

1 Original Research Article

2 Prevalence of Soil-Transmitted ~~Helminthes~~ Helminths among School Pupils in the 3 Upper East Region of Ghana Using Direct Wet Mount technique and Formol-Ether 4 Concentration Technique

5 Abstract

6 The prevalence of soil-transmitted ~~helminthes~~ helminths has been reported to be high among
7 residents especially school pupils in small communities located in Ghana and Sub-Saharan
8 Africa as a whole. Gia is one of the communities in the Kassena-Nankana district of the
9 Upper East Region of Ghana where the prevalence of soil-transmitted ~~helminthes~~ helminths
10 has been reported in 2007 to be 10% by the direct wet mount method. The study sought to
11 determine the current prevalence of soil-transmitted ~~helminthes~~ helminths among primary
12 school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct
13 wet mount and the formol-ether concentration techniques and to also compare the
14 performance of the two techniques employed in the study.

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

20 **Results:** Out of the 394 stool samples examined in the study, ~~2.79 %~~ 2.79% (Hookworm 0.25
21 %, *Strongyloides stercoralis* ~~2.54 %~~ 2.54%, *Ascaris lumbricoides* ~~0.00 %~~ 0.00% and *Trichuris*
22 *trichiura* 0.00%) prevalence was observed with the direct wet mount method whilst ~~9.40~~
23 ~~%9.40%~~ (*Strongyloides stercoralis* 5.08%, Hookworm 3.30%, *Ascaris lumbricoides* ~~1.02~~
24 ~~%1.02%~~ and *Trichuris trichiura* 0.00%) prevalence was observed with the formol-ether

Comment [DLBH1]: Plural for helminth is helminths, without the 'e'. This is a common mistake because 'es' is often use for noun which end with 'h'. However, plural for helminth is helminths as used by all British, American and Australian helminthologists

Comment [DLBH2]: Plural for helminth is helminths, without the 'e'

Comment [DLBH3]: Remove the space between 2.79 and %.

concentration technique. The formol-ether concentration technique demonstrated a very high sensitivity as compared to the direct wet mount technique.

Conclusion: There is high soil-transmitted helminthes-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.

Key words: prevalence, Soil-transmitted helmintheshelminths, formol-ether, wet mount, school-age children, Ghana

1.0 INTRODUCTION

Soil-Soil-transmitted helminthes-helminths (STHs) are these-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 helminthes-helminths that are important in terms of public health are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1]. Soil-Soil-transmitted helminthes-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, Annan et al. (1986) have reported up to 63% infections among school age children [2]. The major soil-soil-transmitted helminthes-helminths in Ghana are *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms *Ancylostoma duodenale* and *Necator americanus* and *Strongyloides stercoralis* [4].

More than one billion people are infected with intestinal helminthes-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

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49 such as stunting growth, anemia and deficient cognitive functions. The disease burden is
50 particularly high in developing countries [2].

51 Over 35.4 million school aged children in Africa are infected with *Ascaris-A. lumbricoides*,
52 40.1 million with *Trichuris-T. trichiura* and 41.1 million with hookworm [1]. Since many
53 children have multiple infections, it is estimated that 89.9 million are infected with ~~soil-soil-~~
54 transmitted ~~helminthes-helminth~~ species.

55 About 44% of the infections are concentrated mainly in four African countries; Nigeria, the
56 Democratic Republic of Congo, South Africa and Tanzania [5]. Previous estimates have
57 suggested that 53 million school aged children (5-15years) are infected with *Ascaris-A.*
58 *lumbricoides*, 50 million with *Trichuris-T. trichiura* and 47 million with hookworm [5].

59 According to the strategic plan for Integrated Neglected Tropical Diseases (2007- 2008) of
60 the Ghana Health Services, there is inadequate prevalence data on ~~soil-soil-~~transmitted
61 ~~helminthes-helminths~~ in Ghana. The World Health Organization has emphasized the need for
62 epidemiological study where up- to—_date information on soil-transmitted ~~helminthes~~
63 ~~helminths~~ is not available. The study will thus provide useful data that will inform policy
64 makers particularly the District Health Management Team (DHMT) to roll out programmes
65 and policies towards the deworming of the pupils in order to eradicate soil-transmitted
66 ~~helminthes-helminths~~. This study of the prevalence of soil-transmitted ~~helminthes-helminths~~ is
67 therefore important for the control and elimination of soil-transmitted ~~helminthes-helminths~~
68 in Ghana as a whole. The study sought to determine the current prevalence of soil-transmitted
69 ~~helminthes-helminths~~ among primary school pupils in Gia and Kajelo communities in the
70 Kassena-Nankana district using direct wet mount and the formol-ether concentration
71 techniques

Comment [DLBH4]: Use abbreviation since this is the second time it is mentioned in the manuscript.

