RESEARCH ARTICLE

Prevalence of intestinal parasites among food handlers attending public health laboratories in Khartoum State, Sudan [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Infections by intestinal pathogens especially protozoans and helminths are considered to pose a real health problem, particularly in the tropics. They cause considerable morbidity and mortality rates in developing countries. The high prevalence of these infections is closely correlated with poverty, poor environmental hygiene, and impoverished health services. This study aimed to detect prevalence and frequency of parasitic infections among food handlers in Khartoum Sudan.

Methods: Three hundred and fifty Food-handlers, attending public health laboratories in Khartoum, Sudan, for an annual medical check-up, were screened for intestinal parasites by four laboratory techniques viz. direct faecal examination, formal-ether concentration, Baermann technique and agar culture method.

Results: The infection rate was 23.7% by Formol-Ether Concentration technique, followed by direct saline stool preparation (7.1%). Out of 83 positive samples the infection rate among different nationalities was as follows: Sudanese 68 (81.9%), Ethiopians 13 (15.7%), Syrians 2 (2.4%) and Egyptians 0 (0%). Intestinal parasites were more prevalent among males (73; 25.1%) than female food handlers (10; 16.9%). Three protozoans, nematodes, two tap worms and one trematode worm were detected among infected population: their frequency were as follows: *Entamoeba histolytica* (7.4%), *Entamoeba coli* (6.86%), *Giardia lamblia* (6%), *Schistosoma mansoni* (1.40%), *Necator americanus* (1.43%), *Hymenolepis nana* (0.68%), *Strongyloides stercoralis* (0.68%), *Taenia*

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Approval Status

	1	2
version 1		
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saginata (0.57%), *Ascaris lumbricoides* (0.57%) and *Trichostrongylus species* (0.29%).

Conclusion: The overall prevalence of protozoan infections among food handler in Khartoum state, Sudan was 20.26% while the helminthic infections was 5.97%. Formol-ether concentration technique is better for detection of intestinal parasites than the direct faecal smear technique. Likewise, Barmann's technique confirms detection of nematodes worms especially hookworms.

Keywords

Food-handlers, Baermann technique, Formal Ether, Agar Culture, Sudan.

This article is included in the **Neglected Tropical Diseases** collection.

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Introduction

In developing countries, intestinal parasitic infections remain a significant cause of mortality and morbidity^{1,2}. Parasitic infections can cause growth delay, iron deficiency anaemia, especially in children, and other psychological and physical health conditions³. The high prevalence of these infections is closely associated with poverty, penurious health services, and poor environmental and personal hygiene⁴.

Soil-transmitted helminth (STH) and other helminth parasites like *Taenia saginata* and *Hymenolepis nana*, represent important causative agents of gastrointestinal infections. Intestinal protozoan dwellers, mainly *Giardia lamblia* and *Entamoeba histolytica*, also, contribute to intestinal disorders¹.

Polluted soil and water sources, and poor personal hygiene are the major factors in the transmission of parasitic infections to humans through the fecal-oral route⁵. Contaminated food caused by inadequate environmental sanitation and insufficient personal hygiene by food-handlers have been implicated in epidemics of protozoan infections in humans⁶. Approximately 500 million people worldwide are diagnosed with amoebiasis, with an annual mortality between 40,000 and 110,000 according to the World Health Organization (WHO)⁷. In 1978 in Geneva the WHO scientific group on the changing pattern of food hygiene problems underlined that many of the hazards related with microbial or parasitic contamination had reduced because of the intensive efforts of food hygiene services and producers⁸. Parasitic infections in food-handlers, which are often asymptomatic, can cause a real threat to immune-compromised

patients⁹. According to the policy of Sudan ministry of health, food-handlers should be screened annually for parasitic infections. The objective of this study is to determine the infection rate and study distribution of intestinal parasite among Sudanese food-handlers in Khartoum, Sudan.

Methods

This was a descriptive cross-sectional study conducted in Khartoum state in the central part of Sudan, during the period from October 2016 to April 2017. Khartoum state is located between longitudes 31.5–34°E and latitudes 15–16°N with an area of approximately 22.142 km² (Figure 1) with a total population of about six million (see [City Population site for Sudan](#)). Stool samples were collected from public health laboratories (the public health lab in the Medical Commission) of Khartoum State; Omdurman locality, Khartoum North locality and Khartoum locality. The samples were analyzed at the Department of Parasitology, University of Science and Technology.

