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REASSESSMENT OF HELMINTH INFECTIONS IN GULU MUNICIPALITY NOTHERN UGANDA AFTER THE TWENTY YEARS OF INSURGENCY : USING THREE DIAGONOSTIC METHODS TO COMPARE THEIR SENSITIVITY
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REASSESSMENT OF HELMINTH INFECTIONS IN GULU MUNICIPALITY NOTHERN UGANDA AFTER THE TWENTY YEARS OF INSURGENCY: USING THREE DIAGONOSTIC METHODS TO COMPARE THEIR SENSITIVITY

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ABSTRACT

***Background*: During the period of insurgency there were several internally displace people camps all over the Northern Uganda. People who lived within ten kilometers from Gulu Municipality were forced to evacuate their villages and re-locate and build huts for themselves in areas identified for them by the government. There were several of such camps within the municipality creating influx of people from the villages to the municipality for security. Now with the situation restored to normal, there is need to re-assess and update information on the prevalence of helminth infections in Gulu municipality where many of the internally displaced people (IDP) settled.**

***Objective*: To find out if S. mansoni and soil transmitted nematode infections are so prevalent and very common in children aged betweenfive to 20 years. In Gulu municipality and that additional preventive and curative measure need to be considered. Further is there a strong need to reconsider more sensitive diagnostic methods at the hospitals or does the standard approach of direct smear examination recognise at least most heavy infected children with any of the parasites.**

***Setting*. The study was carried out in Gulu municipality.**

***Design*: Purposive and random sampling methods were used.**

***Study Population*: Mainly Primary school children aged between five to 20 years randomly selected from four primary schools purposively selected around Gulu municipality were recruited for the study. For control 20 staff of each school randomly selected were also studied**

***Results*: Of the 582 samples tested, 117(20.1%) were found positive for Schistosoma mansoni. Fifteen (2.6%) other samples were found positive for other helminths like Ascaris lumbricoides, Trichuris trichiura H.nana Hookworm. The comparison between the methods showed that the results obtained by the three methods were similar for field research. There is a low intensity of infection with soil transmitted helminths found in the primary schools around Gulu municipality.**

***Conclusion*: We concluded that the prevalence and intensity of infection with soil transmitted helminths was low among the children aged between 5 to 20 years in the four primary school studied (2.6%) but there was medium infection with S.mansoi (20.1%).The sensitivity in detecting the helminthes eggs in the stool specimen were similar. Though the original Kato/Katz method recorded lowest egg count than the Polderman and Odongo-Aginya methods. This could be due to the fact that the slides were read immediately.**

INTRODUCTION

Knowledge of the presence, the distribution and intensity of helminthes infections, is needed at different levels. At the National level, it is a prerequisite for planning, prevention and control strategies. At the hospital level or at the local level in general, it is of paramount importance to decide on diagnostic strategies in the Out Patient Department (OPD) and in the laboratory (1-3). Uganda is one of the few countries in Africa south of the Sahara with extensive control programs for schistosomiasis and soil transmitted helminthes (4-7). However, in spite of the availability of nationwide data, these information are not always accessible for use at the regional hospitals or local health facilities (8, 9). Nevertheless this study has been initiated on the basis of national baseline information. Indeed, a lot of such information is available in Uganda; sometimes unpublished, and sometimes published in the form of internal reports (10-12). The distribution of most helminth infections is highly focal globally .This make information for helminthes infections not necessarily useful for a specific hospital and for the health care facilities of the patients unless the foci of helminth infection is situated in that hospitals catchment area(8,10,11,).

