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% 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 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 very high sensitivity as compared to the direct wet mount technique.

Conclusion: There is high soil-transmitted helminthes 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 helminthes, formol-ether, wet mount, school-age children, Ghana

1.0 INTRODUCTION

Soil transmitted helminthes (STHs) are those that mostly come into contact with the soil through contamination with human feaces from an infected person [1]. Some of the species of soil transmitted helminthes that are important in terms of public health are roundworms (*Ascaris lumbricoides*) whipworms (*Trichuris trichuira*) and hookworms *Ancylostoma duodenale* and *Necator americanus* [1].Soil transmitted helminthes 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 transmitted helminthes 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 helminthes 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, anemia 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 *Ascaris lumbricoides*, 40.1 million with *Trichuris 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 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

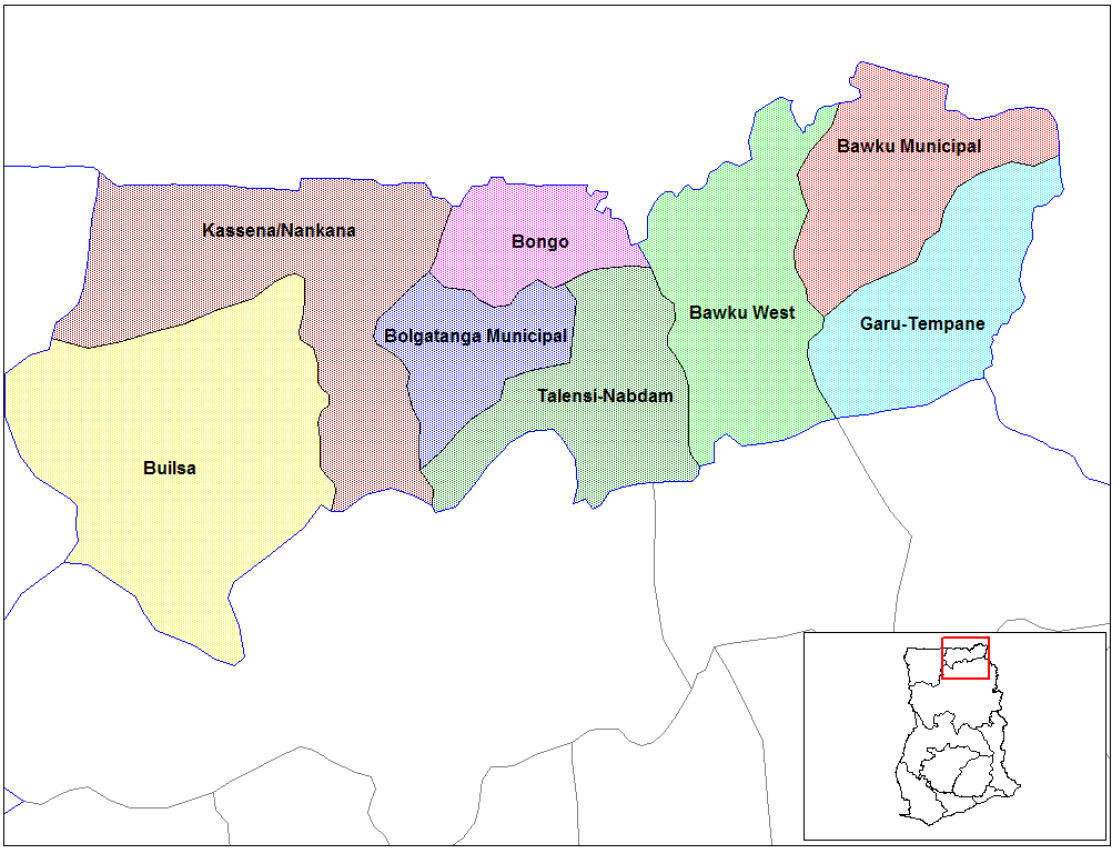
2.0 METHODS

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 the west with Builsa and Sisala Districts. The districts has a total land masses of 1,675km^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 figure 1.0 above 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].

