Contamination of Vegetables with Geohelminths: Prevalence, Intensity and Roles of Hygiene Practices in Samaru-Zaria, Nigeria

(Geohelminthic contamination of vegetables)

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Abstract:

Vegetables are essential for good diet since they supply vitamins, minerals, dietary fiber and phytochemicals. However, they can serve as vehicle for transmission of geohelminths when contaminated vegetables are consumed raw or undercooked. This study assessed geohelminths contamination on some common vegetables. A total of 100 vegetable samples comprising of cabbage, lettuce, spinach, spring onion and waterleaf were sampled from five different locations in Samaru-Zaria. The samples were subjected to sedimentation technique: 200 grams of each vegetable was washed separately in 500ml of normal saline in order to detach parasites' ova, larvae and cysts. The wash water was allowed to sediment overnight and decanted. The sediment was strain into a centrifuge tube through a sieve to remove large matters, followed by centrifugation at 3000 rpm for five minutes to concentrate the parasitic stages. Wet mounts of the sediments were made and examined using the light microscope with 10x and 40x objectives. From result analysis, 75.0% of the vegetable samples were contaminated with various geohelminths: Ascaris lumbricoides 52(52.0%), hookworms 39(39.0%), Dicrocoelium dendriticum 12(12.0%), Giardia lamblia 8(8.0%), Trichuris trichiura 8(8.0%), Enterobius vermicularis 10(10.0%), Strongyloides stercoralis 23(23.0%) and Fasciola species 8(8.0%). Among the five different vegetable types, waterleaf was the most contaminated with Ascarias lumbricoides, hookworms and Enterobius vermicularis eggs. Vegetable samples obtained from ABU Community were most contaminated with Ascaris lumbricoides (65.0%), Dicrocoelium dendriticum (20.0%) and Trichuris trichiura (20.0%); while samples from Samaru-Market had more of hookworms (55.0%). Vegetable samples that were washed by vendors and those displayed on tables for sale were more contaminated than vegetables not washed or displayed on mat. As important as vegetables are to human diet, care must be taken to ensure good hygiene during handling, storage and distribution to consumers.

Keywords—Vegetables; Samaru-Zaria; geohelminths; parasites; contamination

1. **INTRODUCTION**

Geohelminths also called soil-transmitted helminthes (STHs) are widely spread throughout the world, affecting about half of the world's population [1]. Many helminths are free-living organisms (in aquatic and terrestrial environments). However, four species are commonly implicated in human helminthiasis: *Ascaris lumbricoides* (roundworm), *Trichuris triciura* (whipworm), and *Necator americanus* and *Ancylostoma duodenale* (hookworms) [2]. Vegetables are palatable and rich in vitamins, carotene and minerals

elements [3]. Though consumption of vegetables improves health of humans, it may act as passive vehicle for the transmission of parasites through faecal-oral route [4]. Contamination of vegetables with parasites might take place before or after harvest or during storage [5]. Usage of untreated faecal matter (or night-soil) on farmlands in developing countries predisposes vegetables to contamination [6]. Vegetables can also be contaminated when water used during irrigation on farmland or for keeping the vegetables fresh contains infective stages of parasite [7, 8]. The wide spread habit of consuming raw or minimally cooked vegetables increases the chances of handto-mouth transmission of these parasites [9]. Vegetables such as lettuce (*Latuca sativa*), spinach (*Spinacia oleracea*), cabbage (*Brassica oleracea*) and others, which are consumed raw or barely cooked, are cultivated mostly through the use of untreated water supplies containing sewage. The public health risks of using such contaminated water for irrigation are obvious. Nigeria accounts for the highest population of people infected with geohelminth in Sub-Saharan Africa [10, 11]. Geohelminths can impair nutritional status, cause a range of intestinal diseases, and result in complications such as blood loss, iron-deficiency anaemia, protein loss, rectal prolapse, retarded physical and mental abilities. Hence, the need to identify the different or geo-helminthic parasites that pose health risk.