73 2.0 METHODS

2.1 Study Area

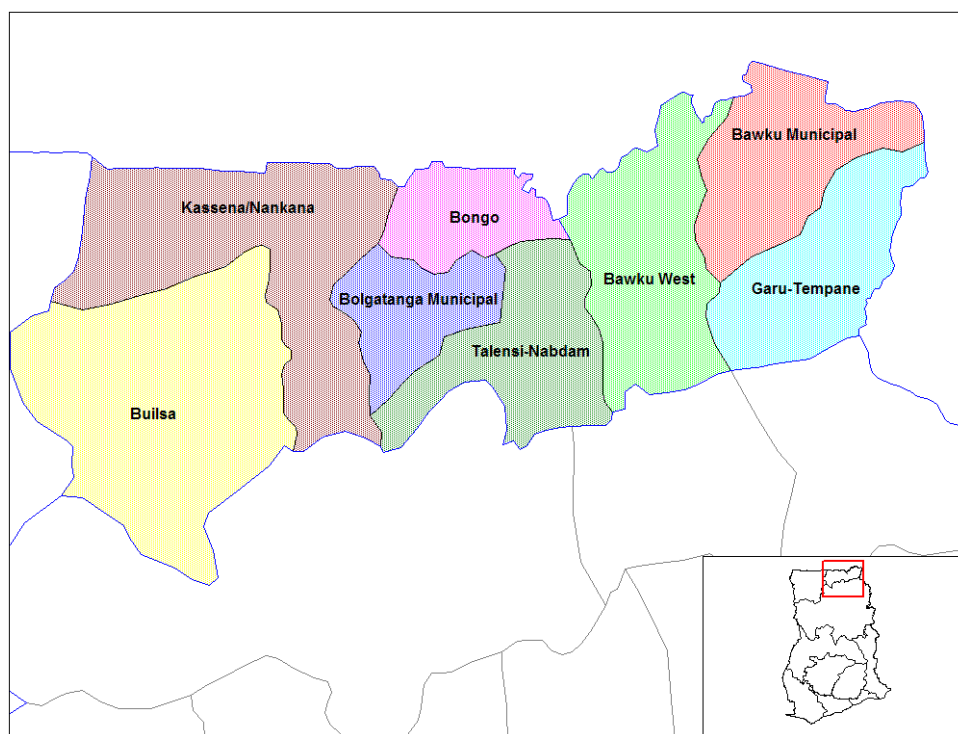
The study areas are Gia and Kajelo communities in the Kassena-Nankana East and West Districts (KND) of the Upper East Region of Ghana in 2011.

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 the west with Builsa and Sisala Districts. The ~~districts~~ district has a total land masses of 1,675 km², 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.

Comment [DLBH5]: Since the district refers to Kassena-Nanakana, then 's' should be removed.

Comment [DLBH6]: Add a space between 1,675 and km²



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

91 **2.2 Study population**

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

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

97
$$SS = [Z^2 \times SD (1 - SD)] \div C^2$$

98 Where SS denotes the sample size, SD denotes the standard deviation, C denotes the
99 confidence interval and Z denotes the Z- score.

100 theThe confidence level was 95% (standard value of 1.96)

101

102 **2.3. Study period**

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

104

105 **2.4 Sample collection**

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

110 Pupils who were unable to produce specimens on the same day in the school were allowed to
111 take the specimen containers home and asked to bring freshly passed stool samples the
112 following day. Each specimen was labelled with a study number, date and time specimen was
113 collected, and time specimen was received. Pupils who delivered inadequate stool specimens
114 (half of the container was considered adequate for all the tests adopted for the study with

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115 enough remaining for preservation) and/or delivered them later than 2 hours after collection
116 were not included in the study [9]. The samples collected were then transferred within one
117 hour to the laboratory for analysis. A semi-structured questionnaire was administered to
118 gather information on age, sex, parental level of education, type of water usage and
119 anthelmintic drug usage in the past one year.

120 **2.4 Laboratory investigations**

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

129 **2.4.1 Direct wet mount technique**

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

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

139 | When organisms or suspicious objects were ~~seen~~detected, the ~~high-dry (40x) object are seen,~~
140 | ~~the high-dry (40x)~~ objective lens was used to ~~see more~~examine the detailed morphology of
141 | the object for confirmation.

142 | 2.4.2 Formol-ether concentration technique

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

Comment [DLBH7]: Unlike percentage (%), in SI unit, there must be a space between 10 and ml.

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

148 | ~~3ml~~Three ml of ether was added to the suspension in the tube, rubber stoppered and shaken
149 | vigorously for 10 seconds.

Comment [DLBH8]: It is a normal convention to start a sentence with a word and not a number.

