




RESEARCH ARTICLE

REVISED Frequencies of gastrointestinal parasites among students of primary school in Al Kalakla Locality, Khartoum State, Sudan: a cross-sectional study [version 3; peer review: 2 approved]

Previously titled: Frequencies of parasite infections among students of primary school in Al Kalakla Locality, Khartoum State, Sudan: a cross-sectional study

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Abstract

Background: Intestinal parasite spread in tropical countries is especially common among primary school students. This study aimed to determine the frequencies of the intestinal parasite by different techniques among school students in Alkalakla locality, Khartoum state.

Methods: This study was conducted in school students in Al-kalakla locality in Khartoum state from period between 20th December 2016 to 5th May 2017. Stool samples were collected from 134 randomly selected students, of whom 67 were males and 67 were females. All samples were examined using the wet preparation technique, formal ether concentration technique and saturated sugar floatation technique.

Results: The frequency of intestinal parasites was 35.5% overall in the students examined; females were more affected than males (38.8% and 32.8%, respectively). The more affected age groups were 12-14 years followed by 9-11 and 6-8 years old (53.8%, 36.3% and 26.4% respectively). The least frequent intestinal parasite was *Taenia* spp. (1.5%) followed by *Giardia lamblia* (3.7%), *Schistosoma mansoni* and *Ascaris lumbricoides* (5.2% each), *Entamoeba coli* (7.5%), *Hymenolepis nana* (10.4%), and *Entamoeba histolytica* (16.4%). In total, 20.9% were infected with single parasite while 14.9% were infected with more

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Invited Reviewers

1 2

version 3

(revision)
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version 2

(revision)
26 Nov 2019



report




report



version 1

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report

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than one parasite. The frequency of parasite by formal ether concentration method was 35.8 %, by wet preparation method was 17.9 % and by the saturated sugar flotation method was 16.4%.

Conclusion: Our data showed that intestinal parasites were common in school students; however, females were more affected than males and the 12-14-years age group was the most affected age group. The formal ether concentration method was the best method for detecting of intestinal parasite.

Keywords

Al-kalakla, *E. histolytica*, *H. nana*, formal ether concentration technique, intestinal parasite, frequencies, Khartoum.

and the Grenadines

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Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Abdalazim Hassan H:** Formal Analysis, Investigation, Methodology; **Abd Alla AB:** Formal Analysis, Writing – Original Draft Preparation; **Elfaki TEM:** Resources, Software, Visualization; **Saad MBEA:** Supervision

Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 2

We modified the aim and objective of the study in this version

In the Material and Methods section, the first subtitle changed from (Study Background) to (Study Area and Design).

In this version we have added that a negative result was obtained when we examined three slides without detecting internal parasite.

In this version we have amended the parasite stages which were detected by the technique used in this study.

In the Results section we edited the spelling mistake of word (occuarance) to the right (occurrence).

Any further responses from the reviewers can be found at the end of the article

Introduction

Intestinal parasites, particularly in tropical and subtropical areas, are a significant health issue¹. Approximately 3.5 billion individuals are estimated to be impacted in developing nations and 450 million are sick as a consequence of these diseases, the majority being children². Approximately one-quarter of the world's population is infected with intestinal parasites and about 80% of all deaths in developing nations are caused annually by infectious and parasitic illnesses³. There is a powerful correlation between the elevated incidence of these diseases and poverty, bad environmental health and insufficient health facilities³. Also involved is poor personal hygiene, an unsafe water supply and an absence of health education⁴. The transmission of intestinal parasites is based on characteristics of the parasite, actions of the individual and ecological and biological factors⁵. Transmission occurs by ingestion of contaminated fecal food or water, by hands contaminated with fecal matter coming into contact with the mouth or by skin penetration by larval stage of the parasite following direct contact with contaminated fecal soil⁶. Children of school-age are especially prone to symptoms, sometimes carrying a greater burden of parasites than adults⁷. Diagnosis is routinely performed using a microscope, with fecal samples prepared for microscopy by direct wet mounting or concentration methods. Although direct wet mounting has low sensitivity^{8,9}, it is still used in low- and middle-income countries⁹. There are many methods to concentrate the stages of intestinal parasites; cysts, eggs and larvae can be analyzed as specimens using techniques such as formal ether sedimentation and flotation¹⁰. These techniques are better than direct wet mounting since they identify more parasites¹¹.

Methods

Study area and design

This study is a cross-sectional study conducted in the locality of Al-Kalakla in the state of West Khartoum. This study was carried out between December 2016 and May 2017.

