [1]. Since many children have multiple infections, it is estimated that 89.9 million are infected with soil-transmitted helminths species.

About 44% of the infections are concentrated mainly in four African countries; Nigeria, the Democratic Republic of Congo, South Africa and Tanzania [5]. Previous estimates have suggested that 53 million school aged children (5-15years) are infected with *A. lumbricoides,* 50 million with *T. trichiura* and 47 million with hookworm [5]. According to the strategic plan for Integrated Neglected Tropical Diseases (2007- 2008) of the Ghana Health Services, there is inadequate prevalence data on soil-transmitted helminths in Ghana. The World Health Organization has emphasised the need for an epidemiological study where up-to-date information on soil-transmitted helminths is not available. The study will thus provide useful data that will inform policymakers particularly the District Health Management Team (DHMT) to roll out programmes and policies towards the deworming of the pupils to eradicate soil-transmitted helminths. This study of the prevalence of soil-transmitted helminths is therefore important for the control and elimination of soil-transmitted helminths in Ghana as a whole. The study sought to determine the current prevalence of soil-transmitted helminths among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the formol-ether concentration techniques.

2. METHODS

2.1 Study Area

The study areas are Gia and Kajelo communities in the Kassena-Nankana East and West Districts (KND) of the Upper East Region of Ghana in 2011.

The Kassena-Nankana District is situated in the North-Eastern part of Ghana. (Fig. 1) It shares boundaries with Burkina Faso to the North, the Bolgatanga Municipality to the South and to the west with Builsa and Sisala Districts. The district has a total land mass of 1,675 km^2, eighty percent, (80%) of which is arable, while the remaining twenty percent, (20%) is covered by forest, rivers, hills, and eroded areas [6].

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

The Fig. 1 depicts the geographical representation of Ghana, The Upper East Region and the District of interest

2.2 Study Population

Pupils between the ages of 5 and 15 years were selected for the study from both schools. 198 of them were from the Gia primary school, and 196 of them were from the Kajelo primary school. Pupils above and below the age group mentioned were excluded from this study [7].

