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Research Article

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Parasitic Contamination of Soil, Irrigation Water and Rawly Consumed Vegetables in Farmlands of Asmara, Eritrea

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Abstract

Background: *About one third of the world population are infected with intestinal parasites. Eating raw vegetables is customary in many parts of the world including Eritrea posing a great risk for transmission of pathogens. Therefore, the aim of this study was to determine the prevalence and risks of parasitic contamination in raw vegetables, soil and irrigation water samples.*

Methods and materials: *A cross-sectional study was carried out on different types of 336 vegetable samples (lettuce, carrot, cabbage and arugula), 60 soil and water samples each using sedimentation technique. Simultaneously, socio demographic and farmer practice data of 60 farmers were collected using structured questionnaire. The obtained data were entered and analysed using SPSS version 20.*

Result: *The magnitude of contamination of vegetable, soil and water samples was 144(42.9%), 21(35%) and 30(50%) respectively. Lettuce was the most contaminated sample (51.2%) and arugula (50.9%), carrot (42.7%) and cabbage (34.1%). The most prevalent parasites detected was S. stercolaris 30.6% followed by A. lumbricoides (11.8%) and hookworm (11.8%). The main findings also include the statistically significant difference in rate of contamination observed among various farmlands, vegetable types, and farmer practices. Also, significant association were observed between vegetable contamination and source of irrigation water (p-value= 0.02) and area of collection (p-value< 0.001).*

Conclusion: *This study conclusively reveals the high burden of vegetable, soil and water contamination and the impact of wastewater irrigation system as major risk factor in transmission of intestinal parasitosis among farmers and communities in Asmara.*

Keywords: Parasitic Infection, Zoonotic Disease, Wastewater, Food-Borne, Strongloides

Introduction

Intestinal parasitic infection is an endemic and serious medical health problem in many developing countries, especially in tropical and subtropical regions of the world where adequate water and sanitation facilities are lacking [1]. Globally, it is estimated that around 3.5 billion people are affected and 450 million are directly sick from intestinal parasite infections, with an estimated 200,000 deaths occurring annually [2].

Geohelminth (soil-transmitted helminth) infection and transmission do not only depend on regional ecological conditions, but also, on local standards of social and economic development of the people [3, 4]. Environmental conditions like contamination of food and water sources with human faeces, sewage contaminated irrigation water, use of night soil as fertilizer and improper human handling of food [2] are also important factors.

Contamination of vegetables can occur during production, transportation, storage and commercialization. More importantly, cultivation conditions involving the quality of irrigation water, fertilizer type, practice of animal husbandry and direct contamination from farm workers are the main risk factors for contamination [5]. Studies have shown that *Ascaris lumbricoides, Cryptosporidium spp. Entamoeba Histolytica, Enterobius vermicularis, Fasciola spp., Giardia intestinalis, Hookworm spp, Hymenolepis spp., Taenia spp., Trichuris Trichuria,* and *Toxocara spp*, can infect humans who consume contaminated, uncooked, or improperly washed vegetables and fruits. In Africa, transmission of intestinal parasitic infection has been considered to increase drastically due to the frequent use of untreated human or animal dung as manure in cultivation by local farmers, which serves as a source of zoonotic parasitic infection [6]. Contamination of soil with animal wastes and increased application of improperly composted manures to soil in which vegetables are grown also play a role in parasite contamination to green vegetables [7].

Irrigation with poor-quality water is also a source of vegetable contamination with food-borne pathogens [8]. Sources of irrigation water include groundwater, surface water (ponds, lakes, rivers, and creeks), and human wastewater (human sewage) [9]. In many developing countries, as a result of rapid urbanization and absence of wastewater treatment facilities, urban farmers often use wastewater either directly from sewage drains or indirectly through waste water-polluted irrigation water. In Sub-Saharan Africa (SSA), it is estimated that 10% of the population in cities are involved in the practice of wastewater irrigation, with 50% to 90% of urban dwellers reported to consume vegetables irrigated with wastewater or polluted surface water within or close to the cities [10].