Sampling and sample size

A total of 350 stool samples were collected from food-handlers who attended for annual check-ups during the study period. About 101 stool samples were collected from participants in the public health lab in Medical Commission in Khartoum North locality, 160 stool samples were collected from participants in the public health Lab in Medical Commission in Omdurman locality and 89 stool samples were collected from participants in the public health Lab in the Medical Commission in Khartoum locality. The sample size was calculated using this formula¹⁰.

$$n = Z^2 PQ/d^2 \quad \text{or} \quad N = Z^2 P(p-1)/d^2$$

Figure 1. Map of Khartoum State, Sudan.

Where n = sample size

P = prevalence rate

Z = 1.96 at $\alpha = 0.05$ (α = desired confidence level)

d = desired width of confidence (precision)

Q = 100-P

There for the sample size (n) was determined as:

$$\frac{1.96^2 \times .05 \times .05}{10^2}$$

$$n = 96.04 \approx 96$$

$$N = (1.95)^2 \times (100 - 50) / 5^2 = 380$$

$$N = 380$$

Methods of samples examination

Four different methods were used for examination of stool samples: direct faecal examination, the formal-ether concentration technique, Barmenn's apparatus technique and Agar culture method.

Direct faecal examination. Direct microscopic (Olympus CX22 Microscope, Japan) examination of the sample was carried out in a systematic manner using a 4× objective lens to select the area to be screened, followed by a 10× objective lens to locate any parasitic objects. Suspicious objects were identified under a 40× objective lens. Then 2 drops of Lugol solution (Cat. No:09.0004.0500, dop®, Turkey) were added to facilitate identification of undifferentiated protozoan cysts and specimens were re-examined 5 minutes later¹.

Formal-ether concentration technique. The formal-ether concentration technique was performed by adding 1 g of faeces to 5 mL of formalin (10%), which was emulsified and strained, and the filtrate centrifuged for 2 min at 3000 rpm. Then 1 mL of sediment faeces and 9 mL of 10% formalin solution (cat no. F-04202, Oxford laboratory reagent, India,) were added to 3 mL of ethyl acetate and centrifuged further for 2 min at 2000 rpm. The upper 3 layers were decanted by inverting the tube and the last drop was allowed to fall back into the tube. Next, the filtrate was allowed to sediment by gravity for 15 min, prepared, examined and identified as in the direct smear technique¹.

Barmenn's apparatus technique. Baermann's technique was performed as described Garcia & Bruckner¹¹ by adding 5 g of fresh faeces placed in the bottom of the strainer. The strainer was placed in the funnel. Warm water (40° C) was added to cover the faeces in the strainer. It was left undisturbed for 1–2 hours to give time for Strongyloides larvae to emerge from faeces into the water. The tip of the tube was opened and 7–10 ml of the fluid was collected into a centrifuge tube. A plastic bulb pipette was used to discard the suspension fluid into a container of disinfectant. The sediment was transferred to the slide and covered with a coverslip and was examined for motile larvae using 10× magnification¹¹.

Agar culture method. The procedure of agar plate culture was performed by adding 2g of fresh stool into the center of agar plate. Area approximately 1 in diameter was placed.

The lid was replaced and the plate was sealed with cellulose tape. The agar plate was maintained (right side up) at room temperature for 2 days. The sealed plates were examined after 2 days through the plastic lid under the microscope for microscopic colonies that develop as random tracks on the agar, indicating larvae at the ends of tracks away from the stool. A hole in the top of plastic petri dish was made with hot of forceps. 10 ml of 10% formalin was added gently through the hole onto the agar surface and swirled to cover the surface. The agar plate was then rinsed and allowed to stand for 30 min. The tape and lid of agar plate were removed. The 10% formalin was poured through a funnel into a centrifuge tube. The formalin rinse fluid was centrifuged for 5 min at 500xg. A wet smear preparation was prepared from the sediment and was examined at the 10× objective (low power) for the presence of larvae if larvae were found they were then identified with a 40× objective (high dry power)¹¹.

Data analysis

The data were analyzed using SPSS (21.01) and Microsoft Excel 2010. Descriptive statistics for categorical data were formulated as frequency and percentage. Chi-Square test was used to compare of categorical data, while independent sample t-test was used to compare of numerical data. The significance level was considered at P-Value of < 0.05.

Ethical considerations

Samples were collected from participants after explanation of the importance of the study and signing the consent form. The ethical approvals were obtained from the National Committee for research, at the ministry of health. Results of direct wet preparation of samples collected were donated for treatment of all participants included in the study and some results were dispatched to a physician for treatment prescription.

