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Detection of Parasitic Contamination of Commonly Consumed Vegetables Sold at Obollo-Afor Market, Nigeria

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ABSTRACT

Background and Objective: Vegetables are plant edible components that are eaten whole or in pieces, raw or cooked as part of a main dish or salad. The parasitic fauna of ten different vegetables distributed at Obollo-Afor Market, Enugu State, Nigeria, were investigated. The objective was to determine the prevalence of parasitic contamination of different commonly consumed vegetables sold at Obollo-Afor Market. **Materials and Methods:** The samples were centrifuged after being cleaned with normal saline and left to stand for 10-12 hrs to allow for sedimentation and the sediments were then inspected under a microscope. Using a modified Zieehl-Nelsen approach, the oocysts of the parasite *Cryptosporidium parvum* were found in vegetables. A questionnaire was used to gather information about the vegetables. **Results:** The overall prevalence of parasites found in the vegetables was 44 (36.7%). *Ascaris lumbricoides* ova (18.3%) was the most prevalent parasite whereas *Trichuris* ova (0.8%) was the least. False cubeb leaf (60%) was the most contaminated vegetable while African spinach (10%) was the least. As 17 (41.5%) vegetable sellers used clean water for irrigation, 1 (2.4%) used wastewater, 11 (26.8%) used animal (human) faeces as manure, 3 (7.3%) used organic manure whereas 4 (9.8%) used inorganic manure. **Conclusion:** There is a moderate prevalence of parasitic infestation, probably due to handling procedures from production to sales.

KEYWORDS

Ascaris lumbricoides, hookworm, *Entamoeba histolytica*, *Strongyloides stercoralis*, *Cryptosporidium parvum*, *Taenia*, *Trichuris*, prevalence, vegetables

INTRODUCTION

The relative nature of the host-parasite relationship varies and this made it possible to illustrate a list of parasite species that show an increasing degree of metabolic dependence on their hosts. Poulin and Morand[1] drew a hypothetical scale in which at one end of the scale is zero dependence, implying a free-living organism and on the other end is 100% or total parasitism. In between these two extremes lies a range of organisms that satisfy their metabolic requirements to a varying extent at the expense of the host.

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Vegetables are planted edible components that are eaten whole or in pieces, raw or cooked as part of a main dish or salad[2]. A vegetable includes leaves, stems, roots, flowers, seeds, fruits, bulbs, tubers and fungi[3]. Vegetables are an essential part of a healthy human diet owing to their nutritional value[4]. To prevent chronic diseases like heart disease, cancer, diabetes and obesity as well as to prevent and treat several micronutrient deficiencies, the World Health Organization recommends consuming at least 400 g of fruits and vegetables daily, especially in less developed countries[5]. Regular consumption of vegetables helps to prevent the human body against a number of diseases by providing nutrients such as vitamins, minerals, proteins and fibers, it also plays a vital role in body weight regulation and related conditions such as diabetes and hypertension[6]. Ononogbu[7], reported that vegetable fats and oils lower blood lipids thereby reducing the occurrence of disease associated with the damage of the coronary artery. Vegetable fats and oil serve as precursors of prostaglandins which are known to perform the role of vasoconstriction and vasodilation of the blood vessels. Vegetable fats and oil are known to serve as precursors of thromboxane which facilitate blood clotting in humans[7].

However, some vegetables are consumed raw in other to maintain their natural taste and to prevent heat liable nutrients[8]. Vegetables are potential sources of transmission for intestinal parasites[6]. This means of parasitic contamination is a bigger issue in resource-constrained nations like Nigeria, where feces in undesignated areas by scavenging animals and people as well as the use of dirty water for vegetable irrigation are all common[9]. For instance, helminths that are spread by eating raw vegetables, such as *Hymenolepis nana*, *Toxocara canis*, *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Enterobius vermicularis*, are well recorded among Nigerians[10]. The high levels of contamination with harmful parasites in many poor nations have been attributed to the use of untreated sewage to irrigate vegetables[11]. Due to aging, hunger, HIV infection and other underlying medical problems, many people are extremely vulnerable to parasite infections[12]. Fresh veggies have been associated with an increase in cases of foodborne illness that have been reported[8]. Research has shown that humans can become infected with parasites like *Ascaris lumbricoides*, *Cryptosporidium* species, *Entamoeba histolytica*, *Enterobius vermicularis*, *Fasciola* species, *Gardia intestinalis*, *Trichuris trichiura*, hookworm, *Hymenolepis*, *Taenia* and *Toxocara* species by eating tainted, raw, or improperly cleaned vegetables[13]. It has been known for at least 100 years that raw vegetables can spread sickness to people[14]. The usage of untreated manure, primarily composed of pig and poultry feces and other practices connected to vegetable contamination are widespread in the Obollo-Aforaxis[13-15].