Despite the fact that intestinal parasitosis is common, assessment of the burden of vegetable contamination with parasites of medical and zoonotic importance is least studied in Eritrea. If our target is to control intestinal parasitic diseases, it's not enough to depend merely on the therapeutic interventions of identified cases. Rather, the need for concerted effort to reduce and eliminate the potential source of infection is equally essential. The current study was therefore carried out to determine the parasitic contamination of rawly consumed vegetables, soil, irrigation water and their association with farmer practices.

Methods

Study design and setting

A cross sectional study was conducted on samples of raw vegetables, irrigation water and soil collected from different farmlands in and around Asmara, capital city of Eritrea. The study was conducted in the time frame of March to May 2019 and included six major farmlands principally supplying the vegetables targeted in the current study setting.

Sampling technique

Four types of vegetables including lettuce (*Lactuca sativa*), carrot (*Daucus Carota*), cabbage (*Brassica Deracea*) and arugula (*Eruca sativa*) and samples of irrigation water and soil were collected from the six pre-identified farmlands found in study area. A total of 336 vegetable samples were collected using area based disproportionate random stratified sampling technique according to the general vegetable production reports provided by Ministry of Agriculture. Sampled vegetables which were present with any damaged or visible opening were excluded. Moreover, a total of 60 soil and water samples (10 samples from each study area) which are used for growing those vegetables in the selected farmlands were included. Soil samples were collected from different spots including the centre and boundaries of the farm. Water samples were collected randomly from the farmlands sources of water like wells, irrigation pipes and springs.

Data collection

A structured questionnaire was developed and employed to capture socio-demographic characteristics of respondent farmers including age, sex, educational level, marital status and farmer practice related questions like hand washing and place of defecation.

Sample collection and analysis

Raw vegetable samples were collected, labelled and transported within 2 hours in plastic bags to the parasitology laboratory at Orotta College of Medicine and Health Sciences. A portion of 200g of fresh vegetable was chopped into small pieces and washed by tap water to remove the dirt. Then, the vegetables were gently and thoroughly washed by physiological saline solution (0.85% NaCl) which is left for overnight sedimentation. The supernatant was, then, decanted leaving 15ml of sediment which was transferred to centrifuge tube. For concentrating the parasitic stages, the tube was centrifuged at 3000rpm for five minutes [11]. The supernatant was decanted without shaking to collect the remaining pellet.

Moreover, water samples were collected and labelled in clean plastic bottles using clean beakers. The samples were filtered using double layer of gauze into a clean container and left overnight to settle. The supernatant was removed without disturbing the sediment and carefully the sediment was transferred into a conical tube and centrifuged at 3800 rpm for 15min [12]. The supernatant was removed to sustain the final pellet. Similarly, using a small spatula, soil samples were collected in a labelled clean plastic bags. Each sample was mixed with 30 ml of 1M glycine and homogenized with the aid of a stirrer for 30 minutes. It was kept at rest for five minutes for sedimentation. The pellet was then discarded after removing the supernatant into another clean holder. The supernatant was centrifuged at 3800 for 15 minutes [13]. The supernatant was then decanted to retain the final pellet.

Each tube with the retained pellets from vegetable, soil and water samples was suspended in an equal volume of diethyl ether and 9 ml of formalin. It was mixed vigorously and centrifuged at 3800 rpm for 15 minutes. Finally, the pellet was used to prepare a smear in a clean slide and examined under light microscope with X10 and X40 magnification after applying a drop of Lugol's iodine solution. A smear was prepared from the pellet to be stained with Modified Zeihl-Neelson stain for detection of coccidian protozoal oocysts including *Cryptosporidium spp., Isospora belli, and Cyclosporine cayetanensi.* The stained smear was observed under X100 magnification with application of oil immersion.

Quality control
Validation of English and Tigrigna (local language) version of the questionnaire and the sample collection and analysis process was carried out by clinical laboratory science senior year students with the guidance of experts in medical parasitology and public health. The pH, expiry date, and concentration of reagents were checked periodically to ensure the activity of the reagents. Pre-test was conducted on 2 farmlands prior to actual data collection period.

