

Original Research Article

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

Abstract

The prevalence of soil-transmitted helminthes 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 helminthes has been reported in 2007 to be 10% prevalent by the direct wet mount method. The study sought to determine the current prevalence of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the formol-ether concentration techniques and to also 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 carried out from to 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 trichiura* 0.00%) prevalence was observed with the direct wet count method whilst 9.40 % (*Strongyloides stercoralis* 5.08%, Hookworm 3.30%, *Ascaris lumbricoides* 1.02 % and *Trichuris trichiura* 0.00%) prevalence was observed with the formol-ether concentration technique. The formol-ether concentration technique demonstrated a very high sensitivity as compared to the direct wet mount technique.

26 **Conclusion:** There is high soil-transmitted helminthes infection among primary school pupils
27 in Gia and Kajelo communities in the Kassena-Nankana district of the Upper East Region of
28 Ghana. However, this is being misdiagnosed due to the use of the direct wet mount technique
29 in clinical laboratories in this communities

30 **Key words:** prevalence, Soil-transmitted helminthes, formol-ether, wet mount, school-age
31 children, Ghana

32 1.0 INTRODUCTION

33 Soil transmitted helminthes (STHs) are those that mostly come into contact with the soil
34 through contamination with human faeces from an infected person [1]. Some of the species of
35 soil transmitted helminthes that are important in terms of public health are roundworms
36 (*Ascaris lumbricoides*) whipworms (*Trichuris trichiura*) and hookworms *Ancylostoma*
37 *duodenale* and *Necator americanus* [1]. Soil transmitted helminthes are also known in many
38 endemic communities as common intestinal worms [2].

39 Prevalence of helminthic infections exceeding 70% of the population has been reported in
40 equatorial and tropical countries of West Africa [3]. In Ghana, Annan et al. (1986) have
41 reported up to 63% infections among school age children [2]. The major soil transmitted
42 helminthes in Ghana are *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms *Ancylostoma*
43 *duodenale* and *Necator americanus* and *Strongyloides stercoralis* [4].

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

49 Over 35.4 million school aged children in Africa are infected with *Ascaris lumbricoides*, 40.1
50 million with *Trichuris trichiura* 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 helminthes 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 *Ascaris lumbricoides*, 50 million with *Trichuris 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 helminthes in Ghana. The World Health Organization has emphasized the need for epidemiological study where up- to – date information on soil-transmitted helminthes is not available. The study will thus provide useful data that will inform policy makers particularly the District Health Management Team (DHMT) to roll out programmes and policies towards the deworming of the pupils in order to eradicate soil-transmitted helminthes. This study of the prevalence of soil-transmitted helminthes is therefore important for the control and elimination of soil-transmitted helminthes in Ghana as a whole. The study sought to determine the current prevalence of soil-transmitted helminthes among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the formol-ether concentration techniques