To prevent and control medically important parasitic diseases, assessing the sources of infectious agents and their level of contamination is crucial because these parasites are highly resilient and able to tolerate challenging circumstances[12]. There is little information on the dangers of parasite diseases linked to vegetable consumption in the research domain, therefore, a need to carry out this research work. This study is aimed at the detection of parasitic contamination of commonly consumed vegetables in Obollo-Afor Market, Udenu Local Government Area (LGA), Enugu State, Nigeria and the specific objectives include determining the prevalence of parasitic contamination of different commonly consumed vegetables sold at Obollo-Afor Market, the prevalence of parasite groups in different vegetables sold, the demographic characteristics of respondents using questionnaires and the major sources of parasitic contamination of vegetables in the study area in relation to the educational level of the respondents.

MATERIALS AND METHODS

Study area: The study was carried out during the rainy season from June to August, 2019. The vegetables were purchased from Obollo-Afor Market where other surrounding markets get their supply of vegetables. The administrative centre of Udenu LGA of Enugu State, Nigeria, is located in Obollo-Afor. It is the point at which the Igbo drive turns north. Amalla and Idoma in Benue State, Enugu-Ezike Northwest and the Umundu, Igugu and Imilike people in the South form its Northern, Eastern and Southern borders, respectively. Obollo-Afor is at a good location. Due to its advantageous position, prostitution is prevalent there as it serves as the entrance to both the Northern and Eastern States of Nigeria[16].

Vegetable sample collection: On each day of sample collection, the vegetables were collected randomly from different vendors to obtain a qualitative estimation of the parasitic content in each vegetable. A 50 g sample was taken of each vegetable randomly purchased from different vegetable vendors. The screening of the vegetable samples was carried out in the University of Nigeria, Nsukka, Department of Zoology and Environmental Biology laboratory.

Processing the vegetable samples: The vegetable samples were subjected to parasitological examination according to Endale *et al.*[12]. A portion (50 g) of each vegetable was washed separately in 125 mL of normal saline (0.85% NaCl) for detaching the stages (ova, larvae, cysts and oocysts) of the parasites commonly assumed to be associated with contamination. According to Salavati *et al.*[17] the washing solution was fixed in formalin (95 mL of the solution against 5 mL of 4% formaldehyde) to preserve the different parasitic stages. The washing solution was allowed to stand on the bench for 10-12 hrs for proper sedimentation. The supernatant was discarded, 15 mL of the sediment was transferred to a centrifuge tube. The tube was centrifuged at 3000 rpm for 5 min in order to concentrate the parasite stages. After centrifugation, the supernatant was gently decanted without shaking. To disseminate the parasite stages, the silt was carefully stirred[12].

Parasite detection: As 100 μL of sediment was transferred to a clean glass slide, covered with cover glass and inspected using ×10 and ×40 objectives on a light microscope[12].

Modified Zeihl-Neelsen staining approach: Clarke and McIntyre[18] described a modified Zeihl-Neelsen staining method for identifying *Cryptosporidium* species oocysts. A thin smear was formed straight from the silt and left to air-dry. The slides were fixed in methanol for 5 min before being stained with carbon fusion for 30 min. The slides were then washed with tap water and decolonized with acid alcohol (1 mL concentration HCl in 99 mL of 96% ethanol) for 1-3 min. Finally, the slides were rinsed with tap water and allowed to air-dry. The slides were then examined under a light microscope (the manufacturer: Philip Harris, Location: China, serial No. 0006149) at ×100 magnification with immersion oil.