Statistical analysis
Statistical analysis was performed with help of SPSS software version 20 after data completion and cleaning. Descriptive statistics, chi-square, binary logistic regression and multiple logistic regression analysis were performed to identify factors associated with parasitic contamination of vegetables at statistical significance of p–value < 0.05.

Result
The result of the study showed that 144(42.9%) of vegetable samples were identified to be contaminated with at least one type of parasite. Total contamination rate in soil and water samples were 21(35%) and 30(50%) respectively (Table 1).

Table 1: Prevalence of different parasites among vegetable, soil and water samples, Asmara, Eritrea

	Type of sample		
Parasites	**Vegetables**	**Soil**	**Water**
	N(%)	N(%)	N(%)
Strongloides	55(16.4)	8(13.3)	5(8.3)
A.lumbricoides	19(5.7)	4(6.7)	2(3.3)
G.duodenalis	21(6.2)	3(5)	11(18.3)
E. histolytica/dispar	19(5.7)	1(1.7)	9(15)
Hookworm	23(6.8)	4(6.7)	3(5)
Cryptospordium	20(6)	1(1.7)	7(11.7)
Tanea	2(0)	0(0)	1(1)
E. vermicularis	0(0)	0(0)	2(3.3)
H.nana	1(0.3)	0(0)	1(1.7)
Total prevalence	144(42.9%)	21(35%)	30(50%)

Lettuce (51.2%) was the most contaminated sample and the rest include arugula (50.9%), carrot (42.7%) and cabbage (34.1%). Among the different sources of water, samples collected from sewage irrigated farmlands were present with the highest contamination (90%).

In the current study, the majorly detected parasites species with their stages were larva of *Strongloides* like parasite and hookworm, ova of *A. lumbricoides*, Hookworm, Taenia spp, *E. vermicularis, H. nana* and *T. trichuria*, cysts of *G. duodenalis* and *E. histolytica/ dispar* and oocyst of *Cryptosporidium spp*. As depicted in Table 1, Strongloides like species (16.4%) was the most prevalent parasite in the contaminated vegetables followed by Hookworm spp (6.8%), *G. duodenalis* (6.2%), *Cryptosporidium spp* (6%), *A. lumbricoides* (5.7%), *E. histolytica/dispar* (5.7%), *Taenia spp* (0.01%).

The highest frequency of *Strongloides* like parasite was identified in sample of arugula (27.3%) and the lowest were present in cabbage (7.9%). Ova of Hookworm was mainly recovered from lettuce (11.2%) and arugula (10.9%). The parasitic contamination frequency was significantly different among different vegetable types (p=0.048). Further analysis with binary logistic regression revealed, compared to cabbage, lettuce was significantly contaminated (aOR=2.1 95%CI: 1.1-4.01). Multi-parasitic contamination of vegetables was also observed in this study. 16(4.8%) of vegetable samples were contaminated with two parasite species.

Assessment of the level of parasitic contamination on water and soil samples was also attempted in this study. S. stercolaris (13.3%), *Hookworm* (6.7%), *A. lumbricoides* (6.7%) were the key type of parasites discovered among the soil samples. *G. duodenalis* (18.3%) and *Cryptospordium spp* (11.7%) were the most retrieved parasites from the water samples. The water and soil samples were also both present with one triple and quadruple parasitic contamination including *Taenia spp* to aforementioned parasites.

There was a statistically significant contamination rate among samples collected from different farmlands (Table 2). The results revealed that samples collected from Adi-segudo (55.6%) had the highest contamination rate, followed by Tselot(32.7%), Godaif (25.7%), Adinfas(19.4), Gejeret(18.8) and Sembel (6.2%). Likewise, Adi-segdo had the highest contamination rate (28.5%) of soil samples in which samples were recorded to harbor four parasite species.

