

RESEARCH ARTICLE

REVISED Frequency of parasitic infections in *Arachishypogaea L* (groundnuts), *Citrulluslanatus* seeds (watermelon seeds), and *Ziziphusspina-christi* (nabag) sold by street vendors in Khartoum State, Sudan: a cross-sectional study [version 3; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Plant products, including seeds are an important source of vitamins, minerals, proteins, and energy. This study aimed to assess parasitic contaminations in roasted groundnuts,

nabag, and tasali (watermelon seeds) sold by street vendors in Khartoum State, Sudan.

Methods: The frequency of parasitic contaminations among all crop products was detected by washing the plants with saline, and then conducting an examination using a formal ether

concentration technique (FECT), followed by a saturated sugar floatation technique.

Results: The detected parasites belonged to two species: *Entamoeba histolytica* (33.3%) and *Giardia lamblia* (15.6%). No helminthic parasites were detected. Mixed contamination of the mentioned parasites was also observed (11.1%). The most contaminated crop was nabag, followed by groundnut, and finally tasali.

Conclusion: No relation was established between the positivity of samples for parasites and crop type, Khartoum State city, or seller sex. FECT was more sensitive than the saturated sugar floatation technique as a detection method.

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1. Samar Al Nahhas, Damascus University,

Damascus, Syria

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Arachis hypogaea L , Groundnuts, Citrullus lanatus, Nabag, Tasali

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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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REVISED Amendments from Version 2

We've made changes in the conclusion section.

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

Introduction

Intestinal parasitic infections can be transmitted orally through the ingestion of infective agents from infected food, water, or contaminated hands^{1,2}. Food contamination and food borne parasitic diseases frequently occur globally. These are estimated to amount to 23.2 million cases and 45,927 deaths annually³. Fresh fruits and vegetables could be a source of dissemination of foodborne parasitic diseases^{4,5}. A study in Ghana revealed *Ascaris lumbricoides* (roundworm), *Ancylostoma duodenale* (nematode), *Necator americanus* (hookworm), and *Strongyloides stercoralis* (threadworm) contaminations in tiger nuts⁶. Another study on tiger nuts reported other contaminants such as animal droppings, fungi toxins and bacteria⁷. Groundnuts, *Ziziphus spina-christi* (nabag), and watermelon seeds (tasali) are widely consumed in Africa and the Middle East⁸. Nabag and tasali are widely eaten in Sudan.

These crops are natural sources of carbohydrates, proteins, fats, -iron, calcium, ascorbic acid, thiamine, riboflavin, and niacin^{9–12}. Groundnuts in Sudan are mainly used for oil extraction, but can be eaten as a snack: raw, or after roasting without its external cortex envelop¹³. Nabag is eaten after the sweet pulp of the fruit is dried to produce fine flour¹⁴. The flour is placed in small metal cups and steamed. Dried pulp flour and water are also mixed with sesame and shaped into small balls¹⁵. Fruit pulp prepared in these two ways can be consumed either immediately or stored for future use. In addition to groundnuts, nabag, and tasali¹⁶, many crop products represent an important and for some, the only source of income in Sudan¹⁵.

Intestinal parasitic infections are very common worldwide. They are often not diagnosed and hence not treated, leading to harmful effects which can be lethal in some cases. Food contamination may occur when food is prepared, stored, or handled; this is a common phenomenon in public places like the streets¹⁵. Identifying parasitic contamination will help fight these infections, because knowing contamination rates allows to take the necessary preventive measures. The objective of this study was to identify parasitic contamination rates in *Arachis hypogaea L* (groundnuts), *Citrullus lanatus* seeds (watermelon seeds), and *Ziziphus spina-christi* (nabag) sold by street vendors in Khartoum State, in Sudan.

Method

Study design

This descriptive cross-sectional study was conducted between July and October 2019 in Khartoum State, Sudan. The study included the Khartoum cities Khartoum, Bahri, and Omdurman.

Ethical considerations

Ethical approval for this study was received from the Sudan University of Science and Technology's Committee of Medical Laboratory Science, with the ethical approval number (MLS – IEC – 03 - 18). All participants in this study issued written informed consent for participation and data publication; for participants under 18 years old, consent was obtained from their guardians.