Perhaps even more important in helminth infections, infection rates and intensity tend to vary over time and in different patients that is particularly important in and around Gulu after the twenty years of insurgency. Last prevalence data were collected before the beginning of the insurgency in Gulu district and are likely to have changed considerably during and after the years of insurgency (personal communication from The Government Vector Control Department Wandegeya to Professor Odongo-Aginya). This study area is poverty stricken and has undergone important ecological changes that are likely to have resulted in important changes in helminth transmission. Research into the present-day prevalence and intensity of infection for the purpose of improving the quality of health care in Gulu hospitals requires that diagnostic methods to be used should be reliable and accurate to be used in day to day diagnosis (4-6). Only then will results of the research be of recognizable relevance for day to day practice. To create data base that are suitable to complement data generated in the field by the national control programs, it is essential to make use of comparable methodology (13-16). We used the three methods firstly to compare their reliability, sensitivity and ease of using them in the field research for the diagnosis of intestinal helminths. Secondly the newly developed Odongo-Aginya-staining method for thick-smear examination should be used and evaluated in more depth to assert the claim that its fast, eggs of parasites are well preserved and even that of hookworm eggs can be recovered using the method after a long period of slides incubation and finally that the larvae of hookworm and Strongyloides stercoralis can be recover by Odongo-Aginya-staining method which are most of the time missed in the Kato/Katz and Polderman method (14,15). Because strongyloides infections are generally missed or greatly underestimated with other thick-smear examination, the Odongo-Aginya method need proper attention, too (7, 16-18). Data from the period before the war in Northern Uganda suggest that Schistosoma mansoni could be highly prevalent in certain parts of Gulu towns itself and probably in villages around as well (18). The poor socio-economic conditions in the area have undoubtedly resulted in high prevalence of at least some of the soil transmitted helminths and virtually no information is available for the prevalence of *strongyloides stercoralis* in the Gulu area.

MATERIALS AND METHODS

Study site: The study was carried out in Gulu municipality which is situated approximately 330 kilometers North of Uganda's Kampala capital city. Gulu is the capital city of the Northern districts of Uganda comprising of Amuro, Gulu, Kitgum, Lamuo, Pader and Nwoya. The administrative head offices of Gulu District are housed in Gulu municipality. Nevertheless all the Northern districts were affected by the 20 years old insurgency, the influx of internally displaced people (IDP) were highest in Gulu Municipality. Gulu Municipality has both peri-urban and urban settlements at the suburb. There are few civil servants living in semi and permanent resident in the Municipality. For their earning, the majority of the residents do odd jobs like carpentry, brick lying, metal fabrication, and small scale cultivation at the suburb. There are several wild tropical fruits trees and domesticated ones like mangoes, guavas and they fruit seasonally mostly during rainy season from April to September but some wild verities fruit during the long dry season from October to March. With this good tropical climate and environment the majority of the primary school children who comes from these homes are at high exposure risks of acquiring infections with the soil transmitted helminthes,. The settlement in the IDP camps was crowded round these ares. Hence the need to established the new situation of helminth infections in and around Gulu Municipality. For convenience four Primary schools situate to the North (Laro), South (Baptist) East (Pece) and West (Kasubi) of Gulu Municipality were purposively selected and the pupils recruited in the study.

Recruitment of the pupils and sampling: Permission to study these primary schools was sought from the District Education Officials. After the consents from the parents of the minors in the lower primary classes

and from the Head Teacher of each of the primary schools, the pupils from each class were randomly selected by a lucky pick of a folded piece of paper written in YES or NO. These identical folded pieces of paper were always equal to the number of pupils in each class so that every pupil had the chance of picking the YES to be recruited in the study. These pieces of paper were first mixed in a container and spread out on top of a table. Only the pupils who picked YES were recruited and later registered in the study.

Sampling: Stool container labeled with individual code number, name, age and sex was given to each pupil during each of the school visit and they were requested to return the stool containers with about five to ten grams of stool at the time of the visit. Those who could not void the specimens had their containers kept at the school until the following day.

Materials: The two newly developed methods; the Polderman and Odongo-Aginya methods are attempts to overcome some of the short coming of the standard method, the Kato/Katz recommended by World Health Organisation (WHO) and adopted internationally for field study. The Kato-Katz method requires not less than six hours before the glycerine clears the background of the stool smear on the slide for accurate visualization of most helminth eggs. During the long waiting clearing time some fragile eggs like those of Hookworm collapse under the cellophane cover slips and disappear or become difficult to recognise under the Microscope. Furthermore the larvae of *strongyloides stercoralis*, cysts of protozoa are most of the time missed in Kato/Katz (19). The cellophane cover slips, the template for measuring the sieved feacal materials on the slides and the nylon sieves are used in all the three methods.