Results

The total number of screened food-handlers included in the study was 350; the age of participant ranged from 16 to 68 years with an average age of 32 years, 46% of the participants were less than 29 years old compared with 54% of being 29 or above (Figure 2). The majority of participants were males (83.1.9%) and (16%) were female (Figure 3).

Distribution of samples according to the residence data showed that 101 participants were from Khartoum north (28.9%), 160 participants were from Omdurman (45.7%) and 89 participants were from Khartoum (25.4%) (Table 1). The majority of the participants were Sudanese (83.1%), followed by the Ethiopian (13.4%), Syrian (3.1%) and the Egyptian (0.03%) (Figure 4).

Direct microscopy detected intestinal parasitic infection in 7.1% of the food-handlers, by using the Formal-Ether Concentration technique (the most sensitive) the study found intestinal parasite's infection 23.7% of the food-handlers. The result of Baermann's

Figure 2. Samples distribution according to age group.

Figure 3. Samples distribution according to gender.

technique was used to detect larva of *Strongyloides stercoralis* and Hookworm (Figure 5). The Agar culture method show in (Table 2). Parasitic infection in stool samples were found by the Formal-ether and direct saline stool preparation, the most prevalent protozoan parasite was *Entamoeba coli* and for helminthic parasites was *Ascaris lumbricoides* in Khartoum north locality (Table 3). The most prevalence protozoan parasite in Omdurman locality was *Entamoeba coli* (9.4%) and for helminth parasites *Schistosoma mansoni* (1.87%) (Table 4). The most prevalence protozoan parasite in Khartoum locality was *Giardia lamblia* (3.3%) and the most prevalent helminth infection was *Necator americanus* (3.3%) (Table 5). The overall prevalence of parasitic infections among food handler in Khartoum state are demonstrated in Figure 6.

Residence and occupation were found to have a significant association with the result of the direct wet examination. The OR indicated that those respondents who working in restaurants were 2.25 times more likely to have positive test compared with others (Table 6). Occupation was found to have a significant association with the result of formal ether and the OR indicated

Table 1. Distribution of study population according to the residence.

Residence	Frequency	Percent
Khartoum north	101	28.9%
Omdurman	160	45.7%
Khartoum	89	25.4%
Total	350	100%

Figure 4. Samples distribution according to Nationality.

Baermann's technique **Figure 5. Frequency of intestinal parasites by Barmann's tech.****Table 2. Frequency of *Strongyloides stercoralis* detected by Agar culture.**

Number examined	Frequency	Percent
Negative	348	99.4
Positive	2	0.6
Total	350	100

Table 3. Prevalence of parasitic infections among food handler in Khartoum north locality.

Type of Parasite recovered	Frequency	Percent in Kh. North	In Khartoum state
<i>Giardia lamblia</i>	7	6.9%	2%
<i>Entamoeba coli</i>	8	7.9%	2.29%
<i>Entamoeba histolytica</i>	3	2.97%	0.86%
<i>Ascaris lumbricoides</i>	2	1.98%	0.57%
<i>Necator americanus</i>	1	0.99%	0.29%
<i>Strongyloides stercoralis</i>	1	0.99%	0.29%
Total	n = 101	n = 101	N=350

Table 4. Prevalence of parasitic infections among food handler in Omdurman locality.

Type of Parasite recovered	Frequency	The percentage in Omdurman locality	The percentage in Khartoum state
<i>Giardia lamblia</i>	11	6.88%	3.1%
<i>Entamoeba coli</i>	15	9.4%	4.29%
<i>Entamoeba histolytica</i>	16	10%	4.57%
<i>Schistosoma mansoni</i>	3	1.87%	0.86%
<i>Hymenolepis nana</i>	2	1.25%	0.57%
<i>Taenia spp</i>	1	0.62%	0.29%
<i>Strongyloides stercoralis</i>	1	0.62%	0.29%
Total	n = 160	n = 160	N=350

Table 5. Prevalence of parasitic infections among food handler Khartoum locality.