Statistical analysis: Data were analyzed using the Statistical Packages for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA). The prevalence of vegetable contamination by parasites was compared using Chi-square analysis. Frequency distribution was used to summarize questionnaire responses. Chi-square analysis was used to compare the frequency of responses relative to respondent characteristics, as questionnaire responses were not matched to contamination status. Only where responses to the questionnaire relate significantly to respondents' demographic characteristics was the result displayed in the result section. The level of significance was set at 95% probability (i.e., $p<0.05$).

RESULTS

A summary of the distribution of the prevalence of parasitic contamination in the vegetable types is presented in Table 1. A total of 120 vegetable samples consisting of 10 types of vegetables commonly consumed were examined for parasitic contamination. Overall, 44 (36.7%) of the 120 samples were contaminated with parasites. The prevalence of parasite contamination ranged from 10.0-60.0%, though the difference in parasite contamination between the vegetable types was not significant statistically (χ^2 = 9.187, p = 0.420). False cubed leaf was the most contaminated vegetable (60%) while the least contaminated vegetable in this study was African spinach (10%).

From Table 2, the most prevalent parasite in pumpkin leaf was *Ascaris* ova (13.3%), histolytica cyst, hookworm ova and *Cryptosporidium* oocyst had equal contamination rates (6.7%). The most prevalent parasite in bushbuck was *Ascaris* ova (40%), followed by C*ryptosporidium* oocyst (10%). In scent leaf, *Ascaris* ova happened to be the only parasite species (30%). The most prevalent parasite in curry leaf was hookworm ova (26.7%), followed by *Ascaris* ova (20%), *E. histolytica* cyst (13.3%) and *Strongyloides* larva

Table 1: Prevalence of parasitic contamination of different commonly consumed vegetables sold at Obollo-Afor Market

Vegetable	Number examined	Number infested (%)
Pumpkin	15	5 (33.3)
Bushbuck	10	5 (50.0)
Scent leaf	10	3 (30.0)
Curry leaf	15	7 (46.7)
African spinach	10	1 (10.0)
Garden egg leaf	15	7 (46.7)
Waterleaf	15	5 (33.3)
Cabbage	10	3 (30.0)
False cubeb	10	6 (60.0)
Bitter leaf	10	2 (20.0)
Total	120	44 (36.7)
		χ^2 = 9.187, p = 0.420

Table 2: Prevalence of parasite groups in different vegetables sold by retailers at Obollo-Afor Market

Vegetable	No. of Examined	*Entamoeba histolytica* cyst (%)	*Ascaris* ova (%)	Hookworm (%)	*Strongyloides* larva (%)	*Cryptosporidium Parvum* oocyst (%)	*Taenia* spp. egg (%)	Percentage (%)
Pumpkin	15	1 (6.7)	2 (13.3)	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)
Bushbuck	10	0 (0.0)	4 (40.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)
Scent leaf	10	0 (0.0)	3 (30.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Curry leaf	15	2 (13.3)	3 (20.0)	4 (26.7)b2	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
African spinach	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
Garden egg leaf	15	4 (26.7)	4 (26.7)	2 (13.3)	4 (26.7)	0 (0.0)	1 (6.7)	1 (6.7)
Waterleaf	15	0 (0.0)	2 (13.3)	1 (6.7)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cabbage	10	0 (0.0)	2 (20.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
False cubeb leaf	10	0 (0.0)	1 (10.0)	3 (30.0)	1 (10.0)	1 (10.0)	0 (0.0)	0 (0.0)
Bitter leaf	10	1 (10.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	120	9 (7.5)	22 (18.3)	11 (9.2)	9 (7.5)	3 (2.5)	2 (1.7)	1 (0.8)
		χ^2 = 12.853, p = 0.169	χ^2 = 8.460, p = 0.489	χ^2 = 16.314, p = 0.061	χ^2 = 13.333, p = 0.148	χ^2 = 7.862, p = 0.548	χ^2 = 8.136, p = 0.521	χ^2 = 7.059, p = 0.631