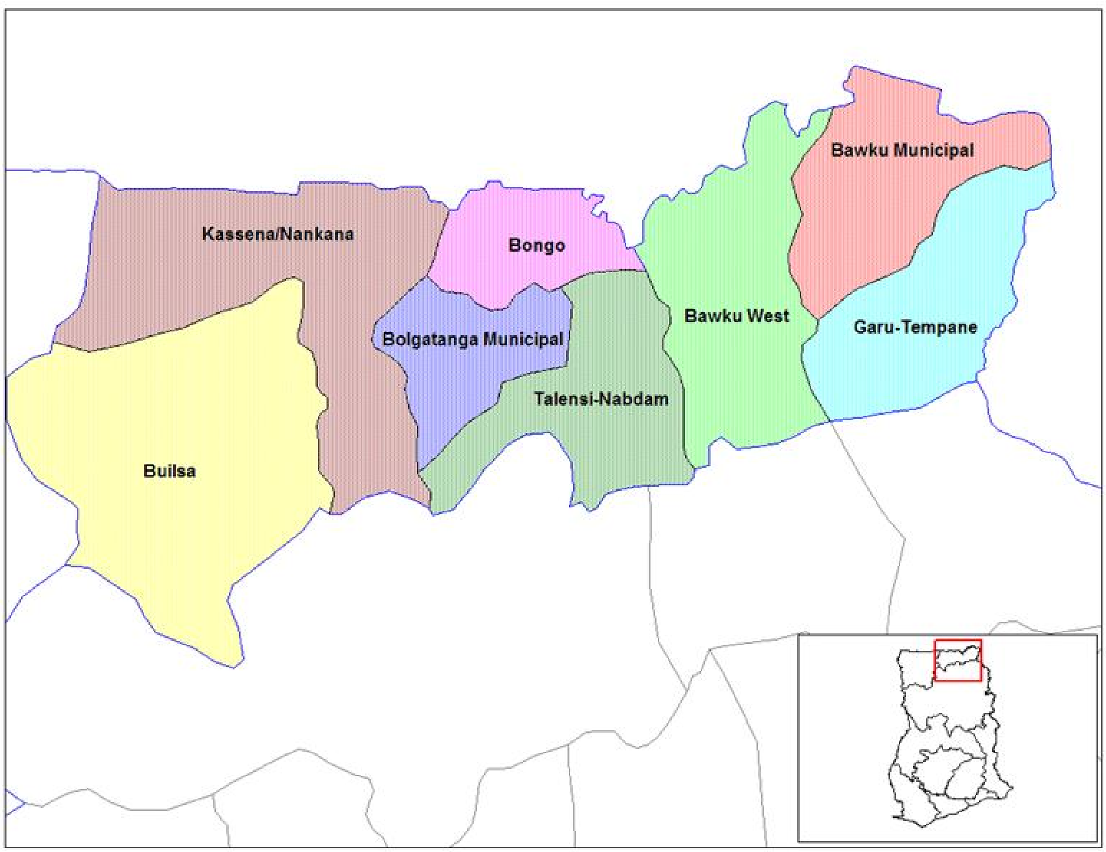

Fig. 1. Ghana map depicting the Upper East region and the Districts of Interest

A total of 394 school pupils were recruited for the study using convenient sampling technique. The sample size was calculated using the formula;

$$SS = [Z^2 \times SD\ (1- SD)] \div C^2$$

Where SS denotes the sample size, SD denotes the standard deviation, C denotes the confidence interval and Z denotes the Z- score. The confidence level was 95% (standard value of 1.96)

2.3 Study Period

The study was carried out from October 2010 to March 2011.

2.4 Sample Collection

Consented subjects were provided with clean, dry, leak-proof and wide-mouthed plastic specimen containers. They were given instructions on how to avoid contamination of stool sample with urine and further instructed to collect about half of the stool in the containers provided and to deliver them to school within 2 hours after collection [8].

Pupils who were unable to produce specimens on the same day in the school were allowed to take the specimen containers home and asked to bring freshly passed stool samples the following day. Each specimen was labelled with a study number, date and time specimen was collected. Pupils who delivered inadequate stool specimens (half of the container was considered adequate for all the tests adopted for the study with enough remaining for preservation) and/or delivered them later than 2 hours after collection were not included in the study [9]. The samples collected were then transferred within one hour to the laboratory for analysis. A semi-structured questionnaire was administered to gather information on age, sex, parental level of education, type of water usage and anthelminthic drug usage in the past one year.

2.5 Laboratory Investigations

Two parasitological methods were used for this study, namely the direct wet mount and formol-ether concentration techniques. Each specimen was first examined macroscopically, and its consistency was recorded as formed (F), semi-formed (SF), bloody-mucoid (BM), loose (L) or Watery (W), in accordance with the description by Ash and Orihel [10]. Samples were analysed fresh, in batches, as soon as they are received; none was preserved in the refrigerator or any preservative added prior to processing, as this will kill ova and larvae of the organism. The test procedures were carried out following standard protocols as described by Garcia [11] and WHO [12]. The steps have been outlined below;

2.5.1 Direct wet mount technique

With a marker pen, the study identification number was written at one end of the slide, and a drop of physiological saline was placed in the centre of the slide. With a wooden applicator stick, a small portion of stool specimen was picked and thoroughly emulsified to make a thin uniform saline suspension- not too thick that faecal debris may obscure organism, and not too thin that blank spaces may be present.

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

When organisms or suspicious objects were detected, the 40x objective lens was used to examine the detailed morphology of the object for confirmation.

2.5.2 Formol-ether concentration technique

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

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

Three ml of ether was added to the suspension in the tube, rubber stopper was used and shaken vigorously for 10 seconds.