Type of Parasite recovered	Frequency	The percentage in Khartoum locality	The percentage in Khartoum state
<i>Giardia lamblia</i>	3	3.3%	0.86%
<i>Entamoeba coli</i>	1	1.1%	0.28%
<i>Entamoeba histolytica</i>	7	7.86%	2%
<i>Necator americanus</i>	3	3.3%	0.86%
<i>Schistosoma mansoni</i>	2	2.2%	0.86%
<i>Hymenolepis nana</i>	1	1.1 %	0.57%
<i>Taenia saginata</i>	1	1.1%	0.29%
<i>Strongyloides stercoralis</i>	1	1.1%	0.29%
<i>Trichostrongylus</i>	1	1.1%	0.29%
Total	n = 89	n = 89	N=350

that those respondents who working in restaurants were 4.23 times more likely to have positive test compared with others (Table 7). The infection rate among different nationalities was as follows: Sudanese 68 (81.9%), Ethiopians 13 (15.7%), Syrians 2 (2.4%) and Egyptians 0 (0%) (Figure 7). Intestinal parasites were more prevalent among males 73 (25.1%) over female food handlers 10 (16.9%) (Figure 8).

Dataset 1. Demographic and parasitic infection data for participants

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Discussion

For this study we select a descriptive cross-sectional and analytical facility bases study to be conducted in public health lab(s) of Khartoum State. The study was conducted between the years 2015 and 2018. This study is subject to several limitations, gastrointestinal parasitic infections are frequently reported among different sectors in Khartoum state especially in the territories of the town where hygienic conditions are poor. Most of the targeted participants do not seek any medical or health advice unless they have been to enforce to do so during routine annual medical check up. This study found that formal-ether concentration technique is the most sensitive method for diagnosing the intestinal parasites. The level of infection with intestinal helminthes in food-handlers in Khartoum state, Sudan (5.97%) was much more than that reported by Babiker *et al.* who found that 2.7% of Sudanese food-handlers were infected with intestinal helminths⁹. It also higher than that reported by Tomaso *et al.* (0.2%) among food-handlers in Austria¹². This may be the result of the migration of foreign nationals to

the capital, migration of individuals from the rural areas to urban areas, lack of personal hygiene, poor management of waste

Figure 6. The overall prevalence of parasitic infections among food handler in Khartoum state.

Table 6. Socioeconomic Correlates of the test direct wet Examination.

variable	Chi-square value	P value	OR
age	0.452	0.785	1.05
residence	8.145	0.018	-
gender	1.510	0.220	1.20
nationality	0.452	0.501	1.06
occupation	14.254	0.046	2.25

Table 7. Socioeconomic Correlates of the Formal ether concentration.

variable	Chi square value	P value	OR
age	0.211	0.616	1.09
residence	3.241	0.197	-
gender	1.790	0.182	1.09
nationality	0.155	0.724	1.02
occupation	22.140	0.032	4.23

and poor sanitization. In food-handlers of Khartoum Sudan, the infection of intestinal helminths was much lower than non-Jordanian food-handlers working in Jordan as described by

Al Lahham *et al.* who found that 13.5% of them were infected with intestinal helminthes¹³. It is also much lower than the infection rate for intestinal helminthes (18.3%) that was described by Costa-Cruz *et al.* in Brazil¹⁴.

The intestinal protozoal infection ratio of our study was low (20.26%) among food handlers in Khartoum State, Sudan. This finding was strongly supported by similar study conducted In Northwest Ethiopia, 29.1%⁵.

Our study has a low infection rate when comparing with the study conducted in 2009 by Babiker *et al.* (29.4%)⁹. This could be attributed to difference of subjects participated in the two studies. For this study, examining Ethiopians, Egyptian and Syrians gave a very low infection rate that affects the overall prevalence. Similar variation was also reported when comparing this study with other research done else were, for example, in Jordan, the frequency of infection by intestinal protozoal was 30.2%¹³.

The current study revealed that Infection with *G. lamblia* was lower than that reported in Somalia (77%)¹⁵.

Furthermore, a similar study in Jordan presented by Al-Lahham *et al.*,¹³ showed that the high rate of infection by intestinal protozoal (30.2%) which is higher than our conducted study. It has been previously reported that about 15% of food-borne disease epidemics are the consequence of infection by food-handlers¹⁶. 6.5% of food handlers in Bahir Dar town in Ethiopia had protozoal and helminthic infection¹⁷. Infected food-handlers have been involved in parasitic transmission, resulting in an

Figure 7. Infection rate among different nationalities.

Figure 8. Infection rate among Gender.

epidemic in food eateries with poor hygiene posing hazards to travelers^{18,19}. Not all of the participants had access to proper health education on food safety, although education levels could have predisposed the arrangement of infection²⁰. Numerous food-handlers did not give the impression of being aware of simple safety and health necessities to work with food and food products. The economic situation may have impact in food production and it can be affected by the foodborne disease, hence large amounts of emolument may be lost by individuals due to reduced efficiency and costs on medical care²¹. Multi-sectoral attitude to food safety at all levels cannot be overstated²².