Significance level was at p = <0.05

6.7%). African spinach had only one parasite species, *Taenia* ova (10%). Garden egg leaf had *E. histolytica* cyst, *Ascaris* ova and *Strongyloides* larva in equal rates of contamination (26.7%), followed by hookworm ova (13.3%), *Taenia* and *Trichuris* ova in equal rates of contamination (6.7%). Waterleaf had *Ascaris* ova and *Strongyloides* larva in equal proportion of contamination (13.3%) and hookworm ova (6.7%). In cabbage, *Ascaris* ova were the predominant parasite species (20%), followed by *Strongyloides* larva (10%). Hookworm ova was the most prevalent parasite in false cubeb leaf (30%), followed by *Ascaris* ova, *Strongyloides* larva and *Cryptosporidium* oocyst in equal proportion (10%). Bitter-leaf had a contamination rate of (20%), with *E. histolytica* cyst and *Ascaris* ova (10%) each.

The distribution of helminth contamination by vegetable types is also shown in Table 2. Parasitic groups isolated were four nematodes (*Ascaris* ova, hookworm, *Strongyloides* larva and *Trichuris* ova), one cestode (*Taenia* spp.) and two protozoa (*Entamoeba histolytica* cyst and *Cryptosporidium parvum* oocyst). Nematodes were the most prevalent while cestode was the least. Among the nematodes, *Ascaris* ova 22 (18.3%) predominated while *Trichuris* ova 1 (0.8%) was the least. *Taenia* species 2 (1.7%) was the only cestode examined. *Entamoeba histolytica* 9 (7.5%) predominated among the protozoan while *Cryptosporidium parvum* oocyst 3 (2.5%) was the least. Generally, there was no significant difference in the prevalence of each parasite type between vegetable types (p>0.05).

A summary of the demographic attributes of questionnaire respondents is provided in Table 3. The respondents were from 11 towns. The majority of the respondents were from Obollo-Afor, 16 (39.0%). Imilike and Eha-Alumona had 6 (14.6%) representatives each. The majority of the respondents were females. Only 1 (2.4%) out of the 41 respondents was male. All the respondents were adults. The majority had primary and secondary school education (36.6 and 43.9%) respectively, compared to those that had non-formal.

Table 3: Demographic characteristics of respondents summarized by frequency

Characteristics	Frequency (%)
Town	
Ede-Oballa	2 (4.9)
Obollo-Afor	16 (39.0)
Ikem	1 (2.4)
Imilike	6 (14.6)
Enugu-Ezike	4 (9.8)
Ovoko	2 (4.9)
Eha-Alumona	6 (14.6)
Uzo-Uwani	1 (2.4)
Udi	1 (2.4)
9th Mile	1 (2.4)
Ogbodo	1 (2.4)
Gender	
Male	1 (2.4)
Female	40 (97.6)
Age	
Adult	41 (100.0)
Children	0 (0.0)
Education	5 (12.2)
Non-formal	15 (36.6)
Primary	18 (43.9)
Secondary	3 (7.3)
Tertiary	
Total	41 (100.0)

Table 4: Summary of questionnaire responses of vegetable sellers at Obollo-Afor Market

Responses	Frequency (%)
Wash before display	
Washed	27 (65.9)
Unwashed	14 (34.1)
Types of water	
Not washed	14 (34.1)
Unclean	5 (12.2)
Clean	22 (53.7)
Display pattern	
Ground	18 (43.9)
Table	23 (56.1)
Market type	
Open market	41 (100.0)
Grocery	0 (0.0)
Irrigation water	
Not irrigated	23 (56.1)
Clean water	17 (41.5)
Wastewater	1 (2.4)
Type of manure	
None	23 (56.1)
Animal feces	11 (26.8)
Organic	3 (7.3)
Inorganic	4 (9.8)

A summary of the responses provided to the questions asked is shown in Table 4. Those who washed their vegetables were almost two times more than those who did not wash (65.9 vs 34.1%), respectively. The type of water commonly used for washing the vegetables was clean water 22 (53.7%) and unclean water 5 (12.2%). Display on the table and the ground had a close frequency in the population (56.1 vs 43.9%) respectively. All respondents were open-market sellers. The majority of the farmers 23 (56.1%) did not use manure, but those who did use animal dung 11 (26.8%).