A total of 394 school pupils were recruited for the study using convenient sampling technique. 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 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, and time specimen was received. 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.

2.4 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 (10x) objective so that the entire cove slip area is scanned for parasite ova, cysts, larvae and trophozoites.

When organisms or suspicious objects were seen, the high dry (40x) object 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 shaked 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 (x^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

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 full explanation about the purpose and the techniques of the study was given.

3.0 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 below 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 (57.87%) were semiformed or soft in Nature, whereas mucoid stool was less frequent, 5 (1.27%).

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

3.3 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 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
Strongyloides stercoralis	5	3.03	7	3.54
Ascaris lumbricoides	0	0.00	4	2.02
Trichuris trichuira	0	0.00	0	0.00

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 prevalence of 8.16% also in the Kajelo community

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

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

(n=196)				
	№	Percent (%)	№	Percent (%)
Hookworm	0	0.00	3	1.53
Strongyloides stercoralis	5	2.25	13	6.63
Ascaris lumbricoides	0	0.00	0	0.00
Trichuris trichuira	0	0.00	0	0.00

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

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

Helminthes	Direct wet mount (n=394)		Formol ether concentration (n=394)	
	№	Percent (%)	№	Percent (%)
Hookworm	1	0.25	13	3.30
Strongyloides stercoralis	10	2.54	20	5.08
Ascaris lumbricoides	0	0.00	4	1.02
Trichuris trichuira	0	0.00	0	0.00

3.6 Performance of direct wet mount method against the formol- ether concentration 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 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% *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.

Ancylostosomiasis 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, *Ancylostosoma duodenale* is

able to infect both percutaneously and by the oral route and also causes infantile ancylostosomiasis, through transplacental or lactogenic transmission. *Ancylostosoma duodenale* is also reported to differ in susceptibility to the same anthelminthic and dosage regimen [1997]. Consequently, the efficacy of anthelminthic therapy depended on the infecting species of hookworm [18].

The low prevalence of *Ascaris lumbricoides* (1.02%) and the absence of *Trichuris trichuira* can be attributed to the very low average annual rainfall of 850 mm and the extreme average annual temperature range of (18-45 ^{0}C) in the Kassena-Nankana district. The eggs of both species require an optimal temperature of about 31 ^{0}C for embryonation whilst temperature of 38 ^{0}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 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 *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, 24], 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.

Disclaimer regarding Consent/Ethical Approval:

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

REFERENCES

1. Ahmadi AN, Gachkar L, pakdad K, Ahmedi O (2007). Potency of wet mount, formalin-acetone and formalin-ether methods in detection of intestinal parasitic infections. Iranian journal of infectious Disease on Tropical Medicine 12:43-47.
2. World Health Organisation (2002). Prevention and Control of Schistosomiasis and soil-Transmitted Helminthiasis. WHO Technical Series Report 912. Geneva; WHO.
3. Brooker S, Clements ACA, Bundy DAP (2000). Towards an atlas of human helminthes infection in sub-sahar an Africa; the use of geographical information systems (GIS). Parasitology Today. 16, 303-307.
4. Ghana Health service report (2010). Soil-Transmitted infection.
5. De silva NR, Brooker S, Hotez PJ, Montresor A, Engels D and Savioli L.(2003). Soil-Transmitted helminthes infections: updating the global picture, Trends in parasitolology. 547-551.

