



Evaluation of Vegetable Contamination with Medically Important Helminths and Protozoans in Calabar, Nigeria

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Authors' contributions

The work was carried out in collaboration among all authors. Author IEE designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors EOI, OAC and ABC managed the analyses of the study. Author EOI managed the literature review searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Vegetables are usually exposed to parasitic ova, larvae or oocyst during cultivation, irrigation, storage, transportation or while processing for consumption. Consumption of raw, unwashed and improperly cooked vegetables is considered a risk factor for transmission of intestinal parasites. This study assessed the parasitic contamination of vegetables and also determined factors associated with parasitic contamination of vegetables in selected markets in Calabar, Cross River State.

Place and Duration of Study: Sample: Markets within Calabar, Cross River State, Nigeria between September and October, 2018.

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Methodology: A pre-tested semi-structured questionnaire was used for collecting data on factors associated with parasitic contamination of vegetables. 300 vegetable samples were purchased and processed from three different markets in Calabar for examination of parasitic contamination using direct wet mount and zinc sulphate floatation techniques.

Results: 15.7% (47/300) of the vegetables sampled were positive for at least one parasite. The species and stages of parasites detected were larvae of *Strongyloides*, ova of *Trichuris trichiura* and Hookworm, and cysts of *Entamoeba histolytica* and *Giardia lamblia*. Larvae of *Strongyloides* (11.3%) were the most frequently detected followed by ova of *T. trichiura* (1.7%) and Hookworm (1.7%) ($P = 0.000$). Among the five vegetable types sampled, waterleaf (40%) had the highest level of parasitic contamination followed by pumpkin (21.7%). Washing vegetables before display and the means of display were significantly associated with parasitic contamination ($P = 0.000$).

Conclusion: Prevention of contamination is important in reducing food-borne parasitic infections and can be achieved through improved personal hygiene practices of farmers, vendors and consumers, proper washing of vegetables and improved sanitary conditions especially around markets where these vegetables are sold.

Keywords: Vegetable; gastro-intestinal parasites; Soil-transmitted helminths; *Strongyloides* sp; Markets; Nigeria.

1. INTRODUCTION

Parasitic infections are widely distributed throughout the world, causing serious problems to public health [1]. Infections are linked with conditions of poverty, crowded living conditions, unsafe water, poor personal hygiene and environmental sanitation [2,3]. Consumption of raw or improperly washed/cooked vegetables such as Waterleaf (*Talinum triangulare*), Cabbage (*Brassica deracea*), Tomato (*Lycopersicon esculentum*) and Carrot (*Daurus carota*) has been shown to be risk factors for human parasitic infections [4].

Globally, food borne diseases continue to be a serious threat to public health and these diseases are a major cause of morbidity [5]. Studies have discovered that *Ascaris lumbricoides*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Enterobius vermicularis*, *Fasciola* spp., *Giardia intestinalis*, hookworm, *Hymenolepis* spp., *Taenia* spp., *Trichuris trichiura*, and *Toxocara* spp., can infect humans who consume contaminated, uncooked, or improperly washed vegetables and fruits [6,7,1]. As a result, an estimated 3.5 billion people are infected with parasitic helminths, with an estimated 200,000 deaths each year [8]. These parasitic infections have been shown to cause iron deficiency anaemia, growth retardation in children amongst other physical and mental health problems [9,3].

Vegetables are usually exposed to parasitic ova, larvae or oocyst during cultivation, irrigation, storage, transportation or while processing for consumption [10]. Bad hygienic practices during production, transport, processing and preparation

by handlers and also consumers contribute in vegetable contaminations [11].

In Calabar and other parts of the country, vegetables form an everyday component of the menu, which in most cases are eaten unwashed or improperly washed. They serve as the main sources of vitamins and fibres for our humans [12]. Vegetables are an important route of transmission of intestinal parasites and have shown to be an important source of food borne parasitic disease outbreak in developing countries [10]. Consumption of raw, unwashed and unhygienically prepared vegetables is considered a risk factor for human parasitic infections [4,12]. As a primary source of essential nutrients, vitamins, minerals and fibre for our bodies, vegetables and fruits form a vital part of our daily diet. However, when eaten uncooked, fruits and vegetables can act as vehicles for infection with protozoan parasites, particularly *Cryptosporidium*, *Cyclospora* and *Giardia* [12].