Table 5: Classification of irrigation and manure type by town and level of education of vegetable sellers at Obollo-Afor Market

(a) Irrigation type

	Irrigation type		
Parameter	Not irrigated (%)	Clean water (%)	Wastewater (%)
Town			
Edeballa	0 (0.0)	1 (50.0)	1 (50.0)
Obollo-Afor	11 (68.8)	5 (31.3)	0 (0.0)
Ikem	1 (100.0)	0 (0.0)	0 (0.0)
Imilike	3 (50.0)	3 (50.0)	0 (0.0)
Enugu-Ezike	3 (75.0)	1 (25.0)	0 (0.0)
Ovoko	2 (100.0)	0 (0.0)	0 (0.0)
Eha-Anumona	0 (0.0)	6 (100.0)	0 (0.0)
Uzo-Uwani	1 (100.0)	0 (0.0)	0 (0.0)
Udi	1 (100.0)	0 (0.0)	0 (0.0)
9th Mile	1 (100.0)	0 (0.0)	0 (0.0)
Ogbodo	0 (0.0)	1 (100.0)	0 (0.0)
			χ^2 = 36.439, p=0.014
Education			
Non-formal	0 (0.0)	5 (100.0)	0 (0.0)
Primary	9 (60.0)	6 (40.0)	0 (0.0)
Secondary	13 (72.2)	5 (27.8)	0 (0.0)
Tertiary	1 (33.3)	1 (33.3)	1 (33.3)
			χ^2 = 21.624, p = 0.001

(b) Manure type

	Type of manure			
Education	None (%)	Animal feces (%)	Organic (%)	Inorganic (%)
Non-formal	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)
Primary	9 (6.0)	2 (13.3)	1 (6.1)	3 (20.0)
Secondary	13 (72.2)	4 (22.2)	0 (0.0)	1 (5.6)
Tertiary	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
			χ^2 = 18.353, p = 0.031	

Classification of respondents' behavior by demography indicates three cases of statistical significance which are presented in Table 5a. The distribution of irrigation types in relation to towns was statistically significantly different (χ^2 = 36.439, p = 0.014). Out of 16 sellers from Obollo-Afor, 11 (68.8%) did not practice irrigation farming, while 5 (31.3%) used clean water for irrigation purposes. Eha-Alumona practised 100% clean water irrigation farming. At Imilike, it was 50% each non-irrigation and irrigation farming. Enugu-Ezike followed the same pattern of irrigation farming as Obollo-Afor.

Level of education was another important determinant of irrigation pattern. All the farmers who had non-formal education practised clean water irrigation farming 5 (100%). This contrasts strongly with those that had primary 6 (40.0%), secondary 5 (27.8%) and tertiary education 1 (33.3%) who practised clean water irrigation while 1 (33.3) tertiary respondents practiced wastewater irrigation (Table 5a). The difference in irrigation pattern between those who had non-formal education compared to other levels of education was observed significantly (χ^2 = 21.624, p = 0.001). Those vegetable farmers who had non-formal education always used either animal feces 4 (80.0%) or other organic manure 1 (20.0%). Those that had formal education sometimes used manure ranging from animal feces to organic and inorganic types. This was responsible for the significant difference observed (χ^2 = 18.353, p = 0.031) (Table 5b).