Kato-Katz: The Kato-Katz thick smear technique is the standard technique recommended by World Health Organisation (WHO) for the quantitative diagnosis of *Schistosoma mansoni* and other intestinal helminth infections. The glycerine in Kato Katz's technique, functions as a clearing-agent while the malachite green, besides being a dye, is bactericidal. Essentially each stool specimen initially strained through stainless steel sieve 250 μ mesh size to remove artifacts was used to fill a hole in a template measuring 41.7 milligram of stool. Cellophane cover slips presoaked in 50% glycerin to which 1% malachite green is added is placed on the feacal materials on the slide, and inverted on absorbent paper and pressed down to spread the feacal matter evenly under the cellophane cover slips and slides are left to clear for at least six hours before the slides are read under the microscopes using ×10 and ×40 objectives (13).

Polderman: Polderman direct smear method is similar to the Kato-Katz method but instead of using 41.7 mg of sieved stool sample this method only uses 25 mg. Also it does not use the malachite green as used in the standard Kato-Katz. The cellophane cover slips are pre-soaked directly in 50% glycerine as in Odongo-Aginya method. The rest of the procedure is the same as that for Kato/Katz method .With this modification, the prepared slides take a shorter time to clear due to the fact there is less faecal material is used and no stain used. This method claims to lose no sensitivity even though it only uses half of the faeces used in the Kato-Katz. However light infections could be missed due to the volume of the specimen measured on the slides

Odongo-Aginya : The Odongo-Aginya method is a modification of the classic Kato-Katz method. Where the Kato-Katz method has problems showing the eggs of hookworms, Strongyloides larvae and protozoa cysts. This method replaces the malachite green with a solution that contains 5% eosin yellow in 10% formalin, mixed 1:1 with 7.5% negrosin in 10% formalin. With this change, the method claims to conserve the hookworm eggs for a prolonged time and show the Strongyloides larvae in a microscope slide where the Kato-Katz is not able to. Essentially each stool specimen initially strained through stainless steel sieve 250μ mesh size to remove artifacts was used to fill a hole in a template measuring 41.7 milligram of stool. A drop of compound stain consisting of eosin 5% in 10% formalin and negrosin 7.5% in 10% formalin measuring about 10μl in volume was added to each measured stool smear on the slide. The stain was stirred in the stool smear on the slide. A wettable cellophane cover slip cut 32 x 41 mm pre soaked in 50% glycerin was placed on the stained stool smear and pressed down. The prepared slides can be examined immediately using x10 and x40 objectives.

Study Procedures: Three separate measured weights were delivered on three slides each containing 41.7mg of sieved stool for Kato/Katz and Odongo-Aginya methods but 25.0 milligram of the sieved stool was used in Polderman method. There were four microscopists and each of them prepared three slides for every specimen and for each method. Therefore nine slides were prepared for each specimen and method.

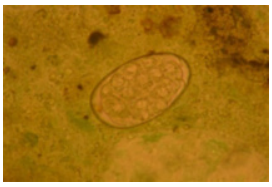
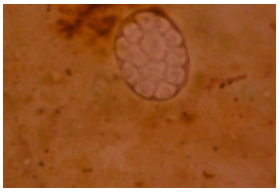
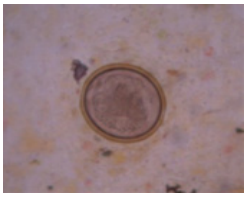
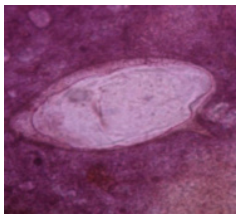
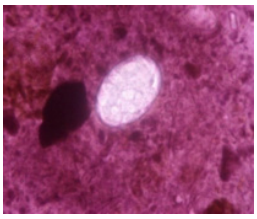
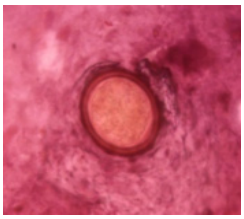
RESULTS