2.0 MATERIALS AND METHODS

2.1 Study area and item

The study was conducted in Samaru-Zaria. The area is located on Longititude 7° 43' 11.8020" E and Latitude 11° 5' 7.9476" N with an elevation of 641 meters above sea level [12]. Five different locations were considered which included: Market Area, A.B.U. Community, Hayin-Dogo, Hayin-Danyaro and Danraka. Five different fresh leafy vegetables including: cabbage, spinach, spring onion, waterleaf and lettuce were sampled at various sale points. Four (4) samples were collected for each vegetable type per location giving a total of 100 samples. The samples were placed in clean well-labeled plastic bags and transported to the laboratory for analysis at Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

2.3 Microscopic detection of parasites by sedimentation method

Two hundred grams of each vegetable was washed separately in 500ml of normal saline for detaching parasitic stages: ova, larvae and cysts. After overnight sedimentation of the wash water, the sediment was then strain into a centrifuge tube using a sieve to remove large matters. The tubes were centrifuged at 3000 rpm for five minutes to concentrate the parasitic stages [13, 14, 15]. After which the supernatant was decanted carefully without shaking, then the sediment was agitated gently to redistribute the sediments [16]. Wet mount preparations of the sediments were made and examined under the light microscope for parasitic ova cysts and larvae with 10x and 40x objectives. Where the sediments could not be contained in a single wet mount, multiple wet mounts will be made and the parasitic counts pooled together. Atlases of Parasitology were used to aid identification of the parasites.

2.4 Data analysis

The data obtained from the study were subjected to analysis of variance (ANOVA), Pearson Chi-square (χ^2) and Odd

Ratio (OR) analyses at 95% confident interval and $P \leq 0.05$ using IBM SPSS version 21.

3.0 RESULTS

Out of 100 vegetables samples examined for parasitic contamination from Samaru-Zaria, 75(75.0%) were contaminated with different parasites as shown in Figure 1. The various parasites found in the vegetables are presented in Table 1.

Ascaris lumbricoides 52(52.0%) was the most prevalent parasite, followed by hookworms 39(39.0%), *Strongyloides stercoralis* 23(23.0%), *Dicrocoelium dendriticum* 12(12.0%) and *Enterobius vermicularis* 10(10.0%). However, *Trichuris trichiura, Giardia lamblia* and *Fasciola* species had equal occurrences of 8(8.0%) each (Table 1). Among these contaminated samples, 20(26.7%) had single parasitic contamination each, while 55(73.3%) had at least two different parasites contaminations.

All the five different vegetable types examined were most contaminated by *Ascaris lumbricoides*. But *Strongyloides stercoralis* was associated (P = 0.020) with spring onion contamination. *Fasciola* species also significantly (P = 0.003) contaminated lettuce and spinach (Table 2).

In terms of distribution of parasites based on sampling locations of vegetable samples in Table 3, *Ascaris lumbricoides* was the most occurring parasite on vegetables across all the locations, but it peaked in ABU Community 13(65.0%). Vegetables from Samaru Market had the highest hookworm contaminations 11(55.0%), while *Trichuris trichiura* occurred mostly in ABU Community. Samples from Hayin Dogo had significantly high contamination by *Strongyloides stercoralis* 9(45.0%).

Out of the 100 vegetable samples examined, 50 samples were washed before sale while the other 50 were not washed before sale. The washed vegetable samples were more contaminated with geohelminths than those not washed. *Ascaris lumbricoides* (OR = 2.253, P = 0.045) and *Enterobius vermicularis* (OR =10.756, P = 0.008) were significantly found on the washed vegetables (Table 4).

Most of the vegetable samples displayed on tables during sale had geohelminthic contaminations. Chances that any vegetable displayed on the ground would be contaminated by *Ascaris lumbricoides* or *Trichuris trichiura* were 100% each (Table 5).

The highest intensity of hookworms (2.15 ± 1.486) was found on cabbage, followed by *Ascaris lumbricoides* (2.05 ± 0.550) on waterleaf. However, the high intensities of *Ascaris lumbricoides* on waterleaf and *Fasciola* species on spinach were significant (Table 6).