Study population

The study population was children at a primary school (Saeed primery boys school, Ebaid Khatiem primery boys school, the

martyr Abdulsalam primary girls school and Ebaid Khatiem primery girls school), between the ages of 6 and 14. The purpose of the study was explained to the guardians, students and head of school and a total of 134 participants agreed to participate in this study, they were selected randomly. The children were split into three age groups (6–8, 9–11, 12–14 years) and the same number of male and female participants was selected (n=67 of each). A labeled, large-mouth stool container was given to each selected student for collection of fecal samples. Samples were collected during the school day, all samples were subjected to examine by direct smear examination, formal ether concentration technique and Saturated sugar flotation technique.

Direct smear examination

Wet preparation was achieved by blending a small part of the stool sample taken with a wooden applicator with a drop of normal saline on a slide. The sample was enclosed with a cover slip and systematically examined under a microscope using a 10X lens and elevated magnification 40X lens for further detail observation, after searching for three slides without detecting intestinal parasite, it was considered a negative result.

Formal ether concentration technique

Approximately 1 g of feces from separate areas of the specimen was gathered and emulsified in glass beaker in 5 ml of formal saline. A further 5 ml were added and mixed from the same solution. The resulting suspension was strained through a strainer. The filtered sample was poured back into a centrifuge tube and an equal quantity of ether was added. The tube was shaken by hand for 1 min and then centrifuged at 2000 rpm for 5 minutes. The upper three layers were removed and the sediment was transported to a slide covered with a cover slip and examined at 10X and 40X magnification under a light microscope.

Saturated sugar flotation technique

Approximately 100 ml of saturated sugar solution (dextrose) was put into a glass measuring cup and approximately 1 g of stool sample was added. The fecal sample was blended with the saturated sugar solution for flotation. The fecal debris was filtered into another cup and the remaining fluid was drawn out. The preparation that was filtered was poured into a glass test tube. To the top of the tube was added fecal flotation solution. A cover slip was placed on the top of the tube. The pipe remained unchanged for 15 to 30 minutes and the covering glass was closely put on a slide and microscopically examined¹².

Identification of parasites

E. histolytica, *G. lamblia* and *Entamoeba coli* were identified by presence of a cyst or trophozoite in the stool. *Taenia* spp., *Schistosoma mansoni*, *Ascaris lumbricoides*, and *Hymenolepis nana* were identified by presence of eggs of each helminth in the stool.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (version 16). Using Chi square test, the significant obtained when $P < 0.05$. Data were presented in tables.

The sensitivity and specificity of each technique was calculated using the two formulae below:

$$\text{Sensitivity} = \frac{\text{positive cases of tested technique}}{\text{positive cases of reference technique}} \times 100$$

$$\text{Specificity} = \frac{\text{negative cases of tested technique}}{\text{negative cases of reference technique}} \times 100$$

Ethical consideration

Ethical clearance for this study was obtained from Committee of medical laboratory science, Sudan University of Science and Technology, ethical approval number (MLS – IEC – 08 – 16). Written informed consent for participation and publication of the data was obtained from the guardians of all participants included in this study.

Results

Overall occurrence of intestinal parasites

Analysis showed that 48 of the 134 stool samples collected from participants were positive for gastrointestinal parasites in Al-kalakla locality, Khartoum state. This was an overall prevalence rate of 35.8%. Infection statuses of all participants, alongside the methods used to identify infection, are available as *Underlying data*¹³.

Occurrence of intestinal parasite by variables

The research disclosed that in females the occurrence of gastrointestinal parasites was 38.8% while in males it was 32.8%. The difference in gender rates was found to be statistically insignificant ($P=0.471$; [Table 1](#)). Findings showed that age groups 6–8, 9–11 and 12–14 years had parasite prevalence rates of 26.4%, 36.3% and 53.8%, respectively. These variations in rates at $P = 0.057$ ([Table 2](#)) were statistically insignificant. The prevalence of different parasites was found as follows: *E. histolytica* (16.4%), *H. nana* (10.4%), *E. coli* (7.5%), *A. lumbricoides* (5.2%), *S. mansoni* (5.2%), *G. lamblia* (3.7%) and *Taenia* spp. (1.5%) ([Table 3](#)). The results showed that 28 (20.9%) were infected with single parasite and that there were 20 infected with more than one parasite (14.9%) ([Table 4](#)).

Correlation between parasitological techniques

The frequency of gastrointestinal parasites by various parasitological methods was as follows: 35.8% by formal concentration method, 17.9% by the wet preparation method and 16.4% by the saturated sugar flotation method. A comparison between the detection rate of each method found that detection rates were significantly different ($P = 0.000$; [Table 5](#)).

Table 1. Prevalence of intestinal parasites among gender.

Gender	Number examined	Number positive	Prevalence (%)
Males	67	22	32.8
Females	67	26	38.8
Total	134	48	35.8

$P = 0.471$

Sensitivity and specificity of techniques compared with formal ether

Assuming that the formal ether concentration technique was the gold standard, the sensitivity and specificity of the wet preparation technique was 50% and 100% respectively, and the sensitivity and specificity of the saturated sugar flotation technique was 45% and 100%, respectively ([Table 6](#)).