The results of this research defined the current distribution; intensity and prevalence of the soils transmitted helminth and Schistosoma mansoni infections in school children in Gulu municipality using the three methods. The usability of the three methods in field research has also been compared. The

percentage of positive specimens in total is 132 out of 582 (22.7%) of this 22.7% Kasubi was the highest with 10.7%; Baptist 2.6% Pece 3.6% and Laro 5.8%. Schistosoma mansoni infection was the most common parasite found (20.1%). Boys were more infected 12.9% of the 20.1% than the girls. Other intestinal parasite had low grade of infections. This was due to the fact that School deworming programme had taken place with albendozole six months prior to our study.

Plates 1

The morphological appearances of different parasite eggs in Kato/Katz, Polderman and Odongo-Aginya method are illustrated.

Methods used	S.mansoni (× 400)	Hookworm (× 400)	A.lumbricoides (× 400	Hookworm larva × 400
Kato/Katz pictures				
Polderman pictures				
Odongo-Aginya pictures				

Distribution helminth infections according to school and gender

Table 1

Shows the number of boys and girls recruited in the study and the number of stool specimens collected from each school.

Results	Kasubi	Baptist	Pece	Laro	Total
Participants recruited	160	155	149	139	603
Participants Sampled	158	151	141	132	582
Boys	68	71	71	63	273
Girls	90	80	70	69	309

Table 2

Shows the number of boys and girls found infected with different intestinal parasites in relation to the schools. As seen the distribution of infections was very different per school. Kasubi had a positive percentage of 62 out of 132(47.0%) whereas the other schools (Baptist), had 15 out of 132(11.4 %); (Pece) had 21 out of 132(15.9%) and Laro 34 out of 132(25.7%).

Positivity of boys and girls infected with different parasites					
School Locations	Kasubi	Baptist	Pece	Laro	Total
Parasite	Boys/Girls	Boys/Girls	Boys/Girls	Boys/Girls	Boys/Girls
Schistosoma mansoni	35/23	10/1	11/7	19/11	75/42
Ascaris lumbricoides	0/1	1/0	0/0	1/0	2/1
Trichuris trichuria	0/0	0/0	1/1	1/0	2/1
Hookworm	0/0	2/0	0/0	0/0	2/0
Strongyloides stercoralis	0/0	0/0	0/0	0/0	0/0
H. nana	2/1	0/1	1/0	2/0	5/2
Total Boy/Girl	37/25	13/2	13/8	23/11	86/46
Combined total	62	15	21	34	132

Since there were very few pupils infected with other soil transmitted helminth, Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana and hookworm,the intensity of S.mansoni infections in the four primary schools in Gulu Municipality was used to compared the sensitivity of the three diagnostic methods. Using the 2 × 2 table the arithmentic means of the intensity of S.mansoni eggs obtained by each method Kato/Katz; Poldermann; and Odongo-Aginya per age group in the four primary schools were compared against the gold standard Kato/Katz method.The result is show in Table 3 below.