Figure 1: Level of parasitic contamination of vegetables sold in Samaru-Zaria

Parasite	Number positive	Percentage (%)
Ascaris lumbricoides	52	52.0
Dicrocoelium dendriticum	12	12.0
Enterobius vermicularis	10	10.0
Fasciola species	8	8.0
Giardia lamblia	8	8.0
Hookworms	39	39.0
Strongyloides stercoralis	23	23.0
Trichuris trichiura	8	8.0
Fasciola species Giardia lamblia Hookworms Strongyloides stercoralis Trichuris trichiura	8 8 39 23 8	8.0 8.0 39.0 23.0 8.0

Table 1: Prevalence of geohelminths on vegetables sold in Sama
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Table 2: Distribution of geohelminths on different vegetables sold in Samaru-Zaria.

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Sample type	Number	Ascaris	Hookworm	Dicrocoelium	Giardia	Trichuris	Enterobius	Strongyloides	Fasciola
	examined	No. (%)	No. (%)	No. (%)	No.(%)	No. (%)	No. (%)	No. (%)	No. (%)
Cabbage	20	6(30.0)	8(40.0)	1(5.0)	0(0.0)	1(5.0)	1(5.0)	3(15.0)	0(0.0)
Lettuce	20	11(55.0)	3(15.0)	4(20.0)	2(10.0)	2(10.0)	2(10.0)	3(15.0)	4(20.0)
Spinach	20	12(60.0)	7(35.0)	4(20.0)	2(10.0)	2(10.0)	2(10.0)	2(10.0)	4(20.0)
Spring onion	20	10(50.0)	10(50.0)	1(5.0)	2(10.0)	1(5.0)	0(0.0)	10(50.0)	0(0.0)
Waterleaf	20	13(65.0)	11(55.0)	2(10.0)	2(10.0)	2(10.0)	5(25.0)	5(25.0)	0(0.0)
Chi-square(χ^2)		5.849	8.155	4.356	2.174	0.815	7.778	11.632	13.043
df		4	4	4	4	4	4	4	4
P-value		0.211	0.086	0.360	0.704	0.936	0.100	0.020	0.003

Table 3: Distribution of geohelminths on vegetables based on sampling locations in Samaru-Zaria

Location	Number	Ascaris	Hookworm	Dicrocoelium	Giardia	Trichuris	Enterobius	Strongyloides	Fasciola
	examined	No. (%)	No. (%)	No. (%)	No.(%)	No. (%)	No. (%)	No. (%)	No. (%)
Abucom	20	13(65.0)	7(35.0)	4(20.0)	2(10.0)	4(20.0)	1(5.0)	1(5.0)	1(5.0)
Danraka	20	9(45.0)	7(35.0)	0(0.0)	2(10.0)	1(5.0)	2(10.0)	4(20.0)	0(0.0)
Hayin Danyaro	20	7(35.0)	6(30.0)	4(20.0)	3(15.0)	0(0.0)	4(20.0)	6(30.0)	3(15.0)
Hayin Dogo	20	11(55.0)	8(40.0)	1(5.0)	1(5.0)	1(5.0)	3(15.0)	9(45.0)	4(20.0)
Samaru Market	20	12(60.0)	11(55.0)	3(15.0)	0(0.0)	2(10.0)	0(0.0)	3(15.0)	0(0.0)
Chi-square (χ^2)		4.647	3.11	6.250	3.533	6.250	5.556	10.503	8.967
df		4	4	4	4	4	4	4	4
P-value		0.325	0.539	0.181	0.473	0.181	0.235	0.033	0.062

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Washing of	Number	Ascaris	Hookworm	Dicrocoelium	Giardia	Trichuris	Enterobius	Strongyloides	Fasciola
vegetables	examine	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
before sale	d								
Not Washed	50	21(42.0)	21(42.0)	5(10.0)	3(6.0)	4(8.0)	1(2.0)	12(24.0)	2(4.0)
Washed	50	31(62.0)	18(36.0)	7(14.0)	5(10.0)	4(8.0)	9(18.0)	11(22.0)	6(12.0)
Chi square(χ^2)		4.006	0.378	0.379	0.543	0.000	7.111	0.056	2.174
df		1	1	1	1	1	1	1	1
P-value		0.045	0.539	0.538	0.461	1.000	0.008	0.812	0.140
Odd Ratio		2.253	0.777	1.465	1.741	1.000	10.756	0.893	3.273