Table 2. Prevalence of intestinal parasite according to age group.

Age group (years)	Number examined	Number positive	Prevalence (%)
6–8	53	14	26.4
9–11	55	20	36.3
12–14	26	14	53.8

$P = 0.057$

Table 3. Prevalence of intestinal parasite according to species.

Parasite	Number examined	Number positive	Prevalence (%)
<i>E. histolytica</i>	134	22	16.4
<i>H. nana</i>	134	14	10.4
<i>E. coli</i>	134	10	7.5
<i>A. lumbricoides</i>	134	7	5.2
<i>S. mansonia</i>	134	7	5.2
<i>G. lamblia</i>	134	5	3.7
<i>Taenia</i> spp.	134	2	1.5

Table 4. Prevalence of intestinal parasite according to type of infection.

Type of infection	Number examined	Number positive	Prevalence (%)
Single	134	28	20.9
Mixed	134	20	14.9

Table 5. Correlation between parasitological techniques.

Parasitological technique	Number examined	Number positive	Prevalence
Wet preparation technique	134	24	17.9%
Formal ether concentration technique	134	48	35.8%
Saturated sugar flotation	134	22	16.4%

$P = 0.000$

Table 6. Sensitivity and specificity of techniques compared with formal ether.

Technique	Formal ether concentration technique		Total	Sensitivity	specificity
	+ve	-ve			
Wet preparation technique	+ve	24	0	50%	100%
	-ve	24	86		
Total		48	86		
Saturated sugar floatation technique	+ve	22	0	45%	100%
	-ve	26	86		
Total		48	86		

Discussion

It was evident from the results that the overall prevalence of gastrointestinal parasites among children's schools (Saeed primary boys school, Ebaid Khatiem primary boys school, the martyr Abdulsalam primary girls school and Ebaid Khatiem primary girls school), in Al-kalakla was high (35.8%) and was found to be higher than the rate reported by Muhajir *et al.* (2017) in Alkalakal (30%)¹⁴. Nevertheless, our rate was found to be lower than that reported by Gabbad and Elawad (2014) in Elengaz, Sudan (64.4%)¹⁵. The study found that females had a slightly higher occurrence of gastrointestinal parasites (38.8%) than males (32.8%). This finding was not aligned with Muhajir *et al.* (2017) in Al-kalakla, which found higher rates of infection in males (16.5%) compared to females (13.5%)¹⁴.

The highest prevalence rate (53.8%) among the 12–14 age group was reported in this study. This rate did not agree with that of Magambo *et al.* in Southern Sudan, who reported the most affected age group was those 6–10 years¹⁶. The higher occurrence of gastrointestinal parasite may be due to student's hygiene and lack of adequate water cycle in these schools.

The findings of this study indicated that the common gastrointestinal parasites in children's schools were *E. histolytica* (16.4%), *H. nana* (10.4%), *E. coli* (7.5%), *A. lumbricoids* (5.2%), *S. mansoni* (5.2%), *G. Lamblia* (3.7%) and *Taenia* spp. (1.5%), while Muhajir *et al.* reported *E. histolytica* the most common gastrointestinal parasites in Al-kalakla (15.5%), *G. lamblia* (12.5%), *H. nana* (1.5%) and *S. mansoni* (0.5%)¹⁴. However, Gabbad and Elawad in Elengaz, Sudan, revealed that *G. lamblia* was the predominant gastrointestinal parasite (33.4%), followed by *H. nana* (26.4%), *T. saginata* (8.6%), *Enterobius vermicularis* (6.2%), *S. mansoni* (4.4%) and *E. histolytica* (3.6 %) ¹⁵.

As far as the detection levels for the three methods used were concerned, it was evident that the best detection rate (35.8%) was recorded for the formal ether concentration method and the lowest rate (16.4%) was recorded for the saturated sugar floatation method, while the wet preparation method showed a detection rate of 17.9%. Our results on formal ether were not in agreement with Eisa in Keryab Village, Sudan, who reported a 90% detection rate¹⁷. However, the detection rate reported in our study was lower than the detection rate reported by Eman (44%)¹⁸.

The study found that the detection rate for wet preparation (17.9%) was lower than the detection rate reported by Eman (41.4%) in Southern Sudan¹⁸. Furthermore, detection rate for saturated sugar floatation technique (16.4%) was lower than the detection rate for Duria in Khartoum, Sudan, reported (58.6%)¹⁸.

Surprisingly, the findings showed a sensitivity of 50% and a high specificity (100%) of the wet preparation method and a sensitivity of 45% and a high specificity (100%) of the saturated sugar floatation method. This could probably be attributed to severe intestinal parasite infections among subjects studied in this study.