318 6. Ghana Health Service Center for Integrated Neglected Tropical Diseases (2007).
319 Prevalence of soil-Transmitted Helminthes in the kassena-Nankana District.
320 Unpublished.
321 7. Chan MS, Bradley M, Bundy DA (1997).Transmission patterns and the epidemiology
322 of hookworm infection. International Journal of Epidemiology (26):1392-1400.
323 8. Booth M, Vounatsou P, N'Goran EK ,Tanner M and Utzinger J (2003). The influence
324 of sampling effort and the performance of the kato-katz technique in diagnosing
325 schistosoma mansoni and hookworm co-infections in rural cote d'Ivoire. Parasitology
326 127: 525-531.
327 9. Behnke JM, De Clerq D, Sacko M, Quattara DB, Vercrysse J (2000). The
328 epidemiology of human hookworm infections in the southern region of Mali. Tropical
329 Medicine and International Health (5): 343-354.
330 10. Ash LR. Orihel TC (1991). Parasites: A Guide to Laboratory procedures and
331 Identification. Chicago: American society of clinical Pathologists.
332 11. Garcia LS (2001). Diagnostic Medical Parasitology, 4th ed. ASM Press , Washington,
333 D.C.
334 12. World Health Organisation, WHO (1994). Bench aids for the diagnosis of intestinal
335 parasites. World Health Organisation. Geneva.
336 13. Pearson RD (2002). An Update on the Geohelminthes: Ascaries lumbricoides,
337 Hookworms, Trichuris trichuira and Strongyloides stercoralis. Current Infectious
338 Disease Reports 4:59-64.
339 14. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME,Loukas A, Xiao S (2004). Hookworm
340 infection. English Journal of Medicine (351): 799-807.

341 15. Barnabas MM and Aboi. JKM (2005). Missed diagnosis of schistosomiasis leading to
342 unnecessary surgical procedures in Jos university Teaching Hospital. Tropical Doctor.
343 35:96-97.
344 16. Albonico M, Stoltzfus RJ, Savioli L, Tielsch JM, Chwaya HM, Ercole E, Cancrini
345 G (1998). Epidemiological evidence for a differential effects of hookworm species,
346 *Ancylostom duodenale* or *Necator americanus*, on iron status of children. International
347 journal on Epidemiology 27:530-537.
348 17. Reynoldson J A, Behnke JM, Pallant LJ, Macnish MG, Gilbert F, Giles S, Spargo RJ
349 and Thompson RC (1997). Failure of pyrantel in treatment of human hookworm
350 infections (*Ancylostoma duodenale*) in the Kimberley Region of Northern West
351 Australia. Acta Tropica 68,301-312.
352 18. Horton J (2003). Global anthelminthic chemotharepy programs : learning from history.
353 Trends in parasitology. (19): 405-409.
354 19. De clerq D, Sacko M, Behnke JM, Traore M, Vercruysse J(1995). Schistosoma and
355 geohelminth infections in Mali, West Africa. Annales de la Société Belge de Medicine
356 Tropicale. 75: 191-199.
357 20. Urbani C, Toure A, Hamed AO, Albonico M, Kane I, Cheikna D, Hamed NO,
358 Montressor , Savioli L (1997). Parasitoses intestinales et Schistosomiasis dans la vellee
359 du fleuve Senegal en Republique Isamique de Mauritanie, Medicine Tropicale. 57:
360 157-160.
361 21. Goodman D, Haji HJ, Bickle QD, Stoltzfus RJ, Tielsch JM, Ramsan M, savioli L, and
362 Albonico M (2007). A comparison of methods for dedecting the eggs of Ascaris ,
363 Trichuris, and hookworm in infant stool, and the epidemiology of infection in
364 zanzibar infants, American journal of Tropical Medicine & Hygiene (76): 725-731.

365 22. Akujobi CN, Iregbu KC and Odugbemi TO (2005). Comparative evaluation of direct
366 stool smear and formol-ether concentration methods in the identification of
367 Crytosporidiumm species. Nigerian Journal of health and Biomedical sciences. 4 (1):
368 5-7.
369 23. Oguama VM and Ekwunife CA (2007). The need for a better method: comparison of
370 direct smear and formol-ether concentration techniques in diagnosis of intestinal
371 parasites. The internet journal of Tropical Medicine 3(2)