Table 4: Role of washing of vegetables before sale

Table 5: Role of display methods during sale of vegetable in relation to geohelminthic contamination

Method	of	Number	Ascaris	Hookworm	Dicrcoelium	Giardia	Trichuris	Enterobius	Strongyloides	Fasciola
display		examined	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
Table		69	36(52.2)	28(40.6)	8(11.6)	7(10.1)	5(7.2)	9(13.0)	16(23.2)	6(8.7)
Mat		30	15(15.0)	11(36.7)	4(13.3)	1(3.3)	2(6.7)	1(3.3)	7(23.3)	2(6.7)
Ground		1	1(100.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)
Chi square(χ	²)		0.972	0.780	0.198	1.406	11.626	2.303	0.302	0.205
df			2	2	2	2	2	2	2	2
P-value			0.615	0.677	0.906	0.495	0.003	0.316	0.86	0.903

Table 6: Intensity of geohelminths on vegetables sold in Samaru-Zaria

Vegetable	Number	Ascaris	Hookworm	Dicrocoeliu	Giardia	Trichuris	Enterobius	Strongyloides	Fasciolasp.
type	examine			т					
	d								
Cabbage	20	0.50 ± 0.224	2.15 ± 1.486	0.05 ± 0.050	0.00 ± 0.000	0.05 ± 0.050	0.05 ± 0.050	1.10±0.764	0.00 ± 0.000
Lettuce	20	1.00 ± 0.281	0.20 ± 0.117	0.20 ± 0.092	0.15 ± 0.489	0.10 ± 0.069	0.10 ± 0.069	0.30±0.206	0.20 ± 0.092
Spinach	20	1.35 ± 0.350	0.55 ± 0.185	$0.20\pm0.0.092$	0.10 ± 0.308	0.15 ± 0.109	0.10 ± 0.069	0.20 ± 0.156	0.25±0.123
Spring	20	0.70±0.193	0.95 ± 0.266	0.05 ± 0.050	0.10 ± 0.308	0.05 ± 0.050	0.00 ± 0.000	1.25±0.403	0.00 ± 0.000
onion									
Waterleaf	20	2.05 ± 0.550	1.50 ± 0.478	0.10±0.069	0.10 ± 0.308	0.15 ± 0.109	0.25 ± 0.099	0.90 ± 0.561	0.00 ± 0.000
Anova (F)		3.156	1.171	1.082	0.573	0.371	2.003	0.997	3.291
P-value		0.018	0.329	0.370	0.683	0.829	0.100	0.413	0.014

4.0 DISCUSSION

Vegetables constitute a very important part of human diet. There is no doubt that vegetables are good sources of nutritional vitamins and minerals [17]. However, poor hygienic practices during cultivation, processing, distribution or storage can contribute to spread of pathogens or infections. A very good fractions of vegetables sold in Samaru-Zaria had geohelminths, which is a direct evidence of faecal contamination or poor hygiene. This study found out that 75.0% of the vegetable samples had geohelminths; similarly, 76.5% prevalence of geohelminths had been reported by Simon-Oke *et al.* [18]. Other studies by Leon *et al.* [19] and Damen *et al.* [20] had reported lower prevalence of geohelminths in vegetables. The main reasons for the differences are largely due to prevailing environmental and sanitation factors. It is important that people who buy

vegetables must consider some measures of safety like washing, peeling or cooking before consumption.

Ascaris lumbricoides was the most prevalent geohelminth (52.0%) on the vegetables in this study. It is known that eggs of this nematode are capable of withstanding harsh soil/environmental conditions for months in the tropics [21]. Ascaris lumbricoides had always been the most prevalent geohelminth infesting vegetables [15, 18, 22].