Sampling

The study was conducted on street vendors in Khartoum State, sampling groundnuts, nabag, and tasali. In total, 69.8 g of nabag, 50 g of tasali, and 69.9 g of groundnuts were purchased from 15 sellers in Sudan's Khartoum state (five sellers from each city of interest, i.e. Bahri, Khartoum, and Omdurman), using a cluster random sampling technique, for a total of 45 samples (15 samples from each crop product type). The items were brought to the laboratory and tested under a microscope for parasitic agents. Sellers' location, gender, and age group were observed.

Sample processing

Each product purchased from the same seller was put separately in clean, dry glass bottles after labelling, which were thenfilled up to the surface with distilled water. Bottles were left for one hour, and then the nabag, tasali, and groundnut were removed from the liquid using a plastic sieve; the washes were collected and then examined using a formal ether concentration technique (FECT) and saturated sugar floatation technique.

Formal ether concentration technique

Nabag and tasali washes were added to 4 ml of 10% volume per volume formal saline contained in a conical centrifuge tube. The contents were well-mixed by centrifuging for 20 seconds. After centrifugation four layers of ether, plant debris, formal saline, and deposit were discarded using a sterile plastic Pastier pipette. The deposits of sieved wash liquid were examined under a microscope using 10× and 40× magnification, to detect parasitic agents such as cysts,trophozoite larva, helminth eggs, and species *G. lamblia, E. histolytica, A. lumbricoides, Trichuris trichiura, A. doudenale, N. americanus* and *S. stercoralis*)¹⁷.

Saturated sugar solution floatation technique

Approximately 1 ml of each previously prepared crop wash was put into a glass tube; afterthat, the floatation solution (saturated sugar) was added gradually until a convex surface was formed on the top of the glass tube, which was covered with a glass cover. The tube was left for 15–30 min, after which the glass cover was removed; the solution was put on a microscope slide, and examined under the microscope to detect parasitic agents¹⁷.

Statistical analysis

The statistical package for social science (SPSS, IBM) version 20 program, was used for data analysis. Statistical tests were performed at a 5% significance level (P < 0.05) and a

confidence interval (CI) of 95%. The measured frequencies were computed. The statistical significance of relationships between variables was determined using Pearson's Chi-squared test.

Results

The participants in this study were 15 street vendors selling groundnuts, nabag, and tasali. The population was divided into six age groups: 10-20, 21-30, 31-40, 41-50, 51-60, and over 61 years. The most common age group was 31-40 (46.7%), followed by 41-50 (20%), 21-30 (13.3%), 10-20 (6.7%), 51-60 (6.7%), and more than 61 (6.7%). Eight (53.3%) of the 15 subjects were women, while seven (46.7%) were men.

The total parasitic contamination rate was 60% (27 samples), divided as follows: 20% (n = 9) for groundnut, 22.2 % (n = 10) for nabag, and 17.8 % (n = 8) for tasali. A Chi-squared test was used to test the relationship between crop type and detection of parasites, revealing an insignificant relationship with P = 0.757 (Table 1). The presence of both *E. histolytical E.disparl E. moshkovskii* and *G. lamblia* accounted for 11.1% of the positive results.

The contamination rate assessed using FECT was 53.3% (24 samples), while the sugar floatation technique detected a contamination rate of 48.9% (22 samples); the correlation between detection and technique used was found to be highly significant at P = 0.000 (Table 2). Using FECT, the detected prevalence of *E. histolytical E.disparl E. moshkovskii, G. lamblia*, and mixed contamination of *E. histolytical E.disparl E. moshkovskii* and *G. lamblia* were 33.3% (15), 11.1% (5), and 8.9% (4), respectively; when using the sugar floatation technique, the detected prevalence of *E. histolytical E.disparl E. moshkovskii, G. lamblia*, and mixed contamination of *E. histolytical E.disparl E. moshkovskii, G. lamblia*, and mixed contamination of *E. histolytical E.disparl E. moshkovskii, G. lamblia*, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, and mixed contamination of *E. histolytical E.disparl E. moshkovskii*, G. lamblia, were 37.8% (17), 8.9% (4), and 2.2% (1), respectively (Table 2). The prevalence rates of *E. histolytical E.disparl E. moshkovskii* in groundnuts, nabag, and tasali were 15.6% (7), 8.9% (4), and