In our study, a relationship was found in parasitic infection with occupation and residence, but no association was found between the frequency of parasite infection and age, gender, and nationality. We find that the annual monitoring of food-handlers in Khartoum is insufficient to screen parasitic infections and that more frequent screening, for example, monthly screening, should be supported. Our recommendations are that health education and personal hygiene should be included in the annual check of food handlers. The use of the most sensitive technique for diagnosing intestinal parasitic infections should be established.

Data availability

Dataset 1: Demographic and parasitic infection data for participants
<http://doi.org/10.5256/f1000research.14681.d204800>²³

Competing interests

No competing interests were disclosed.

Grant information

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Guéladio Cissé

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The paper addresses the very interesting topic of food borne diseases risks in developing countries. It presents an estimation of the prevalence of intestinal parasites among a specific exposed group in the food chain (the handlers).

The literature is not enough recent. An effort could be made by the authors to search for some more recent studies on the same topic.

The study needs further clarifications at the level of Methods(page 3):

- Using the term "a cross-sectional study" and indicating a period between October 2016 and April 2017 (6 months) is questionable. Can a background on the season(s)over the period be provided?

Sampling and sample size (page 3)

- If it is the team itself that did all the examinations with the same 4 standardized laboratory methods applied to samples from the 3 indicated laboratories, then the calculated (expected) sample size should be presented first and then the strategy to reach the number from the 3 centers be presented.
- How to reach the sample size in a rigorous and balanced way between 3 targeted laboratories?;
- Are there any exclusion criteria?
- Was there a limit of days or weeks during which the number of handlers to enroll should be reached?
- How is the team alerted about a food handler coming to the screening of a laboratory?...If possible, more explanation on the selection process could be interesting.
- It is better to not present in the first sentence the effective sample size reached, but rather provide the number under Results

Methods of samples examination

- After being presented all the 4 methods, the reader may expect a clear indication of what

method has been found more appropriate for which result;

Results

- Effectively more appropriate to indicate here the final number of handlers who successfully provided samples.
- A table could be helpful to present the major results from each method
- Please complete all the titles of figures and tables, to make sure that each title is self standing. Example: Figure 2 should better read like this: "Samples of food handlers distribution according to age in Khartoum, (October 2016 - April 2017)".
- Please do this for all titles
- More specifically for Figure 6 (page 7), maybe helpful to recall in the title the methods used for detecting these parasites.
- Figure 7: Not very much sure that this is reflecting the real percentage of infection among the different nationalities. If the figure is about the distribution of the total number of cases between the different nationalities, it does not make much sense as the Sudanese group is far much larger; it would be much more interesting to present the % among the same group (number of cases of the group/total number of the group); with this the figure could be completely something else; please check this; maybe a statistician can assist also on this

Discussion

- The first sentence is a little bit confusing. Maybe "We presented a descriptive cross sectional study ..." And therefore you should avoid a repetition of "study" in the sentence. Please rework this sentence.
- The second sentence is also with an error about dates; under Methods it was stated between October 2016 and April 2017, here it is now stated "... between years 2015 and 2018". Please correct or clarify.
- "This study is subject to several limitations". Where are they presented? Please state them clearly.
- "gastrointestinal parasitic infections ..." this sentence is starting without a capital letter and it seems completely disconnected from the sentences above. please check this
- A clear limitations paragraph is needed

Conclusion

- A clear conclusion paragraph is needed.

English

- There are a few grammatical corrections needed, and an additional careful reading may be helpful

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?

I cannot comment. A qualified statistician is required.

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

Yes

Are the conclusions drawn adequately supported by the results?

Partly

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, however I have significant reservations, as outlined above.

Reviewer Report 23 July 2018

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Mubarak M. Abdlrahman

Tropical Medicine Research Institute (TMRI), Khartoum, Sudan

Good simple study highly needed for the country like Sudan. The work is original and reflect the health situation of the foreign labor that working in the restaurants and food industry in Sudan. I have some comments on the paper:

1. In paragraph one it is Khartoum State, not just Khartoum.
2. In the third paragraph of the Introduction if the Author change the word polluted with the word contaminated is better.
3. In Table 3 if the Author use the word sentence percentage in Khartoum North in the 3ed upper cell of the table is better. And the sentences percentage in Khartoum State in the 4th upper cell is also better.
4. In Table 4 if he delete the word (the) from the upper cells is better.
5. Figure 6 should come before Table 3.

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?

I cannot comment. A qualified statistician is required.

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: Parasitology, Molecular biology, Epidemiology

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.

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