Among the five different leafy vegetables examined in this study, waterleaf was the most contaminated with eggs of *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis* and *Strongyloides stercoralis*. Similar findings had been reported by Eneaya and Njom [22], Dauda *et al.* [23] and Akinseye *et al.* [24]. The waterleaf provides a good surface for geohelminthic eggs and larvae to attach. *Ascaris lumbricoides* and *Trichuris trichiura* were the most prevalent geohelminth in vegetables sold in ABU Community. This

could have been due to local method of using animal wastes to enrich the vast flower beds in the area.

It was also found that vegetables that were washed had more geohelminths than those that were not washed. Washing of vegetable is a good thing and should be encouraged. However, some of the vegetable sellers in the study area used the same water in washing all vegetables [17]. Contamination from a few samples will be transferred to other uncontaminated ones if the same wash water is repeatedly used. The use of running tap and proper draining of the wash water should be encouraged instead. The method of displaying vegetables during sale is not only paramount for customer attraction, but the vegetables should be displayed in a hygienic manner. There was a very great tendency of finding Ascaris lumbricoides and Trichuris trichiura on any vegetable kept on the bare ground for sell. Vegetables are prone to contaminations from a variety of ways: dried faecal matter blown by air, contaminated wash water, irrigation water, transportation and flooding [14, 17].

5.0 CONCLUSION

This study has indicated 75.0% overall geohelminths contamination of vegetables sold in Samaru-Zaria. The various geohelminths found included: *Ascaris lumbricoides* (52.0%), hookworm (39.0%), *Dicrocoelium dendriticum* (12.0%), *Giardia lamblia* (8.0%), *Trichuris trichiura* (8.0%), *Enterobius vermicularis* (10.0%), *Strongyloides stercoralis* (23.0%) and *Fasciola* species (8.0%). Presence of these geohelminths in vegetables poses great risk to health of consumers. Vegetables that were washed had more geohelminthic contamination due to repeated use of the same wash water. When vegetables are displayed on the ground for sale, they are predisposed to direct contamination from the soil.

Safety of any vegetable for human consumption should be put in consideration by the farmers, distributors and final consumers. But the greatest care should be taken by the final consumers to ensure proper washing or cooking before eating.

6.0 Conflict of Interest

The authors declare that there is no any financial, competing or conflict of interest.

7.0 Acknowledgement

We acknowledge the technical assistance of the staff of Helminthology Laboratory at the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

References

1. Hall, A., Hewiit, g., Tuffrey, V., de Silva, N. (2008). A review and meta-analysis of the impacts of intestinal worms on child growth and nutrition. Maternal and Child Nutrition, 4(1):118-236