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

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

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

2.6 Statistical Analysis

The data were analysed using the Statistical Package for Social Scientist (SPSS) Statistical Software (version 17.0, SPSS Inc., Chicago, IL, USA). Chi-square (x^2) test was used for all categorical variables and all other statistical comparisons, the level of significance was set at $p<0.05$.

2.7 Ethical Approval

The study protocol was sent for review and approval from the Committee on Human Research, Navrongo Health Research Centre and Kassena-Nankana District Health Management Team (DHMT). Permission to undertake the study at the War Memorial Hospital and Tongo Health Centre Laboratories was sought and granted by the hospital management and the head of the District Laboratory. The heads of both schools and parents/guardians of the pupils, who were enrolled for the study, gave informed consent after the full explanation about the purpose and the techniques of the study were given.

3. RESULTS

3.1 Demographic Characteristics

Of the 394 studied subjects, 192 (48.7%) were males, and 202 (51.3%) are females. Children aged between 5-10 years constituted 52.1% of the total subjects studied. Children between the ages of 11-15 years also constituted 48.0% of the total subjects studied.

3.2 Macroscopic Examination of Specimen

The Table 1 represents the relative frequencies of the types of consistency of stool samples corrected from the studied patients. It shows that most of the stool specimens, 228 in number (57.87%) were semiformed or soft, whereas mucoid stool was less frequent, only 5 (1.27%) in number.

3.3 Prevalence of Soil-transmitted Helminthes in the Gia Community

According to the table 2, the study revealed that the prevalence of soil-transmitted helminthes in Gia is 3.54% with the direct wet mount method against 10.61% with the formol-ether concentration technique".

Table 1. Relative frequencies of consistency of stool samples

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

3.4 Prevalence of Soil-transmitted Helminthes in the Kajelo Community

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

3.5 Prevalence of soil-transmitted helminths in the Gia and Kajelo Communities

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

3.6 Performance of Direct Wet Mount Method against the Formol- Ether Concentration Technique (the Gold Standard) in Detection of the Soil-transmitted Helminth Parasites

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

Table 2. Prevalence of soil-transmitted helminthes in the Gia community

Helminthes	Direct wet mount (n=198)		Formol-ether concentration (n=198)	
	№	Percent (%)	№	Percent (%)
Hookworm	1	0.51	10	5.05
S. stercoralis	5	3.03	7	3.54
A. lumbricoides.	0	0.00	4	2.02
T. trichuira	0	0.00	0	0.00

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

Helminths	Direct wet mount(n=196)		Formol-ether concentration (n=196)	
	№	Percent (%)	№	Percent (%)
Hookworm	0	0.00	3	1.53
S. stercoralis	5	2.25	13	6.63
A. lumbricoides	0	0.00	0	0.00
T. trichuira	0	0.00	0	0.00

Table 4. Variation of the prevalence of soil-transmitted helminths in Gia and Kajelo communities

Helminths	Direct wet mount (n=394)		Formol ether concentration (n=394)	
	№	Percent (%)	№	Percent (%)
Hookworm	1	0.25	13	3.30
S. stercoralis	10	2.54	20	5.08
A. lumbricoides	0	0.00	4	1.02
T. trichuira	0	0.00	0	0.00

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

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

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

4. DISCUSSION

The higher female ratio can be attributed to most parents now embracing the idea of sending their female children to school [13]. The prevalence of 5.08% *S. stercoralis* detected by the formol-ether concentration technique compared with 2.54% by the direct wet mount method highlighted the lack of sensitivity of the direct wet mount for detection of *S. stercoralis* infection. In the study, 0.25% prevalence of hookworm was detected by the direct wet mount method as compared to the 3.30% by the formol-ether concentration technique. Hookworm infection was found in pupils in almost all the age group examined. This finding is significant because hookworm is not known to be endemic in the Upper East Region probably because of a missed diagnosis or solely because the direct wet mount is the sole diagnostic method used in most laboratories in the Upper East Region. Based on this study and other studies elsewhere [14,15], hookworm infection is best detected in stool samples by the formol-ether concentration technique.