DISCUSSION

A total of 44 (36.7%) out of the 120 samples were contaminated with parasites. The prevalence of contamination of commonly consumed vegetables was not statistically significant. False cubed leaf was the most contaminated vegetable (60%) while African spinach (10%) was the least contaminated vegetable in this study. This study revealed that there was a considerably moderate level of parasitic contamination of vegetables in the Obollo-Afor Market. This is in line with the previous reports from Alexandria, Egypt[8] and Naknon Si Thammarat Province, Southern Thailand[19] where the contamination rates were 31.7 and 35.1%, respectively. However, the present findings contrasted with the reports from Nsukka ecological zone, Nigeria[15], Tripoli, Libya[20], Metro Manila, Philipines[21] and Southern Iran[22] where the contamination rates were 51.8, 58, 51.6 and 43.7%, respectively. A lower contamination rate of 3.5% was observed in North-East Nigeria in a study conducted by Adamu *et al.*[23] whereas, Kozan *et al.*[24], reported a 0% prevalence of helminthic eggs in washed vegetables in Turkey. Also, Saudi Arabia[25] and Bahir Dar City, Northwest Ethiopia[26] reported lower contamination rates of 16.2 and 39.1%, respectively. Vegetables are important means of parasitic contamination, especially intestinal parasites, which represent an important source of food-borne outbreaks in developing countries[27].

In the present study, 10 different vegetable types were screened for different stages of parasites. False cubeb leaf and bushbuck were mostly contaminated (60.0 and 50.0%), respectively. This could be attributed to the creepy nature of these vegetables. They seem to be more vulnerable to parasitic contamination because they creep on soil that may contain contaminated night soil and other contaminated animal dung. On the other hand, African spinach and bitter leaves had the lowest contamination rates of 10 and 20%, respectively. This could be attributed to the fact that they grow taller and therefore, less vulnerable to parasitic contamination.

There are major potential determinants of parasitic contamination revealed from the questionnaire responses. Firstly, the type of water used for irrigation in town, where Obollo town practised only (31.3%) clean water irrigation and (68.8%) unclean water irrigation, Enugu-Ezike practised (50%) clean water and (50%) unclean water for irrigation purposes. The second likely determinant of parasitic contamination of vegetables in these areas could be attributed to the type of manure used. The majority of vegetable farmers used human/animal feces as manure. These types of manure are potential carriers of different stages of parasites. Thirdly, the display patterns of vegetable in the market matters a lot. Those vegetables displayed on the ground have higher tendencies to be contaminated because of dust and dirt from surface runoff. Also, the majority of the vegetable sellers washed the vegetables before display even though some were washed with dirty water. Washing the vegetables before display reduces the chances of parasitic transmission to humans.

Ascaris lumbricoides ova happened to be the most prevalent parasite in this study (18.3%). This is in consonant with research conducted in Mannuthy, India[28] where the *Ascaris* species was the only parasite isolated from the positive samples (2.7%). In another study carried out in West Bengal, India, the *Ascaris* species was the most predominant parasitic ova observed[29]. *Ascaris* species which is a roundworm affects about a quarter of the world's population[30], It is one of the resistant intestinal infections that is frequently employed as a parasitological indicator[31] and is more common in developing countries[30]. The possession of a thick outer covering (cuticle) made *Ascaris* species more resistant to adverse environmental conditions and provides them with a long span in the soil[32].

Therefore, the vegetables should not be eaten raw and/or must be properly washed before consumption. This study is applicable to vegetable handlers or vendors (sellers and consumers), they should always put on hand gloves to avoid being contaminated.

CONCLUSION

There is a moderate prevalence of parasitic contamination of vegetables sold at Obollo-Afor Market, probably due to handling procedures from production to sales. *Ascaris lumbricoides* was the most prevalent parasite. Vendors that washed their vegetables were almost two times more than those that didn’t wash. Information gathered from this research should be notified to the general public of the tremendous increase in the rate of parasitic contamination of vegetables. A policy should be made on how best to manage vegetables from farm to table. The usual home practice of washing vegetables with saline water should be maintained.

SIGNIFICANCE STATEMENT

The purpose of this research was to determine the prevalence of parasites in commonly consumed vegetables sold at Obollo-Afor Market, Nigeria. It revealed vegetables sold at the market were contaminated with parasites of public health importance. The overall prevalence of parasites in the vegetables was 36.7% and *Ascaris lumbricoides* being the most predominated parasite. False cubeb leaf (60%) was the most contaminated vegetable whereas African spinach (10%) was the least. There was no significant difference in the prevalence of each parasite type between vegetable types. This study should be furthered by the use of molecular characterization of parasites of vegetables to confirm identification.

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