- 2. WHO. (2017). Soil-transmitted helminth infection. Factsheet, Updated November, 2017. Accessed 10th April, 2018 from:<u>http://www.who.int/mediacentre/factsheets/fs366/e</u><u>n/</u>.
- Mohamed, M.A., Siddig, E.E., Elaagi[, A.H., Edris, A.M.M., Nasir, A.A. (2016). Parasitic contamination of fresh vegetables sold at Central Markey in Khartoum State, Sudan. Annals of Clinical Microbiology and Antimicrobials, 15(17):1-7
- Beiromvard, M., Akhaghi, L., Fattahi Massom, S.H., Meamar, A.R., Motevalian, A., Oomazdi, H., Razmjou, E. (2013). Prevalence of zoonotic intestinal parasites in domestic and stray dogs in a rural area of Iran. Preventive and Veterinary Medicine, 109(1-2):162-167
- Halablab, M.A., Sheet, I.H., Holail, H.M. (2011). Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon. American Journal of Food Technology, 6:129-139.
- 6. Olayemi, A.B. (1997). Microbiological hazards associated with agricultural utilization of urban polluted river water. International Journal of Environmental Health Research, 7(2):149-154.
- 7. Amoah, P., Drechsel, P., Abaidoo, R.C., Abraham, E.M. (2009). Improving food hygiene in Africa where vegetables are irrigated with polluted water. West Africa Regional Sanitation and Hygiene symposium. Accessed from:http://ir.knust.edu.gh/bitstream/123456789/9758/1/ Improving%20food%20hygiene%20in%20Africa%20w here%20vegetables%20are%20irrigated%20with%20pol luted%20water.pdf
- Ofor, M.O., Okorie, V.C., Ibeawuchi, I.I., Ihejirika, G.O., Obilo, O.P., Dialoke, S.A. (2009). Microbial contamination in fresh tomato wash water and food safety consideration in South-eastern Nigeria. Life Science Journal, 6(3):80-82.
- 9. Mba O. (2000). Detection and enumeration of parasites eggs on irrigated vegetables and salad crops in Plateau State, Nigeria. Journal of Medical Laboratory Science, 9:30-36.
- 10. Hotez PJ, Asojo OA, Adesina AM. (2012). Nigeria: "Ground zero" for the high prevalence neglected tropical diseases. PLoS Neglected Tropical Diseases, 6(7):e1600.
- 11. Federal Ministry of Health [FMH]. (2013) Nigeria master plan for neglected tropical disease (NTDs) 2013-2017. p. 1-142. Accessed 10th April 2018 from: http://www.schoolsandhealth.org/Shared%20Documents /Downloads/Nigeria%20Neglected%20Tropical%20Dis ease%20Control%20Master%20Plan%202013.pdf
- 12. Zaria, Nigeria. <u>https://www.latlong.net/place/zaria-nigeria-21741.html</u>
- Idahosa, O.T. (2011). Parasitic contamination of fresh vegetables sold in Jos Market. Global Journal of Medical Research, 11(1)(1):20-25.

- Tefera, T., Biruksew, A., Mekonnen, Z., Eshetu, T. (2014). Parasitic contamination of fruits and vegetables collected from selected local markets of Jimma Town, Southwest Ethiopia. International Scholarly Research Notices, ID 382715.
- Bekele, F., Tefera, T., Biresaw, G., Yohannes, T. (2017). Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch Town, Southern Ethiopia. Infectious Diseases of Poverty, 6(19):1-17
- Cheesbrough, M. (2009). District Laboratory Practice in Tropical Countries, Part I, 2nd ed. Updated. Cambridge, UK: Cambridge University Press.
- 17. Simon-Oke, I.A., Afolabi, O.J., Obasola, O.P. (2014). Parasitic contamination of fruits and vegetables sold at Akure metropolis, Ondo State, Nigeria. Researcher, 6(12):30-35.
- Bishop, H.G., Okwori, G.O. (2017). Escherichia coli and Staphylococcus aureus contaminations of carrots sold within Zaria, Nigeria and their antibiotic susceptibility profiles. Open Access Journal of Science, 1(4):00022.
- Leon, W.U., Monzon, A.A., Arceo, E.J., Ignacio, G.S. (1992). Parasitic contamination of fresh vegetables sold in metropolitan Manila Philiphines. South-East Journal of Tropical Medicine and Public Health, 23(1):162-164.
- Damen, J.G., Banwat, E.B., Egah, D.Z. Allanana, J.A. (2007). Parasitic contamination of vegetables in Jos, Nigeria. Annals of African Medicine, 6(3):115-118.
- Stephenson, L.S. (1987). Impact of helminths infection on human nutrition. New York: Taylor and Francis. p. 252-260.
- Eneaya, C.I., Njom, V.S. (2003). Geohelminth contamination of some common fruits and vegetables in Enugu, South-east Nigeria. Nigerian Journal of Parasitology, 24(1):123-128.
- Dauda, M.M., Medinat, M.O., Sabiu, T. Parasitic contamination of fruits and vegetables sold at Kaduna Metropolis, Nigeria. Nigerian Journal of Parasitology 2011;32(2):309-315.
- Akinseye, J.F., Ayuba, S.B., Adewuyi, I.K., Agunlejika, R.A. (2017). Isolation of intestinal parasites in vegetables sold in major markets in Akure, Ondo State, Nigeria. International Journal of Health Science and Research, 7(6).