This study therefore seeks to screen vegetables for parasitic contamination and also determine factors associated with parasitic contamination of vegetables in selected markets in Calabar, Cross River State.

2. METHODOLOGY

2.1 Study Area

A cross-sectional study was conducted between September and October, 2018 to determine the level of parasitic contamination of vegetables and also determine risk factors associated with parasitic contamination of vegetables sold in

selected local markets in Calabar Metropolis, Nigeria. Calabar, the capital of Cross-River State, is a moderately populated city located in the South-South region of Nigeria on the longitude 8 19'30"E and latitude 4 57'0"N. Calabar features a tropical climate with a lengthy wet season and a short dry season. The average annual temperature is 28°C, and approximately 3000 mm of precipitation annually. Three markets were randomly selected: Watt market, Marian market and 8miles market.

2.2 Data Collection

A pre-tested semi-structured questionnaire was used for collecting data on factors associated with parasitic contamination of vegetables. These factors included: status of the produce (type of produce, washed before display or not and means of display) and educational status of the vendors. In each market, vegetable samples were purchased from randomly selected sellers. A total of 150 vendors participated in this study.

2.3 Sample Collection

Three major markets; Watt market, Marian market and 8miles market, were selected for this study. The fresh vegetable samples used in this study included Carrot (*Daurus carota*), Pumpkin (*Telfairia occidentalis*), Waterleaf (*Talinum triangulare*), Tomatoes (*Lycopersicon esculentum*) and Cabbage (*Brassica deracea*). These samples were purchased from food vendors for a period of 8 weeks, between September and October, 2018. A total of 300 fresh vegetables frequently consumed were randomly purchased from sellers in three markets. 60 of each vegetable were sampled and 20 each from the selected markets.

2.4 Parasitological Analysis

The samples were transported to the laboratory in sterile polythene bags for parasitological analysis. Each sterile polythene bag was labelled with a unique number and date of collection. Approximately 200g aliquot of each vegetable was washed in 500mL of distilled water and the eluent was filtered through a sterile 0.4 mm² sieve to remove unwanted materials. This was left to sediment for 24 hrs. The filtrate was then dispensed into clean centrifuge tubes and centrifuged at 3000 rpm for five minutes [13]. The supernatant was discarded, and the sediment was placed on a clean glass slide. A clean cover slip was placed softly on the slide to avoid air bubbles and over flooding. Lugol's iodine stain was used

and the preparation was examined under a light microscope (Olympus CHT) using ×10 and ×40 objectives [14, 15].

Using the zinc sulphate floatation technique, 2ml of the sediment was placed in a test tube. This was filled with zinc sulphate solution to the brim and a cover slip was placed on top. The cover slip was removed after fifteen minutes to allow enough time for the cysts and ova to float. The cover slip was placed face downwards on a clean microscope slide and examined under ×10 and ×40 objectives with a drop of Lugol's iodine stain under the cover glass, to identify the ova and cysts [14].

2.5 Data Analysis

Statistical analysis was performed with SPSS software version 22 (IBM, Chicago, IL, USA). P-values equal to or less than 0.05 were considered to be statistically significant. The difference in parasitic contamination among the different categories was compared using the Chi-square test. Univariate and multivariate logistic regression analysis were performed to determine factors associated with parasitic contamination of vegetables.

3. RESULTS

Five different types of vegetables were collected from three local markets and examined for parasitic contamination. Results showed that 47 (15.7%) of the vegetables sampled were positive for at least one parasite (Table 1). The difference in the level of parasitological contamination in the vegetables was statistically significant at $P = .00$.