Table 2: Prevalence of vegetable contamination across different area of collection, Asmara, Eritrea

	Godaif		Adisegdo		Tselot		Sembel		Adinfas		Gejeret		Total	P-value
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)		
Vegetables	37	25.7	26	55.3	17	32.7	9	6.2	28	19.4	27	18.8	144	0.000
cabbage	11	25.6	9	20.9	1	2.3	2	4.7	12	27.9	8	18.6	43	0.001
carrot	11	34.4	0	0	10	31.2	6	18.8	0	0	5	15.6	32	0.136
lettuce	5	12.2	10	24.4	6	14.6	1	2.4	16	39	3	7.3	41	0.05
arugula	10	35.7	7	25	0	0	0	0	0	0	11	39.3	28	0.176
Soil	3	14.3	6	28.6	2	9.5	2	9.5	5	23.8	3	14.3	21	0.313
Water	2	6.7	9	30	6	20	0	0	7	23.3	6	20	30	0.000

The relationship between source of water used for farming and contamination rate was also investigated. The analysis revealed that 57.7%, 50%, and 39.2% of the produce irrigated by sewage, underground water and dam water was contaminated with at least one parasite species, respectively (Table 3). Farmlands which use sewage as source of irrigation water was significantly associated with parasitic contamination (cOR=2.1 95%CI:1.1-4.0). Similarly, a higher odds of parasitic contamination of vegetables if the irrigation water is contaminated (cOR=2.1 95%CI:1.1-4.0) (Table 4).

Table 3: Chi-square test of sociodemographic factors associated with parasitic contamination of vegetables

Variables	Results of parasitic analysis		X^2	P-value
	Total vegetable samples	Positive samples (%)		
Area of collection				
Adi segdo	47	26(55.3)	20.18	0.001
Adi nefas	54	28(51.9)		
Godief	73	37(50.7)		
Gejeret	62	26(43.5)		
Tselot	52	17(32.7)		
Sembel	48	9(18.8)		
Type of produce				
cabbage	126	43(34.1)	7.68	0.05
carrot	75	32(42.7)		
lettuce	80	41(51.2)		
arugula	55	28(50.9)		
Type of irrigation water				
Dam water	36	18(50)	6.22	0.045
Sewage	45	26(57.7)		
Underground	255	100(39.2)		
Contamination of water				
Contaminated	162	80(49.3)	5.439	0.02

Not contaminated	174	64(36.7)		
Contamination of soil				
Contaminated	112	51(45.5)	0.49	0.483
Not contaminated	224	93(41.5)		
Age of farmers				
<20	33	16(48.4)	0.784	0.853
20-40	167	69(41.3)		
40-60	106	47(44.3)		
>60	30	12(40)		
Sex				
Males	282	122(43.2)	0.118	0.732
Females	54	22(40.7)		
Level of Education				
Illiterate	71	28(39.4)	1.437	0.6
Elementary to junior	156	72(46.1)		
High school	98	39(39.7)		
College or above	11	5(45.4)		
Marital Status				
Single	108	50(46.2)	1.688	0.43
Married	217	91(41.9)		
Divorced	11	3(27.2)		
Years of Experience				
<1year	30	17(56.6)	2.59	0.22
1-5 year	110	45(40.9)		
>5 year	196	82(41.8)		
Handwashing after defecation				
Yes	139	55(39.5)	1.047	0.305
No	197	89(45.1)		
Type of fertilizer used				
Human manure	99	43(43.4)	0.019	0.890
Animal manure	237	101(42.6)		
Practice of Animal husbandry				
Yes	68	28(41.1)	0.098	0.754
No	268	116(43.2)		
Have Farm Advisors				
Yes	247	94(38)	8.775	0.003
No	89	50(56.1)		

Table 4: Binary and multiple logistic regression of factors associated with parasitic contamination of vegetables