2.0 METHODS

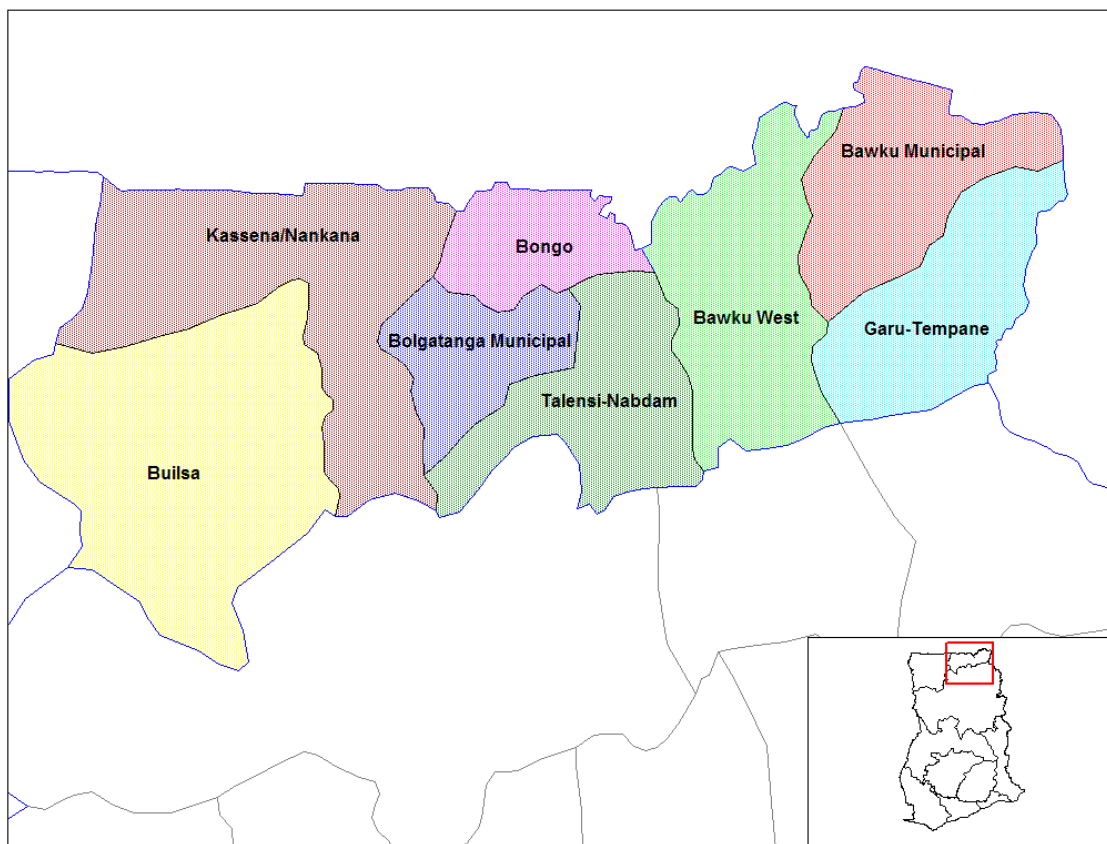
2.1 Study Area

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 in the North-Eastern part of Ghana. (**Figure 1.0**) It shares boundaries with Burkina Faso to the North, the Bolgatanga Municipality to the South and to

76 the west with Builsa and Sisala Districts. The districts has a total land masses of 1,675km²,
77 eighty percent, (80%) of which is arable, while the remaining twenty percent, (20%) is
78 covered by forest, rivers, hills, and eroded areas [6].

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



85 *The figure 1.0 above depicts the geographical representation of Ghana, The Upper East*
86 *Region and the District of interest.*

88 **2.2 Study population**

89 Pupils between the ages of 5 and 15 were selected for the study from both schools. 198 of
90 them were from the Gia primary school and 196 of them were from the Kajelo primary
91 school. Pupils above and below the age group mentioned were excluded from this study [7].
92 A total of 394 school pupils were recruited for the study using convenient sampling
93 technique. Sample size was calculated using the formula;

$$94 \quad n = \frac{t^2 \times p(1 - p)}{m^2}$$

95 Where, 't' is the confidence level at 95% (standard value of 1.96)

96 'p' is the estimated prevalence

97 'm' is the margin of error at 5% (standard value of 0.05)

98

99 **2.3. Study period**

100

101 **2.4 Sample collection**

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

106 Pupils who were unable to produce specimens on the same day in the school were allowed to
107 send the specimen containers home and asked to bring freshly passed stool samples the
108 following day. Each specimen was labelled with a study number, date and time specimen was
109 collected, and time specimen was received. Pupils who delivered inadequate stool specimens
110 (half of the container was considered adequate for all the tests adopted for the study with
111 enough remaining for preservation) and/or delivered them later than 2 hours after collection
112 were not included in the study [9]. The samples collected were then transferred within one
113 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 anthelmintic drug usage in the past one year.

2.4 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 analyzed fresh, in batches, as soon as they are received; none was preserved in the refrigerator or any preservative added prior to processing, as this will kill ova and larvae of the organism. The test procedure were carried out in accordance with standard protocols as described by Garcia [11] and WHO [12]. The steps have been outlined below;

2.4.1 Direct wet mount technique

With a marker the study identification number was written at one end of the slide and a drop of physiological saline was placed in the 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 cover slip in a way as to avoid air bubbles. The slide is then placed on the stage, and the preparation was examined systematically under the low power (10 xs) objective so that the entire cover slip area is scanned for parasite ova, cysts, larvae and trophozoites.

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

2.4.2 Formol-ether concentration technique

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

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

3ml of ether was added to the suspension in the tube, rubber stoppered and 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 xs) objective, and in a systematic manner as to observe the entire cover slip area. If an organism or suspicious objects are seen, the higher magnification (40 xs) objective was used to observe its detailed morphology.

2.5 Statistical analysis

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

Ethical approval

164

165 **3.0 RESULTS**

166 **3.1 Demographic characteristics**

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

170

171 **3.2 Macroscopic examination of specimen**

172 The table 1 below represents the relative frequencies of the types of consistency of stool
173 samples corrected from the studied patients.

174 It shows that most of the stool specimens 228 (57.87%) were semifirmed or soft in
175 Nature, whereas mucoid stool was less frequent, 5 (1.27%).

176 ~~The table 1 below represents the relative frequencies of the types of consistency of 394 stool~~
177 ~~samples corrected from the studied patients.~~

178 Table 1: Relative frequencies of consistency of stool samples.

	Consistency type	Frequency	Percentage (%)
179	Semifirmed	228	57.87
180	Formed	127	32.23
181	Loose	10	2.54
182	Mucoid	5	1.27
183	Watery	24	6.09
	Total	394	100

184

185 **3.3 Prevalence of Soil-transmitted Helminthes in the Gia Community**

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

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

Techniques	Direct wet mount			Formol-ether concentration		
Helminths	n	Positive	Percent. (%)	n	Positive	Percent. (%)
Hookworm	198			198		
<i>Strongyloides stercoralis</i>						
<i>Ascaris lumbricoides</i>						
<i>Trichuris trichiura</i>						