8.9% (5), respectively, and for *G. lamblia* were 2.2% (1), 6.7% (3), and 6.7% (3) (Table 3). Mixed contamination was found in groundnuts, nabag, and tasali in the following proportions: 2.2% (1), 6.7% (3), and 2.2% (1), respectively. Contamination rates for groundnut, nabag, and tasali detected by FECT were 20% (9), 20% (9), and 13.3% (6), respectively, while those detected using the sugar floatation technique were 20% (9), 13.3% (6), and 15.5% (7).

The relationship between crop type positivity to contamination and technique used was negligible for both FECT (P =0.655) and sugar floatation technique (P = 0.591) (Table 4). The 31-40 age group had the highest contamination rate (33.3%), followed by 41–50 (13.3%), more than 61+ (6.7%), 51-60 (4.4%), 21-30 (2.2%), and 10-20 (0%) groups. There was a significant association between seller age group and outcome positivity (P = 0.028). Contamination rates were 24.4%, 20%, and 15.6% in Khartoum state cities, i.e. Khartoum, Bahri, and Omdurman, respectively. There was no relation between city and contamination rate (P = 0.329). The results revealed that E. histolytica/E.dispar/E. moshkovskii was the dominant parasite across all cities, with prevalence rates of 17.8%, 17.8%, and 8.9 % in Khartoum, Bahri, and Omdurman, respectively; while G. lamblia had lower prevalence rates in Khartoum, Bahri, and Omdurman, at 13.4%, 4.4%, and 8.9%, respectively. Relationship testing between city and species detected yielded insignificant results (P = 0.460).

Discussion

Groundnut and watermelon seeds are important cash crops. They respectively represented 43,532 USD (59,620 tons) and 49,355 USD (74,149 tons) of Sudanese exports in 2018¹⁸. To the best of our knowledge, this study was the first to look into the parasitic contamination of groundnuts, nabag, and tasali sold by Sudanese street vendors. Two studies in Ghana and Nigeria estimated parasitic infections in the root plant *Cyperus esculentus L.* (tiger nuts); those studies were similar

Age groups (years)	Frequency		Total	
	Males	Females		
10–20	1	0	1 (6.7%)	
20-30	1	1	2 (13.3%)	
30-40	3	4	7 (46.7%)	
40-50	2	1	3 (20%)	
50–60	0	1	1 (6.7%)	
More than 61	0	1	1 (6.7%)	
Total	7 (46.7%)	8 (53.3%)	15 (100%)	
	15 (100%)			

 Table 1. Frequency of study subjects according to age groups.

Parasite spp.	Technique, % (n)			
	FECT		Sugar	
	Positive	Negative	Positive	Negative
E. histolytica/E.dispar/E. moshkovskii	33.3 (15)	0 (0)	37.8 (17)	0 (0)
G. lamblia	11.1 (5)	0 (0)	8.9 (4)	0 (0)
E. histolytica/E.dispar/E. moshkovskii and G. lamblia	8.9 (4)	0 (0)	2.2 (1)	0 (0)
None	0 (0)	46.7 (21)	0 (0)	51.1 (23)
Total	53.3 (24)	46.7 (21)	48.9 (22)	51.1 (23)
	100 (45)		100 (45)	

Table 2. Comparison between parasite species detected and technique used.

P=0.000

Table 3. Correlation between crop type and detected parasite species.

Crop type	Parasite spp., % (n)			Total
	E. histolytica/E.dispar/ E. moshkovskii	G. lamblia	E. histolytica/E.dispar/ E. moshkovskii & G. lamblia	
Groundnuts	15.6 (7)	2.2 (1)	2.2 (1)	20 (9)
Nabag	8.9 (4)	6.7 (3)	6.7 (3)	22.3 (10)
Tasali	8.9 (4)	6.7 (3)	2.2 (1)	17.8 (8)
Total	33.4 (15)	15.6 (7)	11.1 (5)	60.1 (27)

P=0.757

Table 4. Comparison betweencontaminated crop type and techniqueused.