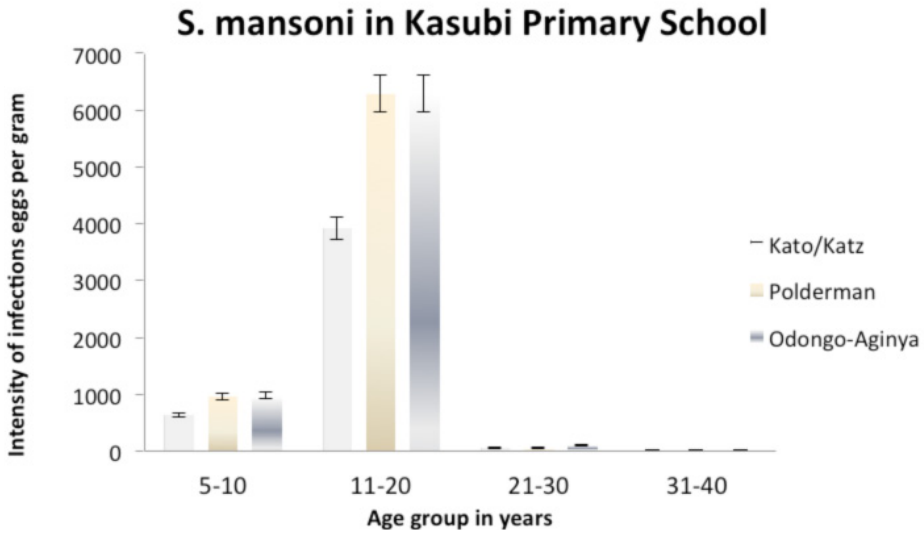
Table 3

Results of the arithmentic means of the S.mansoni eggs obtained by the three methods compared using 2 × 2 tables

Method used	S. mansoni in Kasubi primary school	S. mansoni in Baptist primary school	S. mansoni in Peace primary school	S. mansoni in Laro primary school
	P value	P value	P value	P value
Kato/katz/ Poldermann	0.006*	0.614	0.0002*	0.008*
Kato/Katz/ Odongo-Aginya	0.362	0.771	0.121	0.073
Poldermann/ Odongo-Aginya	0.000*	0.808	0.034*	0.665

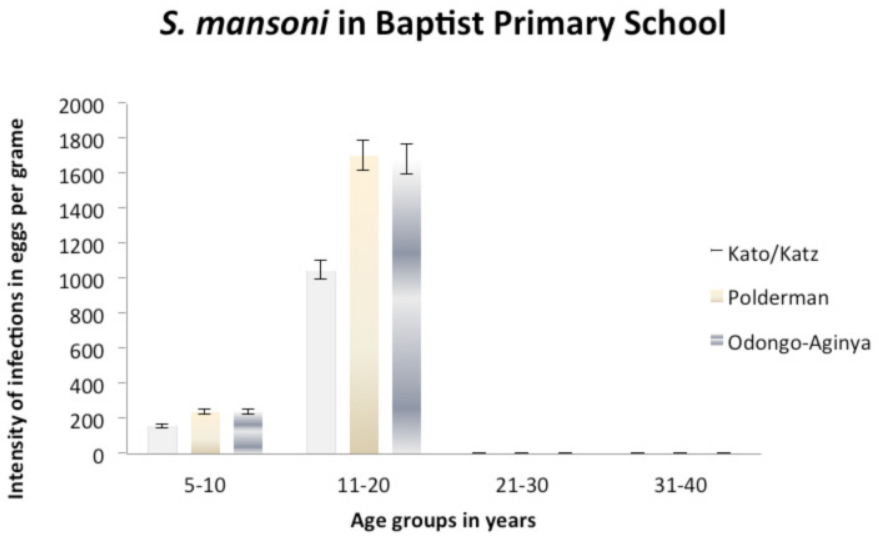
*shows significance difference in the sensitivity of the methods in detecting S.mansoni eggs.

Figure 1
Intensity of S. mansoni in Kasubi Primary School according to age group in years



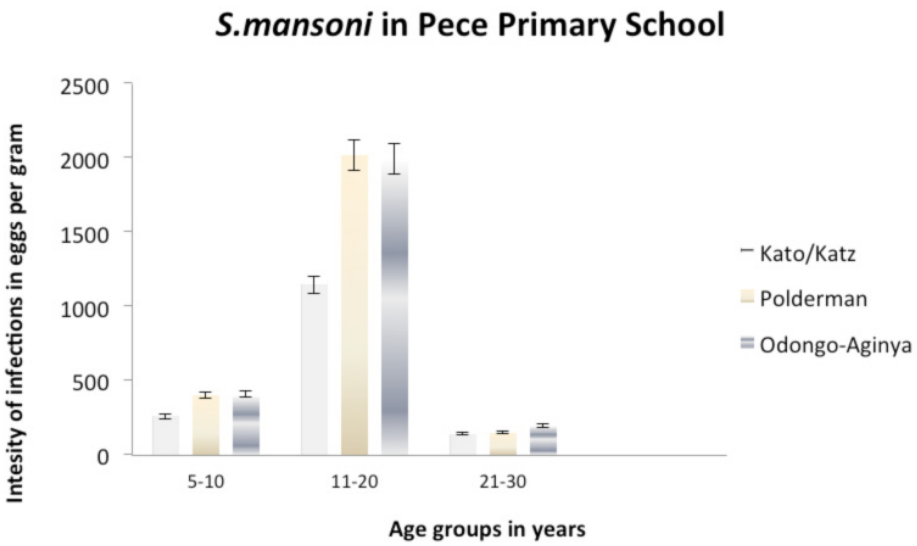
Showing errors bar at 5% values of confident interval