The species and stages of parasites detected were larvae of *Strongyloides*, ova of *T. trichiura* and Hookworm, and cysts of *E. histolytica* and *G. lamblia*. Larvae of *Strongyloides* (11.3%) were the most frequently detected parasite followed by ova of *T. trichiura* (1.7%) and Hookworm (1.7%). The prevalence of cysts of *E. histolytica* (0.3%) and *G. lamblia* (0.7%) were low. The prevalence of *Strongyloides* contamination was significantly higher ($P = .00$) than other parasitic contamination observed in the study (Table 2).

Prevalence of parasitic contamination according to markets surveyed showed that Marian Market (23%) had the highest prevalence of parasitic contamination followed by 8 Miles Market (13%) and Watt Market (11%). This difference was however not statistically significant ($P = .45$) (Fig. 1).

Table 1. Frequency distribution of parasitic contamination of vegetables

Vegetable type	No. of sample examined	No. positive (%)	χ^2	P-value
<i>Darus carota</i>	60	3(5.0)	35.872	.00
<i>Telfaria occidentalis</i>	60	13(21.7)		
<i>Talinum triangulare</i>	60	24(40.0)		
<i>Lycopersicon esculentum</i>	60	4(6.7)		
<i>Brassica deracea</i>	60	3(5.0)		
Total	300	47(15.7)		

Table 2. Prevalence of parasitic contamination according to parasite type

Parasites detected	No. of vegetable sample examined	No. positive (%)	χ^2	P-value
<i>Strongyloides stercoralis</i>	300	34(11.3)	81.830	.00
<i>Trichuris trichiura</i>	300	5(1.7)		
Hookworm	300	5(1.7)		
<i>Entamoeba histolytica</i>	300	1(0.3)		
<i>Giardia lamblia</i>	300	2(0.7)		

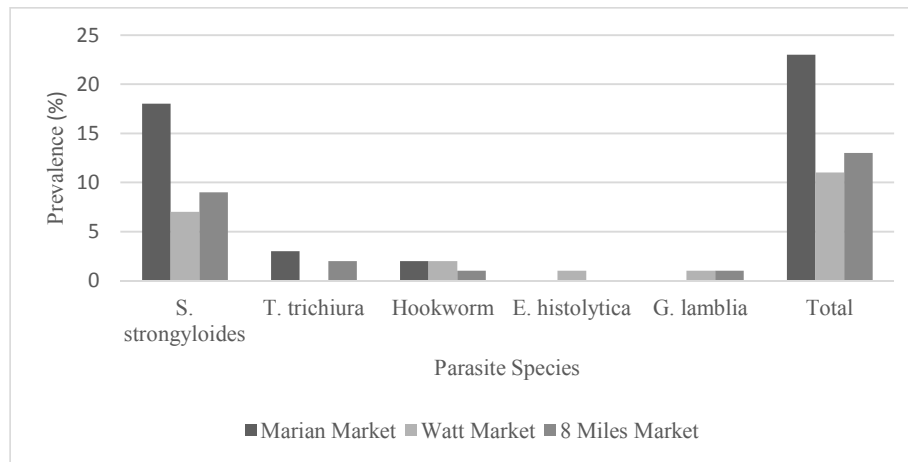


Fig. 1. Prevalence of parasitic contamination according to markets surveyed in Calabar (n=100; P = .045)

Fig. 2 shows the level of parasitic contamination of vegetables. Among the five vegetable types sampled, Waterleaf (40%) had the highest level of parasitic contamination followed by Pumpkin (21.7%). Cabbage (5%) and Carrot (5%) each recorded the least level of parasitic contamination. Larvae of *Strongyloides* were recorded in all vegetable types while cyst of *E. histolytica* was recorded in Waterleaf only. The difference in the level of parasitic contamination according to vegetable type was not statistically significant ($P = .91$).