Ancylostomiasis is associated with greater intestinal blood loss; ingesting 0.15 ml per worm per day and causing severe iron-deficiency anaemia [16] while *N. americanus* infection is acquired almost exclusively by active penetration of the skin, *A. duodenale* can infect both percutaneously and by the oral route and also causes infantile ancylostomiasis, through transplacental or lactogenic transmission. *A. duodenale* is also reported to differ in susceptibility to the same anthelminthic and dosage regimen [1997]. Consequently, the efficacy of anthelminthic therapy is depended on the species of hookworm [17,18].

The low prevalence of *A. lumbricoides* (1.02%) and the absence of *T. trichuira* can be attributed to the very low average annual rainfall of 850 mm and the extreme average annual temperature range of (18-45°C) in the Kassena-Nankana district. The eggs of both species require an optimal temperature of about 31°C for embryonation whilst temperature of 38°C is lethal. Areas where the average annual rainfall falls below 1400 mm, usually demonstrate the absence of transmission [3]. Several studies in Sahalean countries; Mali [19], Mauritania [20] have demonstrated the absence of transmission of these two parasites. In addition, it cannot be attributed to a lack of diagnostic sensitivity because the formol-ether concentration technique was used in this study. The method is well noted for its diagnostic capability and is widely used in epidemiological and clinical studies on the diagnosis of intestinal helminthic infections [11]. The transmission and distribution of *A. lumbricoides* and *T. trichuira* are largely determined by inadequate sanitary practices and the local habits in the disposal of faces [2]. Current opinions suggest that the absence of *A. lumbricoides* is due to the improvement in the prevailing social environment and behaviours of people in communities [21]. Therefore, there is the need for further studies to assess current trends of ascariasis, trichuriasis and other helminths to understand the epidemiological patterns of these helminthic infections.

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

In other words, the direct wet mount method exhibited lower performance; being about three times less sensitive than the formol-ether concentration technique for the detection of intestinal helminths. Indeed, a significant difference ($p<0.05$) observed in the sensitivities of the two methods (formol-ether concentration technique and direct wet mount method) used in this study have been reported in other studies that have compared these methods [22].

Lack of sensitivity of the direct wet mount method is highlighted in this study and others elsewhere [23,24], and hence support the argument that most laboratories in the country underestimate the true prevalence rates of helminthic infections among pupils.

The overall prevalence of 2.79% of intestinal helminthes parasite observed in this study for the direct smear is comparable to the rates that have been reported elsewhere [23,24]. This supports the fact that the direct wet mount is less sensitive in identifying helminths in stools of pupils. The need for introducing stool concentration technique in routine laboratory practice becomes compelling as reliance on the wet mount method alone may miss about three-quarters of helminthic infections.

5. CONCLUSION

The overall prevalence was 2.79% at both Gia and Kajelo communities with the direct wet mount method and 9.40% with the formol-ether concentration technique. The study indicated that the formol-ether concentration method is superior to the direct wet mount methods for routine diagnosis of intestinal helminthic infections. The study concluded that the low prevalence of intestinal helminths is due to lack of sensitivity of the traditional direct wet mount method. Hence, parasitology laboratories in the country have underestimated the 'true' prevalence of intestinal helminthic infections.

6. RECOMMENDATIONS

It is recommended that stool samples that are found negative for parasites by the traditional direct wet mount method should be re-examined by the formol-ether concentration technique as a confirmatory test. The formol-ether concentration technique, when used as a confirmatory test, will increase the detection of intestinal helminthic infections.

CONSENT AND ETHICAL APPROVAL

The study protocol was sent for review and approval from the Committee on Human Research, Navrongo Health Research Centre and Kassena-Nankana District Health Management Team (DHMT). Permission to undertake the study at the War Memorial Hospital and Tongo Health Centre Laboratories was sought and granted by the hospital management and the head of the District

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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