Conclusion

From the outcomes, it can be concluded that gastrointestinal parasites are common among children's schools in Al-kalakla, Khartoum state. Furthermore, prevalence rate was significantly greater among females, the highest rates of infection were recorded in the 12–14-years age group and, lastly, the formal ether concentration method showed the highest sensitivity level for the identification of distinct gastrointestinal parasites.

Data availability

Underlying data

Figshare: Hala and Ahmed file.sav. <https://doi.org/10.6084/m9.figshare.9804869.v1>¹³.

This project contains the following underlying data:

- Hala and Ahmed file.sav (parasites detected in stool samples for each child, with method used to detect them)
- Data dictionary (2).docx

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CCO 1.0 Public domain dedication).

Acknowledgements

We would like to express our immense gratitude and appreciation to the department of parasitology and medical entomology staff of Sudan University of Science and Technology, also to the school manager and the all participant in this study.

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Reviewer Report 28 August 2020

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 **Sandrine Lydie Oyegue-Liabagui**

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Background

The study evaluates the frequencies of the intestinal parasite among primary school students. The authors do not clearly present the objective of their work. Rather, they have the best techniques for identifying more parasites. The question then arises whether the aim of this work is the evaluation of parasite identification techniques or the determination of the prevalence of intestinal parasites among children of school age.

Therefore that the authors clearly indicate the objective of the study.

Materials and Methods

- Study background: This paragraph describes the type, location, and period of study. It might be more suitable to name it "Study area and Design".
- Direct smear examination: in this paragraph, please indicate when the sample is considered negative.
- Identification of parasites: This paragraph "Identification of parasites" should be included in the description of the methods used. e.g.: this method is used to detect eggs, cysts, vegetative forms, etc.

Results:

- Overall occurrence of intestinal parasites: replace "occurrence" by occurrence.
- It would be more interesting to correctly describe the prevalence of each intestinal parasite. Which parasite is most prevalent in the general population?

- Occurrence of intestinal parasite by variables: By age group what types of parasites are found? As far as the analysis of prevalence by age group is concerned, it would also have been interesting to make a pair-wise comparison, analysing the groups two by two using the Mann-Whitney test.
The authors only indicate that there were 20 people infected with more than one parasite. However, they do not make any mention of the different polyinfections observed in the infected study participants. Therefore, a more detailed analysis of the observed polyinfections would be necessary.

Discussion:

- There is not much discussion about the distribution of parasites by age group and why the 12-14 year old age group is the most infected as opposed to the youngest, who seem to be the least attentive to hygiene measures.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 02 December 2019

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The previous comments have been addressed in the second version of the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases (including parasitic diseases), antimicrobial resistance, and medical education

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 23 October 2019

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The study provides basic information about the frequencies of gastrointestinal parasites among children of a school in Sudan. The findings of this study may be useful to people of public health interest in the local region but can not be generalised. The following observations were noted while reviewing this manuscript:

Title:

1. Since the study:
 - a. detects only the intestinal parasites,
 - b. does not reveal anything about the clinical symptoms of parasitic infections and
 - c. involves children of only one school, the title should be changed to 'Frequencies of gastrointestinal parasites among students of a primary school in Al Kalakla Locality, Khartoum State, Sudan: a cross-sectional study'.

Methods:

1. **Study population:** The authors should clearly mention how the sampling was determined and how each of the participants was selected (e.g, random sampling).
2. **Identification of parasites:** The information (whole paragraph) about identification of parasites should be mentioned/moved after the last concentration method (i.e just before data analysis).
3. **Saturated sugar flotation technique:** The first and second sentences can be joined with removal of full stop after first sentence and use of 'and' 3rd line: 'sample of fecal' should be 'fecal sample'.
4. **Data analysis:** specificity should be determined by multiplication of the fraction by **100 (not by 10)**.
5. The authors should clearly mention (under 'Methods') whether all the samples were subject to examination by all the three methods in the study.

Results

1. The term '**occurrence**' would be better than 'prevalence' (which has its specific epidemiological meaning) in the title/contents of first two paragraphs.
2. **3rd line of 2nd paragraph: 'years'** should be added after the age groups mentioned (i.e, 6–8, 9–11 and 12–14 years).

Discussion

1. **First sentence:** The study done at one school can not be generalised. **This sentence can be restructured as** 'It was evident from the results that the overall occurrence of gastrointestinal parasites among children of a school (*school name can be mentioned*) in Al-kalakla was high.....
2. The study needs to discuss the reasons for higher occurrence:

- a. of the parasites in this study compared to that of other studies,
- b. of the parasites in females compared in males
- c. of the parasites in 12–14 years of age group compared in other age groups.

Conclusion:

The study done at one school can not be generalised. The authors can note the first comment made under 'discussion' and conclude accordingly.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases (including parasitic diseases), antimicrobial resistance, and medical education

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

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