Crop type	Positive results, % (n)		
	FECT	Sugar floatation	
Groundnut	20 (9)	20 (9)	
Nabag	20 (9)	13.3 (6)	
Tasali	13.3 (6)	15.5 (7)	
Total	53.3 (24)	48.8 (22)	

to some degree, and found contamination to be significant^{6,7}. The overall contamination rate in the present study was 60%, which is considered significant. This rate was expected, because vendors sold their products unprotected and handled them with their bare hands. Contamination does not necessarily happen at the selling stage; it can happen during crop farming, harvesting, storage and transport, and even at home, according to Idahosa, 2011^{19} and Porter *et al.*, 1990^{20} . Contamination may occur during the planting phase as a

result of polluted irrigational water, as mentioned by Keraita *et al.*, 2002^{21} , which is contaminated as a result of inadequate or insufficient sanitation infrastructures to cope with the rate of urbanization²². In our study, the most contaminated crop was nabag (22.2%), followed by groundnut (20%), and finally tasali. This may be due nabag being sold raw, which also exposes it to the previously mentioned contamination factors, particularly during the growing and harvesting phases; When nuts fall to the ground, they may come into contact with

potentially polluted soil, as well as other external contaminants carried by wind, humans, or animals.

However, because groundnuts grow beneath the soil surface¹³, they might be damaged if the nuts comes into contact with soil that has been contaminated, or with polluted irrigational water.

Despite this, salting and roasting may help to reduce contamination. Tasali had the lowest contamination rate in comparison to others. This could be explained by it being protected by the fruit (watermelon) during growing and harvesting, as well as the washing, salting, and roasting processes that occur before it is eaten. In our study, the parasites detected belonged to two species: E. histolytical E.dispar/ E. moshkovskii (44.4%) and G. lamblia (26.7%). E. histolytical E.disparl E. moshkovskii was the most common of both in all sampled crops (groundnut: 17.8% vs 4.4%, nabag: 15.6% vs 15.6%, and tasali: 11.1% vs 8.9%). Groundnuts had the highest rate of E. histolytical E.disparl E. moshkovskii contamination (44.4%). This finding is consistent with a Nigerian study on tiger nuts, which are similar to groundnuts in their cultivation process, and in which E. histolytical E.disparl E. moshkovskii was identified as the only protozoan parasite (25% contamination rate)^{6,7,13}. The precise prevalence rates of *E. histolytical* E.disparl E. moshkovskii and G. lamblia in Khartoum State are unknown; however, some studies may help to provide a more concise view . E. histolytical E.disparl E. moshkovskii and G. lamblia were found to be common parasites in the following areas: Alhag-yousif (G. lamblia: 46.4% and E. histolytica/ E.disparl E. moshkovskii: 15.50%), Elengaz (G. lamblia: 33.4% and E. histolytical E.disparl E. moshkovskii: 3.6%), and Alkalakla (G. lamblia: 33.4% and E. histolytica/ E.dispar/ E. moshkovskii: 3.6%)^{17,23,24}. These results suggest that contaminated crop products could be a major source of infection.

Between the saturated sugar floatation technique (prevalence rate: 48.9%) and FECT (prevalence rate: 53.3%), the FECT was the found the most efficient technique for crop examination (P = 0.000). This finding was supported by Abdalazim *et al.*, (2019)¹⁷ (P = 0.000). FECT outperformed the saturated sugar floatation technique in detecting both parasites, with FECT detecting 44.2% of *E. histolytical E.disparl E. moshkovskii* and *G. lamblia* while the sugar floatation technique detected

40% of *E. histolytical E.disparl E. moshkovskii* and 11.1% of *G. lamblia.* According to our study, seller sex and location (stationed vehicle or no vehicle) did not affect the positivity of crop samples to contamination (P-value: 0.807 and 0.329 respectively), while the age group played a role in the occurrence of contamination²⁵.

Conclusions

Roasted groundnut, nabag, and tasali seeds sold by Sudanese street sellers were tainted with protozoan parasites. In all seeds examined, *E. histolytical E.disparl E. moshkovskii* was the dominant parasite. Although female vendors had a greater prevalence of contamination, the connection between seller sex and positivity to contamination was not significant.