Figure 2
Intensity of S. mansoni in Baptist Primary School according to age group in years



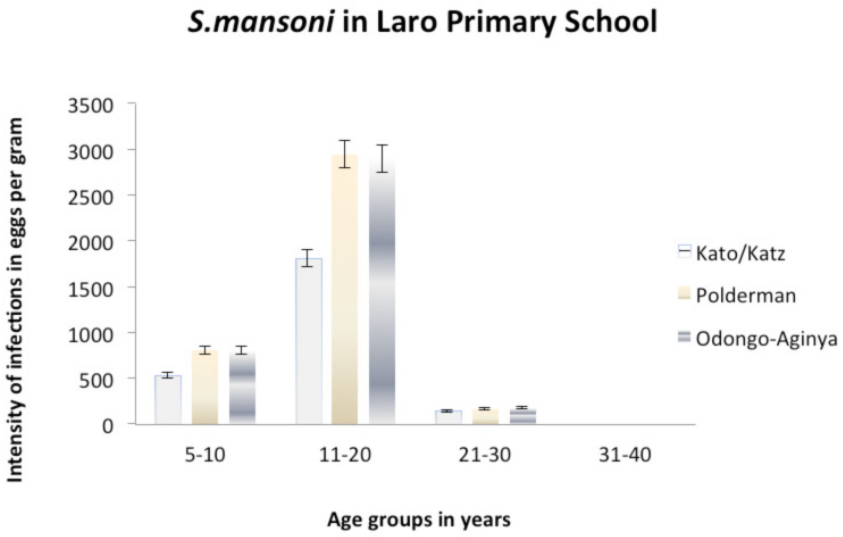
Showing errors bar at 5% values of confident interval

Figure 3
Intensity of S.mansoni in Pece Primary School according to age group in years



Showing errors bar at 5% values of confident interval

Figure 4
Intensity of S.mansoni in Laro Primary School according to age group in years



DISCUSSION

The overall results of this research showed low intensity of infections with all the intestinal helminths detected. From inquiries done at the schools, information gathered was that de-worming programme had taken place in all the schools in Gulu municipality. It was reported that the six monthly de-worming programme which is in primary health care of Uganda Ministry of Health took place using albendazole for soil transmitted helminths and praziquantel at 40mg/kg body weight for S.mansoni infections and was done six months prior to this research (4,5,6,7). Hence, the number of helminths found was low. Consequently the number of positives cases found in this study does not give an accurate indication of the helminth infections in the area because of the de-worming which must be taken into account. All of the children in this research received the treatment. The light infections observed in this study could indicate failure of the drugs used to clear the infections or re-infections (1, 2, 3, 11). Whichever way, this phenomenon indicates that interruption of treatment could result in the buildup of prevalence

and intensity of infections in these schools.