Variables	N	Positive samples (%)	cOR(95% CI)	aOR(95% CI)
Area of collection				
Adi Segdo	47	26(55.3)	5.35(2.12-13.5)*	4.39(1.25-12.4)*
Adi Nefas	54	28(51.9)	4.66(1.89-11.4)*	3.54(0.99-10.18)
Godaef	73	37(50.7)	4.45(1.88-10.5)*	3.97(1.64-10.5)*
Gejeret	62	26(43.5)	3.34(1.38-8..07)*	2.89(0.89-7.29)
Tselot	52	17(32.7)	2.1(0.83-5.32)	1.79(0.67-4.78)
Sembel	48	9(18.8)	Reference	Reference
Type of produce				
carrot	126	32(42.7)	1.43(0.79-2.58)	1.95(1.0-3.78)*
lettuce	75	41(51.2)	2.0(1.14-3.59)*	2.19(1.19-4.03)*
arugula	80	28(50.9)	2.0(1.05-3.81)*	1.7(0.87-3.65)
cabbage	55	43(34.1)	Reference	Reference
Type of irrigation water				
Dam water	36	18(50)	1.55(0.77-3.12)	1.45(0.89-3.25)
Sewage	45	26(57.7)	2.12(1.1-4.0)*	2.01(1.0-6.23)*
Underground	255	100(39.2)	Reference	Reference
Years of experience				
<1 year	30	17(56.6)	Reference	Reference
1-5 year	110	45(40.9)	0.35(0.12-1.65)	0.44(0.17-1.15)
>5 year	196	82(41.8)	0.56(0.23-1.87)	0.49(0.21-1.62)
Do you have farm advisors				
Yes	247	94(38)	0.4(0.2-0.7)*	0.69(0.40-1.19)
No	89	50(56.1)	Reference	Reference

This study also assessed sociodemographic factors of farmers in relation with contamination of vegetables by conducting interviews with participants in respective farmlands. Subjects were asked about their educational status and it was revealed that the majority (46.7%) of the farmers had at least primary education, though, 20% were illiterate. The rest 33.3% had secondary education; the difference was not statistically significant (P = 0.6).

Discussion

Food-borne parasitic infections have received little attention in developing countries in which vegetables are exposed to harbour pathogens primarily during cultivation facilitated through the use of contaminated water and ineffective hygienic practices [14]. This makes vegetables an important route for transmission of intestinal parasites and food-borne outbreaks.

In the current study, the overall parasitic contamination of vegetables was 42.9% which is coherent with findings reported in SSA [11, 15]. However, greater amounts of contamination were reported elsewhere [16]. The observed difference might be attributed to the geographical differences, climatic and environmental variations, type of sample, number of samples examined and techniques used for identification.

An overall 35% and 50% of parasite contamination rate was detected in a total of 60 soil samples and water samples respectively which is similar to several studies in Africa [3, 17]. In contrast, the prevalence was higher than studies conducted in Europe and Asia [5, 18]. This variation might be explained by the differences in source of irrigation water, techniques used for retrieval of parasites and the fact that those studies were carried out throughout the year.

Lettuce (51.2%) was found to be the most dominantly contaminated produce followed by arugula (50.9%), carrot (42.7) and cabbage (34.1%). The variation among these produces is accounted for the fact that these vegetables have uneven surfaces which allow parasite eggs to bind more easily to the outer boundary of the vegetables [7]. Furthermore, some studies show that vegetables with dense foliage such as arugula and lettuce were most contaminated because, the dense foliage would protect the helminth eggs against unfavourable conditions such as sunlight, dryness, and wind [19].

In this study, Larva of *Strongloides* like spp was the highest detected parasite in vegetable (16.4%) and soil (13.3%) samples. The possible reason that might explain this finding is the free living mode of *Strongloides spp* with its self-sufficiency for proliferation in regard to host requirements [20]. The predominance of *Strongloides* like parasite is similar with studies conducted elsewhere

[21, 22]. However, the finding is contrary to studies reported by other investigators where *E. histolytica/dispar, Cryptosporidium spp,* and *Toxocara spp* were the frequent parasites detected [7, 23, 24].