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

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

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

161 | 2.5 Statistical analysis

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

166 Ethical approval

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

Comment [DLBH9]: It would be good to add the REFERENCE NUMBER of the ethical approval in bracket.

174

175

176 3.0 RESULTS

177 3.1 Demographic characteristics

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

181

182 3.2 Macroscopic examination of specimen

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

185 It shows that most of the stool specimens 228 (57.87%) were semifformed or soft in

186 Naturenature, whereas mucoid stool was less frequent, 5 (1.27%).

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

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

193

194 3.3 Prevalence of Soil-transmitted Helminthes in the Gia Community

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

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

	Helminthes	Direct wet mount (n=198)		Formol-ether concentration	
	(n=198)				
		No	Percent (%)	No	Percent (%)
199	Hookworm	1	0.51	10	5.05
200	~~<i>S. strongyloides</i>~~ <i>stercoralis</i>	5	3.03	7	3.54
201	~~<i>A. searis</i>~~ <i>lumbricoides</i>	0	0.00	4	2.02
202	~~<i>T. richuris</i>~~ <i>trichuira</i>	0	0.00	0	0.00

203

204 3.4 Prevalence of Soil-transmitted Helminthes in the Kajelo Community

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

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

Helminthes	Direct wet mount(n=196)	Formol-ether concentration
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(n=196)					
		No	Percent (%)	No	Percent (%)
208	Hookworm	0	0.00	3	1.53
209	<i>Strongyloides S. stercoralis</i>	5	2.25	13	6.63
210	<i>Ascaris A. lumbricoides</i>	0	0.00	0	0.00
211	<i>Trichuris T. trichiura</i>	0	0.00	0	0.00

212

213 3.5 Prevalence of soil-transmitted helminths in the Gia and Kajelo Communities

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

217 Table 4: **Variation** of the prevalence of soil-transmitted ~~helminthes~~ helminths in Gia and
 218 Kajelo communities.

Helminthes					
		Direct wet mount (n=394)		Formol ether concentration (n=394)	
		No	Percent (%)	No	Percent (%)
219	Hookworm	1	0.25	13	3.30
220	~~<i>S. strongyloides</i>~~ <i>stercoralis</i>	10	2.54	20	5.08
221	~~<i>A. scaris</i>~~ <i>lumbricoides</i>	0	0.00	4	1.02
222	~~<i>T. richuris</i>~~ <i>trichiura</i>	0	0.00	0	0.00

223

224 3.6 Performance of direct wet mount method against the formol- ether concentration 225 technique (the gold standard) in detection of the soil-transmitted ~~helminthes~~ helminth 226 parasites.

227 The direct wet mount method detected a total of 20 soil-transmitted ~~helminthes~~ helminth
 228 parasites as against 60 by the formol—ether concentration technique or the gold standard.

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229 The evaluation results gave sensitivity of 33.3% (20/60) and specificity of 100% (334/334),
230 respectively.

231 Table 5: Performance of direct wet mount method against the formol-ether concentration
232 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	Positive	20	40	60
Concentration	Negative	0	334	334
Technique				
Total results		20	374	394

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

234 4.0 DISCUSSION

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

247 | Ancylostomiasis is associated with greater intestinal blood loss; ingesting ~~0.15ml~~0.15 ml
248 | per worm per day and causing severe iron-deficiency anemia [16] while ~~Necator-N.~~
249 | *americanus* infection is acquired almost exclusively by active penetration of the skin,
250 | ~~Ancylostoma-A.~~ *duodenale* is able to infect both percutaneously and by the oral route and
251 | also causes infantile ancylostomiasis, through transplacental or lactogenic transmission.
252 | ~~Ancylostoma-A.~~ *duodenale* is also reported to differ in susceptibility to the same
253 | anthelmintic and dosage regimen [1997]. Consequently, the efficacy of anthelmintic
254 | therapy depended on the infecting species of hookworm [18].

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

Comment [DLBH10]: Should be written as °C and not °C

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

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

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

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

292 | 5.0 CONCLUSION

293 | The overall prevalence was 2.79% at both Gia and Kajelo communities with the direct wet
294 | mount method and 9.40% with the formol-ether concentration technique. The study clearly
295 | indicated that the formol-ether concentration method is superior to the direct wet mount
296 | methods for routine diagnosis of intestinal ~~helminthes~~helminthic infections. The study

concluded that the low prevalence of intestinal ~~helminthes-parasites~~helminths is due to lack of sensitivity of the traditional direct wet mount method. Hence, ~~Parasitological~~parasitological laboratories in the country ~~have underestimate-underestimated~~ the 'true' ~~prevalence's-prevalence~~ of intestinal ~~helminthes-helminthic~~ 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.

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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