190

Helminthes	Direct wet mount		Formol-ether concentration	
	n=198		n=198	
	Positive	Percentage (%)	Positive	Percentage (%)
191 Hookworm	1	0.51	10	5.05
192 <i>Strongyloides stercoralis</i>	5	3.03	7	3.54
193 <i>Ascaris lumbricoides</i>	0	0.00	4	2.02
194 <i>Trichuris trichiura</i>	0	0.00	0	0.00
Total	6	3.54	21	10.61

195

196 3.4 Prevalence of Soil-transmitted Helminthes in the Gia **Kajelo** Community

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

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

Helminthes	Direct wet mount		Formol-ether concentration	
	n=196		n=196	
	Positive	Percentage (%)	Positive	Percentage (%)

200	Hookworm	0	0.00	3	1.53
201	<i>Strongyloides stercoralis</i>	5	2.25	13	6.63
202	<i>Ascaris lumbricoides</i>	0	0.00	0	0.00
203	<i>Trichuris trichuira</i>	0	0.00	0	0.00
	Total	5	2.55	16	8.16

204

205 **3.5 Overall Prevalence of soil-transmitted helminths in the Gia and Kajelo Communities**

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

209 Table 4: Overall **Variation** of the prevalence of soil-transmitted helminthes in Gia and
 210 Kajelo communities.

211	Helminthes	Direct wet mount		Formol-ether concentration	
		n=394		n=394	
		Positive	Percentage (%)	Positive	Percentage (%)
211	Hookworm	1	0.25	13	3.30
212	<i>Strongyloides stercoralis</i>	10	2.54	20	5.08
213	<i>Ascaris lumbricoides</i>	0	0.00	4	1.02
214	<i>Trichuris trichuira</i>	0	0.00	0	0.00
	Total overall prevalence	11	2.79	37	9.40

215

216 **3.6 Performance of direct wet mount method against the formol- ether concentration**
 217 **technique (the gold standard) in detection of the soil-transmitted helminthes parasites.**

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

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 Technique	Positive	20	40	60
	Negative	0	334	334
Total results		20	374	394

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

4.0 DISCUSSION

The high proportion of children in the study is significant because most studies on helminthes have targeted children between this age group [13]. The higher female ratio can be attributed to most parents now embracing the idea of sending their female children to school.

The prevalence of 5.08% *Strongyloides stercoralis* detected by the formol-ether concentration technique compared with 2.54% by the direct wet mount method highlighted the lack of sensitivity of the direct wet mount for detection of *Strongyloides stercoralis* infection. In the study, 0.25% prevalence of hookworm was detected by the direct wet mount method as compared to the 3.30% by the formol-ether concentration technique. Hookworm infection was found in pupils in almost all the age group examined. This finding is significant because hookworm is not known to be endemic in the Upper East Region probably because of missed diagnosis or solely because the direct wet mount is the sole diagnostic method used in most laboratories in the Upper East Region. Based on this study and other studies

elsewhere [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 0.15ml per worm per day and causing severe iron-deficiency anemia [16] while *Necator americanus* infection is acquired almost exclusively by active penetration of the skin, *Ancylostoma duodenale* is able to infect both percutaneously and by the oral route and also causes infantile ancylostomiasis, through transplacental or lactogenic transmission. *Ancylostoma duodenale* is also reported to differ in susceptibility to the same anthelmintic and dosage regimen [1997]. Consequently, the efficacy of anthelmintic therapy depended on the infecting species of hookworm [18].

The low prevalence of *Ascaris lumbricoides* (1.02%) and the absence of *Trichuris trichiura* 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 absence of transmission [3]. Several studies in Sahalean countries; Mali [19], Mauritania [20] have demonstrated absence of transmission of these two parasites. In addition, it cannot be attributed to lack of diagnostic sensitivity because the formol-ether concentration technique was used in this study. The method is well noted for its diagnostic capability and is widely used in epidemiological and clinical studies on diagnosis of intestinal helminthes infections [11]. The transmission and distribution of *Ascaris lumbricoides* and *Trichuris trichiura* are largely determined by inadequate sanitary practices and the local habits in the disposal of faces [2]. Current opinions suggest that the absence of *Ascaris lumbricoides* is due to improvement in prevailing social environment and behaviors of people in communities [21]. Therefore, there is the need for further studies to assess current trends of

ascariasis, trichuriasis and other helminthes to understand the epidemiological patterns of these helminthic infections.

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

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

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

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

5.0 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 clearly

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

Recommendations

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

Statement of ethical approval

Ethical clearance was approved by the Kassena-Nankana District Health Management Team (DHMT). Permission to undertake the study at the Wa Memorial Hospital and Tongo Health Centre Laboratories was sought and granted by the hospital management and the head of the District Laboratory. The heads of both schools and parents/guardians of the pupils, who were enrolled for the study, gave informed consent after full explanation about the purpose and the techniques of the study was given.

Disclaimer regarding Consent/Ethical Approval:

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

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