3.1 Factors Associated with Parasitic Contamination of Vegetables

Washing vegetables before display and the means of display were significantly associated with

parasitic contamination ($P = .00$). Vegetables not washed before display were 6.556 times at higher risk of contamination as compared to those that were washed before display (OR: 6.556; 95% CI: 0.077, 0.403). Also, vegetables displayed on the floor were 2.593 times at a higher risk of contamination as compared to those displayed on tables (OR: 2.593; 95% CI: 0.313, 1.296). Among 150 interviewed vendors, 50 had attended primary school while 78 and 22 had no formal education and secondary/above respectively. However, this was not significantly associated with parasitic contamination of vegetables ($P = .69$) (Table 3).

4. DISCUSSION

Despite efforts by the Federal Ministry of Health and other non-governmental organisations, the

burden of intestinal parasites is still high due to poor sanitation, favourable climatic conditions and poor knowledge on parasitic disease transmission and prevention. The current study sought to assess the level of parasitic contamination of vegetables and determine risk factors associated with parasitic contamination.

Five different types of parasites were identified with prevalence rate of 15.7%. Similar studies have been carried out to evaluate the status of contamination of vegetables with medically important parasites [16,17,18,12,19,20]. The overall prevalence rate of parasitic contamination in this study supports the findings from Egypt [21], Nigeria [22] and Sudan [7] but was lower than previous findings elsewhere [16,23,24]. Nonetheless, it was observed to be higher when compared to a study from Turkey [25] and India [26]. These differences could be attributed to variations in environmental and climatic conditions, sanitary conditions, laboratory techniques employed and types of vegetable sampled which are instrumental in the transmission of parasitic diseases.

In this study, larvae of *Strongyloides* (11.3%) were the most frequently detected. This agrees with the results of [6] and Tefera et al. [27] linked the high prevalence of *Strongyloides* to its complex life cycle – alternating between free-living and parasitic cycles, and thus does not require a host for its proliferation. However, studies conducted elsewhere have reported different results [22,1, 23].

Prevalence of parasitic contamination according to markets surveyed showed that Marian market (23%) had the highest prevalence of parasitic contamination. This could be as a result of the vendors' close proximity with the market's dumpsites. Vegetables left in stores can therefore be contaminated by flies and rodents which visit these dumpsites. Waterleaf (40%), which is a leafy vegetable, had the highest level of parasitic contamination. Rahmati et al. [28] in Iran reported a similarly higher prevalence of parasitic contamination in green leafy vegetables. Avcioglu et al. [29] stated that leafy vegetables enable the parasitic stages attach easily to their surface unlike those having smooth surfaces.

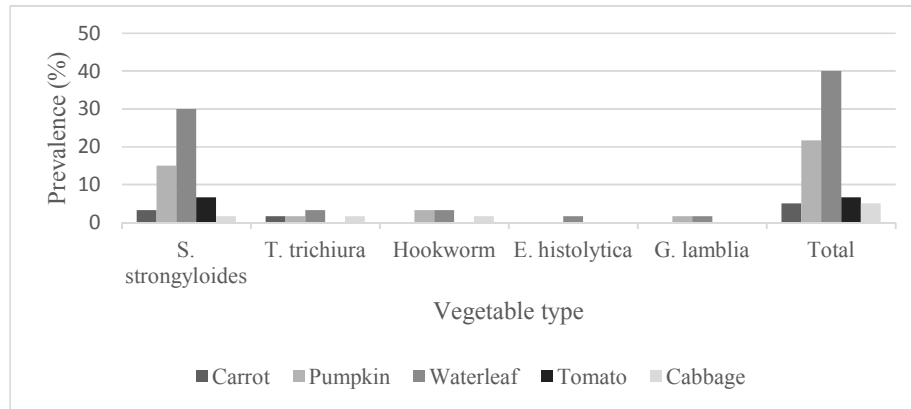


Fig. 2. Parasitic contamination according to vegetable type (n=60; P = .91)

Table 3. Binary Logistic Regression of factors associated with parasitic contamination of vegetables in selected markets in Calabar