Expanding the quantity of soil samples gathered and examined from farmers, central markets, and street vendors. Increasing the sample size and include additional goods from the vendors. Attempting to scan the soil in crop-growing areas and compare the results to those from central markets in order to pinpoint when contamination happened.

Data availability

Underlying data

Figshare: Parasitic Contamination Rate of Arachishypogaea L (Groundnuts), Citrulluslanatus Seeds (Watermelon Seeds), and Ziziphusspina-christi (Nabag) Sold by Street Venders in Khartoum State- Sudan. https://doi.org/10.6084/m9.figshare.14397914.v4²⁵.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Version 3

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Samar Al Nahhas

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After reviewing the revised manuscript, I found it OK. I approve it to be indexed.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, infectious diseases, molecular diagnosis, immuno diagnosis, microbiology, enteric parasites, leishmaniasis, malaria, toxoplasmosis, echinococcusis.

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 21 December 2021

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The authors did not revise the conclusion of the manuscript as suggested, as per my previous comments ("The conclusion seems to be a continuation of the results section. Please make conclusions based on these findings, like the implication of these findings to public health and possible control measures as well as what further studies could focus on.") or given reasons for not revising it.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology, Parasitology and One Health

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.

Version 2

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The authors revised the manuscript according to the comments I raised besides the major issue on the conclusion.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology, Parasitology and One Health

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 30 November 2021

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Samar Al Nahhas

Department of Animal Biology, Faculty of Science, Damascus University, Damascus, Syria

After reviewing the revised paper, the authors did the requested changes.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, infectious diseases, molecular diagnosis, immuno diagnosis, microbiology, enteric parasites, leishmaniasis, malaria, toxoplasmosis, echinococcusis.

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.

Version 1

Reviewer Report 19 November 2021

https://doi.org/10.5256/f1000research.57096.r98829

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? Erastus Mulinge 匝

Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

The manuscript describes intestinal parasite contamination in food items sold by street vendors in Khartoum, Sudan. These parasitic agents detected are significant causes of diarrhea and other gastrointestinal symptoms which are major public health problems and therefore this manuscript deserves indexing.

Major issues

- 1. The conclusion seems to be a continuation of the results section. Please make conclusions based on these findings, like the implication of these findings to public health and possible control measures as well as what further studies could focus on.
- 2. Clarify the difference: the text (page 4) stated that 21 samples were positive for parasites by sugar flotation but in the table, it is 22.

Minor issues

- 1. Replace *Entamoeba histolytica*, address this as *Entamoeba histolytica*/*E.dispar*/*E. moshkovskii* throughout the manuscript.
- 2. Look carefully throughout the manuscript and clear all the spacing mistakes.
- 3. Change the last sentence in page 3 to *G. lamblia*, *E. histolytica*, *A. lumbricoides*, *Trichuris trichiura*, *A. doudenale*, *N. americanus* and *S. stercoralis*.
- 4. Avoid the word "then" in the methods.
- 5. Show p values for correlations in tables 2 and 3.

- 6. Results: the last paragraph, "The results revealed that *E. histolytica* was the dominant parasite across all city, with prevalence rates" change to "all cities".
- 7. Discussion: the previous study was 60% do you mean the present study?

Is the work clearly and accurately presented and does it cite the current literature? $\ensuremath{\mathsf{Yes}}$

Is the study design appropriate and is the work technically sound? $\ensuremath{\mathsf{Yes}}$

Are sufficient details of methods and analysis provided to allow replication by others? $\ensuremath{\mathsf{Yes}}$

If applicable, is the statistical analysis and its interpretation appropriate? $\ensuremath{\mathsf{Yes}}$

Are all the source data underlying the results available to ensure full reproducibility? $\ensuremath{\mathsf{Yes}}$

Are the conclusions drawn adequately supported by the results? $\ensuremath{\mathbb{No}}$

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology, Parasitology and One Health

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.

Author Response 20 Nov 2021

Ahmed Abd Alla, Sudan University of Science and Technology, Khartoum, Sudan

Major issues

 The conclusion seems to be a continuation of the results section. Please make conclusions based on these findings, like the implication of these findings to public health and possible control measures as well as what further studies could focus on.