The egg counts found in this research are authentic in that every specimen was run by each microscopist three times for each of the three methods. Both negative and positive slides were interchanged among the microcopists for validation of the negativity and positivity and the total number of eggs counted in the sample. This was done to achieve true independent results. In this research however, one sample was fully examined by one person after which the, second person checked one slide out of every method used (15). The importance of independent results is shown when the prevalence and intensity is calculated using two or more different laboratory technicians each blinded on each other results (15). All of the four schools selected have permanent streams flowing near them at the distance of about 50 meters away from the school compound. Kasubi School to the West of the Municipality has stream called Ooitino; Baptist to the South of the municipality has Layebi stream, To the East of the municipality Pece school has stream pece and Laro school to the North of the municipality has Onyama stream. These streams in general are thought to be Sources of Schistosoma mansoni infections for the children in these schools as they play in and around them during breaks time at school. Kasubi had the highest infection rate compared to the other schools .Of those 59 positives found in the Kasubi school, 58 were Schistosoma mansoni and one Trichuris trichuria. The contributing factor of high rate of Schistosoma mansoni in this school is thought to be due to the fact that the school shares the stream with the army whose Barack is situated upstream of Ooitino and there was one of the largest internally displace people camp (IDPC) in Kasubi during the insurgency. When the number of positive samples per gender is compared, the percentage of boys infected (29.7%), is much higher than the percentage of girls (14.2%). The explanation to this could be linked to the fact that boys are freer and are more likely to play outside and in the water than the girls who most of the times are kept at home to help with the domestic work (4, 5, 6). This is shown in table 2, where the total number of positive samples is 132 out of the 582 samples collected. The difference between the samples at recruitment and the sample examined is that some children were unable to produce a stool sample. 132 Out of the 582 samples is a prevalence of 21.5%. Out of these 132 helminth positive samples, 117 were Schistosoma mansoni. The low number of other helminths found in this research is probably because the de-worming was carried out with the drug Albendazole and this is very effective against the other helminths that were examined but not against Schistosoma.

Comparing microscopic methods: The Kato-Katz and the Odongo-Aginya methods both use 41.7mg of stool but the Polderman direct smear method used 25.0 mg of sieved stool sample. In theory, this means that the sensitivity of the 25.0 mg should be half of the other two methods (15). In practice this difference in amount of stool sample proves to be less relevant than expected. The possible explanation for the lack of difference is that the 25.0 mg Polderman method is easier to examine under the microscope than the other two Kato methods due to the fact that the smear is thinner. The time it takes for the 25.0 mg Polderman to clear up is much less than the other two methods and the thickness of the sample on the slide make it easier to find the eggs. One of the disadvantages on the Kato-Katz and the Odongo-Aginya was the use of 41.7 mg of stool sample that most of the time proved to be too much and took a long time to clear up especially in Kato/Katz method which requires not less than six hours before the glycerine clears the background of the stool smear on the slide for accurate visualization of most helminth eggs. During the long waiting clearing time some fragile eggs like those of Hookworm collapse under the cellophane cover slips and disappear or become difficult to recognise under the Microscope. These have however been overcame in Odongo-Aginya method (15). Both Malachite green and the negrosin-eosin compound stain used in Kato/Katz and Odongo-Aginya methods respectively at time obscure the good visualization of parasites eggs on the slide especially when dealing with hard stool specimens. Nevertheless these dyes have bactericidal properties especially in the nigrosin-eosin compound stain which has 10% formalin as the base. This helps to fix and kill most microorganisms thus rendering the sample safe to handle especially in this era of HIV (15). Polderman direct smear does not provide this imperfect protection. There were 117 total positive samples for Schistosoma mansoni and that there was no method that showed all of the 117 positive samples is due to the fact that there were very light infections, in some samples which showed one egg out of nine slides and this was variably missed by the three methods used. The single oral dose of 500 mg Albendazole was used to treat infections with soil transmitted helminths in the de-worming programme (3, 7). This is probably the reason why the number of positives soil transmitted helminths found is this study was low. Albendazole is not very effective when treating Schistosoma infections. Praziquantel the drug of choice for schistosommiasis was used in this de-worming program.

CONCLUSION

We concluded that the prevalence of infection with soil transmitted helminths was low among the children aged between five to 20 years in the four primary school studied. Out of 582 pupils only 15 (2.6%) were found to be infected with various helminth like Ascaris

lumbricoides, Trichuris trichiura, Hymenolepis nana and hookworm. Nevertheless there was medium infection with S.mansoni. Out of the 582 pupils studied 117 (20.1%) of them were infected.The sensitivity of the three methods used to detect the helminthes eggs in the stool specimen were similar. The gold standard method Kato/Katz method recorded lowest egg count in all the schools and in all th age group than the Polderman and Odongo-Aginya methods. This could be due to the fact that the slides were read immediately they were pepared.

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