Variables	Category	Number examined	Rate of parasite contamination N (%)	Crude Odds Ratio (95% CI)	P-value
Washed before display	Yes	68	9(13.2)	1	.00
	No	82	38(46.3)	6.556 (0.077-0.403)	
Means of display	On the floor	53	20(37.7)	2.593 (0.313-1.296)	.00
	On table	97	27(27.8)	1	
Handler's level of education	No formal	50	18(36.0)	0.812 (0.291-2.270)	.69
	Primary/Secondary/above	78	20(25.6)	0.498 (0.185-1.341)	

In the present study, washing vegetables before display and the means of display were significantly associated with parasitic contamination ($P = .00$). Vegetables not washed before display were 6.556 times at higher risk of contamination as compared to those that were washed before display. Tefera et al. [27] stated this might be due to the risk of contamination of the produce during production, transportation and other processing activities. Studies have shown that washing vegetables positively impact on the rate of parasitic contamination [29]. However, Alemu et al. [10] reported that pre-washing of vegetables could not inactivate viable infectious parasitic stages. It is therefore suggested that vegetables need to be washed following standard procedures using chemicals such as calcium hypochlorite to effectively remove parasitic stages [30]. Besides, vegetables displayed on the floor were 2.593 times at a higher risk of contamination as compared to those displayed on tables. Means of display of vegetables by vendors have been identified as a risk factor associated with parasitic contamination [27,7].

5. CONCLUSION

This study has revealed that vegetables sold in some markets in Calabar metropolis are contaminated both protozoan and helminth parasites and can serve as a source of transmission to the public especially if they are not properly washed and cooked before consumption. Prevention of contamination still remains a viable route for reducing food-borne parasitic infections. This can be achieved through improved personal hygiene practices of farmers, vendors and consumers, proper washing of vegetables and improved sanitary conditions especially around markets where these vegetables are sold. Furthermore, farmers and vendors should be enlightened on the importance of wearing gloves and washing hands after handling vegetables.

CONSENT

As per international standard or university standard, participant's written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bekele F, Tefera T, Biresaw G, Yohannes T. Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. *Infect Dis Poverty*. 2017;6(19):1-7. DOI:10.1186/s40249-016-0226-6.
2. Wegayehu T, Tsalla T, Seifu B, Teklu T. Prevalence of intestinal parasitic infections among highland and lowland dwellers in Gamo Area, South Ethiopia. *BMC Pub Health*. 2013;13:151.
3. Effanga EO, Imalele EE. Intestinal parasites and anaemia amongst adult patients attending a tertiary hospital in Calabar, Nigeria. *J Global Biosci*. 2018;7(9):5633-5653.
4. Cheesbrough M. *Medical Parasitology. Medical laboratory manual for tropical countries*. 1991;163-411.
5. Blackburn C, McClure, PJ. *Foodborne pathogens: hazards, risk analysis and control*. Washington DC: CRC Press. 2002;18-19.
6. Idahosa OT. Parasitic contamination of fresh vegetables sold in Jos markets. *Global J Med res*. 2011;11(1):5-11.
7. Mohamed MA, Siddig EE, Elaagip AH, Edris AMM, Nasr A.A. Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. *Ann Clin Microbiol Antimicrob*. 2016;15:17-23.
8. Wakid HM. Improvement of Ritchie technique by identifying the food that can be consumed pre-analysis. *J Appl Sci*. 2009;5: 293-296.
9. WHO. De-worming for health and development. In: report of the third global meeting of the partners for parasite control, Geneva; 2005.
10. Alemu G, Mama M, Misker D, Haftu D. Parasitic contamination of vegetables marketed in Arba Minch town, Southern Ethiopia. *BMC Infect Dis*. 2019;19:1-8. Available: <https://doi.org/10.1186/s12879-019-4020-5>
11. Gupta SS, Nayek SS, Garai D. Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. *Environ Monit Assess*. 2010;165(1-4):169-177.
12. Utaaker KS, Kumar A, Joshi H, Chaudhary S, Robertson LJ. Checking the detail in retail: occurrence of *Cryptosporidium* and *Giardia* on vegetables sold across different counters in Chandigarh, India. *Int J Food Microbiol*. 2017;263:1-8.
13. Omowaye OS, Audu PA. Parasites contamination and distribution on fruits and vegetables in Kogi, Nigeria. *CIBTech J Bio-Protocols*. 2012;1(1):44-47.