The second most predominant parasites detected were *A. lumbricoides* and Hookworm species. This outcome is clearly explained by the fact that *A. lumbricoides* eggs are very resistant to harsh environmental conditions. Ascaris spp can lay about 200,000 eggs daily in which each egg may survive in the absence of oxygen and at 5–10 °C remaining infective for up to 6 years. Moreover, A. lumbricoides eggs have been found to attach to soil particles (especially clay) and be unaffected by desiccation for 2-3 weeks [25]. Similarly, the larva form of Hookworm species is capable of vertical migration across contaminated soil which may perforate through the skin in barefooted individuals to cause intestinal infestation and anemia [26].

G. duodenalis and *Cryptosporidium spp* were the most frequent parasites isolated from the water samples. A previous study reported at least 45.33% of the farmers in the study setting were found to be harbouring *Giardia cyst* [27]. The study generally revealed the impact of water contamination and raw consumption of vegetables as major causative factor in the occurrence of Giardiasis among farmers and communities [27].

Moreover, multiple species contamination was observed in all kinds of samples examined in this study. This might indicate the possibility of high level contamination of the vegetables, which perhaps results in multiple parasitic infections in human. It might also indicate the persistence of intestinal parasitic infection in the area [20].

The contamination rate was significantly variable among the samples collected from different farmlands. Both vegetable and soil samples collected from “Adisegudo” had a statistically significant higher prevalence compared to other farmlands (aOR: 4.39; 95%CI: 1.25-12.4). This might be associated with the differences in type of fertilizer applied and source of irrigation water used for cultivation. 95.7% of vegetables grown in “Adisegudo” area is irrigated through wastewater corresponding to the highest prevalence of contamination in all three types of samples. Therefore, untreated wastewater irrigation system and use of human manure as fertilizer is suspected as main cause of these peak contamination in Adisegudo farmlands. This is in agreement with studies that report strong associations between contamination rate and source of irrigation water [28]. Similarly, a study from Brazil reports higher frequencies of parasites in those who used chicken manure, mixed manure and, bovine manure [5].

The direct application of night soil, animal manure and wastewater as an agricultural fertilizer has been gaining prominence in developing countries [29, 30]. Especially, due to the growing scarcity of water, population growth and rapid urbanizations, the health risks related to the use of excreta-polluted irrigation water to grow vegetables is becoming a shared issue. This apart, the current study did not address the effect of seasonal variation on the contamination of vegetables and other samples. The findings of this study also could not underscore the infectivity of the parasitic stages detected as viability study was not conducted.

Conclusion

This study conclusively reveals the high burden of vegetable, soil and water contamination and the impact of wastewater irrigation system as major risk factor in transmission of intestinal parasitosis among farmers and communities in Asmara. This is very concerning in the study setting where raw vegetables raised on untreated sewage forms the basis of a regular diet. Prevention of contamination remains the most effective way to decrease food borne parasitic infection. Comprehensive health education programs should also be initiated regarding the risk involved in the use of untreated wastewater irrigation system. Consumers should always observe the basic principles of food and personal hygiene thorough washing of raw vegetables before consumption. An adequate treatment of the sewage water and banning wastewater use for irrigation of plant intended for human consumption, among others, should be implemented. Moreover, further studies should be conducted on the viability of parasitic contaminants of vegetables. These studies should also be conducted in different regions of the country.

Abbreviation

spp: species; SPSS: Statistical Package for Social Sciences; WHO: world health organization.

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Ethical Approval and Consent to participate

Ethical consent was obtained from Orotta College of Medicine and Health Sciences research ethical committee. Volunteer farmers that participated in this study were ahead informed regarding the full scope and intention of the study. Verbal and written consent were obtained from the farmers upon data acquisition.

Consent for publication

Not Applicable

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

SMS, SA, DA, OS, NM, MW, AT and RT conceived of the study, participated in the design, and performed the laboratory experiments. NF and YK performed the statistical analysis, data interpretation, result presentation and prepared/reviewed and edited the manuscript. All authors read and approved the final manuscript.

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