Author response: I added a paragraph in the conclusion section in response to this.

 Clarify the difference: the text (page 4) stated that 21 samples were positive for parasites by sugar flotation but in the table, it is 22.

Author response: I revised my data and I found the table is right, so I changed it in text.

Minor issues

• Replace Entamoeba histolytica, address this as Entamoeba histolytica/E.dispar/E.

moshkovskii throughout the manuscript.

Author response: I respond to these all throughout the text and I changed all *E. histolytica* with *histolytica*/*E. dispar*/*E. moshkovskii.*

- Look carefully throughout the manuscript and clear all the spacing mistakes.
- Author response: I respond to this.
 - Change the last sentence in page 3 to *G. lamblia, E. histolytica, A. lumbricoides, Trichuris trichiura, A. doudenale, N. americanus* and *S. stercoralis.*
- Author response: I respond.
 - Avoid the word "then" in the methods.

Author response: I respond to this.

• Show p values for correlations in tables 2 and 3.

Author response: I added p-value under Tables 2 and 3.

- Results: the last paragraph, "The results revealed that *E. histolytica* was the dominant parasite across all city, with prevalence rates" change to "all cities".
- Author response: I changed it as the reviewer said.
 - Discussion: the previous study was 60% do you mean the present study?

Author response: I changed 'previous' to 'present'.

I hope I clarify all the things.

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 November 2021

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Samar Al Nahhas

Department of Animal Biology, Faculty of Science, Damascus University, Damascus, Syria

This scientific article addresses the frequency of parasitic infections in some vegetative crops. It addresses an important topic concerning both human and animal nutrition. Raw vegetables and fruits are one of the most essential sources of nourishment. They often contribute to an epidemiological role in the transmission of parasitic food-borne diseases. Usually, fruits and vegetables become contaminated with different parasitic phases (cyst, oocyst, ova, and larvae) during irrigation by waste water or by direct contamination from animals and humans during harvesting, packing, transport processing, distribution, and marketing.

In Sudan as in many countries revealed the presence of different parasites species like soil worms, protozoa, fungus...etc.

Identifying parasitic contamination will help fight these infections, because knowing

contamination rates allows applying strict control of fruit and vegetable cultivation areas, such as keeping animals far from direct contact with agricultural crops, periodic inspection of water sources used in irrigation, as well taking the necessary preventive measures.

I have some points that need to be clarified:

- The authors record the Sellers 'gender and age group, **what is the benefit**? Page 5: last paragraph before discussion, they mentioned "The 31-40 age group had the highest contamination rate outcome positivity". **Have the authors tested stool samples isolated from vendors**?
- It is not possible to distinguish and differentiate *E. histolytica* from the morphologically identical nonpathogenic species *E. dispar* and *E. moshkovskii* using light microscopy. **How did the authors confirm the presence of** *Entamoeba histolytica*?
- The authors applied two techniques: FECT and sugar floatation. What is the benefit?

Is the work clearly and accurately presented and does it cite the current literature? $\ensuremath{\mathsf{Yes}}$

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? $\ensuremath{\mathsf{Yes}}$

If applicable, is the statistical analysis and its interpretation appropriate? $\ensuremath{\mathsf{Yes}}$

Are all the source data underlying the results available to ensure full reproducibility? $\ensuremath{\mathsf{Yes}}$

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: parasitology, infectious diseases, molecular diagnosis, immuno diagnosis, microbiology, enteric parasites, leishmaniasis, malaria, toxoplasmosis, echinococcusis....

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.

Author Response 20 Nov 2021

Ahmed Abd Alla, Sudan University of Science and Technology, Khartoum, Sudan

- 1. Here we need to know the effect of gender and age group in the transmission of parasites through the crops, although some crops are sold by females more than males, like tasali.
- 2. We didn't take stool specimens, but of course, we mean by positive the crops are affected not the person.
- 3. Yes, it's difficult to distinguish between the species by light microscope, so we change all *E. histolytica* to *E. histolytica/E.dispar/E.moshkovskii.*
- 4. Here we want to compare which is the best.

Competing Interests: No competing interests were disclosed.

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