14. Cheesbrough M. District laboratory practice in tropical countries, part 1. NY, USA: Cambridge University Press. 2009;206–207.
15. Duedu KO, Yarnie AE, Tetteh-Quarcoo PB, Attah SK, Donkor ES, Ayeh Kumi PF. A comparative study of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-air markets in Accra, Ghana. BMC Res Notes. 2014;7:836.
16. Maikai BV, Elisha IA, Baba-Onoja EBT. Contamination of vegetables sold in markets with helminth eggs in Zaria metropolis, Kaduna State, Nigeria. Food Control. 2012; 28:345-348.
17. Lalonde LF, Gajadhar AA. Detection of *Cyclospora cayetanensis*, *Cryptosporidium* spp., and *Toxoplasma gondii* on imported leafy green vegetables in Canadian survey. Food Waterborne Parasitol. 2016;2:8–14.
18. Caradonna T, Marangi M, Del Chierico F, Ferrari N, Reddel S, Bracaglia G, et al. Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. Food Micro. 2017;67:67–75.
19. Karshima SN. Parasites of importance for human health on edible fruits and vegetables in Nigeria: A systematic review and meta-analysis of published data. Pathology and Global Health. 2018;112:47–55.
20. Li J, Shi K, Sun F, Lia T, Wang R, Zhang S, Jian F, Ning C, Zhang L. Identification of human pathogenic *Enterocytozoon bieneusi*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* on the surfaces of vegetables and fruits in Henan, China. Int J Food Microbiol. 2019;307:108292
21. Hassana A, Farouka H, Abdul-Ghani R. Parasitological contamination of freshly eaten vegetables collected from local markets in Alexandria, Egypt: A preliminary study. Food Control. 2012;26:500-503.
22. Simon-Oke IA, Afolabi OJ, Obasola OP. Parasitic contamination of fruits and vegetables sold at Akure metropolis, Ondo State, Nigeria. Researcher. 2014;6(12):30-35.
23. Kathleen NO, Yvonne AL, Xiang TG, Vachel GVP. Parasite contamination of freshly harvested vegetables from selected organic and conventional farms in the Philippines. Pertanika J Trop Agric Sci. 2018;41(4):1741-1756.
24. Bekele F, Shumbej T. Fruit and vegetable contamination with medically important helminths and protozoans in Tarcha town, Dawuro zone, South West Ethiopia. Res Rep Trop Med. 2019;10:19–23.
25. Adanir R, Tasci F. Prevalence of helminth eggs in raw vegetables consumed in Burdur, Turkey. Food Control. 2013;31:482-484.
26. Sunil B, Thomas DR, Latha C, Shameem H. Assessment of parasitic contamination of raw vegetables in Mannuthy, Kerala state, India. Vet World. 2014;7(4):253-256.
27. Tefera T, Biruksew A, Mekonnen Z, Eshetu T. Parasitic contamination of fruits and vegetables collected from selected local markets of Jimma town, Southwest Ethiopia. International Scholarly Research Notices. 2014;20(4):7.
28. Rahmati K, Fallah M, Maghsood AH, Shamsi-Ehsan T, Matini M. The Prevalence of Parasitic Contamination of Vegetables Consumed in Malayer City, West of Iran, in 2014. Avicenna J Clin Microbiol Infect. 2017;4(2):e42380.
29. Avcioglu H, Soykan E, Tarakci U. Control of helminth contamination of raw vegetables by washing. Vector-Borne Zoonotic Dis. 2011;11(2):189–191.
30. Fallah AA, Pirali-Kheirabadi K, Shirvani F, Saei-Dehkordi SS. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. Food Control. 2012;25(2):617–620.

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