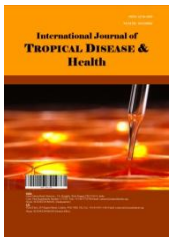

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Parasitic Contamination of Fresh Fruits and Vegetables Sold in Port Harcourt Metropolis Markets, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Parasitic contamination of fresh fruits and vegetables sold in selected markets in Port Harcourt Metropolis, Rivers State, Nigeria. was investigated. Oil mill and Creek Road were the two major markets in Port Harcourt metropolis area selected for this study. A total of 216 samples of different types of fruits and vegetables were randomly sampled for parasitological examination using normal saline and zinc sulphate floatation techniques. The results showed that out of the 216 samples of vegetables and fruits examined, 87 (40.3%) were infected. The results were statistically significant (P< 0.05). The parasites identified included *Entamoeba coli; Entamoeba histolytica, Giardia lamblia, Balantidium coli, Ascaris lumbricoides*, *Ancyclostoma* spp and *Fasciola* spp. *Entamoeba coli* was the most prevalent parasite (83.15%) while *Ascaris lumbricodes* (1.12%), *Ancyclostoma* spp (1.12%) and *Fasciola* spp (1.12%) had least prevalence. There was statistical no difference (P>0.05) in prevalence of parasites genus in the two markets. Tomatoes had the highest contamination rate of (10.6%) followed by waterleaf, bitter leaf, green leaf, pumpkin leaf, pear, orange, and scent leaf with the prevalence rate of 9.3%, 8.8%, 4.6%, 3.2%, 1.9%, 1.4% and 0.5%

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respectively. Produce contaminated with intestinal parasites poses a serious health challenge to the consumers if they are not properly washed before consumption. Personal hygiene and proper washing of fresh fruit and vegetable before consumption will reduce the prevalence of food-borne parasitic infections.

Keywords: Fruit; vegetable; market; parasites; Port Harcourt.

1. INTRODUCTION

Fruits and vegetables are important components of a healthy diet, and their daily consumption plays a key role in the prevention of certain nutritional deficiencies, cardiovascular diseases and stroke (Hartley et al., 2013); [1]. Reduction in the consumption of fruits and vegetables is linked to poor health and increased risk of non-communicable diseases [2,3,4]. According to 2010 reports, globally, about 6.7 million deaths were attributed to poor consumption of fruits and vegetables [2,5,1]. Nutraceuticals are useful to the human body especially in the prevention and/or treatment of some infections. Vegetables and fruits are a major source of nutraceuticals, which are natural ingredients found in them. These components can be in the form of antioxidants, natural colourant (e.g., carotenoids), minerals, and vitamins, which often have added advantages [6,7]. Recommended 400g (five portions), daily intake of fruits and vegetables provide sufficient dietary fibres and nutrients lacking in other food items, thus reduce the risk of noncommunicable diseases like diabetes, heart disease, stroke and cancer [4]. High level of water and dietary fibres in vegetables and fruits may help in lowering the possibility of obesity.

However, fruits and vegetables have been implicated as a key epidemiological factor in the transmission of parasitic food-borne infections particularly when not adequately washed [8,9,10]. Globally various occurrences of protozoan infections in humans have been attributed to the consumption of improperly washed fresh vegetables and fruits. This is also seen prevalent in developing countries [9]. Vegetables and fruits contaminated with eggs and cysts of parasites via handling, processing, and transportation, serve as potential vessels for the transmission of diseases [11-15].

They are two main types of parasites mostly associated with food produce which causes different intestinal infections, these are the helminths and the protozoan parasites [16]. The common helminth reported are *Enterobius vermicularis* (pin worm), *Trichuris trichura* (whip worm), *Ascaris lumbricoides* (roundworm), *Necator americanus* and *Ancyclostoma duodenale* (hookworm), *Fasciola* spp (flatworm) and *Strongyloides stercoralis*. The common intestinal protozoan parasites include *Giardia lamblia*, *Cryptosporidium* spp, *Entamoeba coli*, *Entamoeba histolytica* and *Balantidium coli* [17]. Some of the helminths and protozoan parasites can infect different tissues and organs of the host body causing mechanical injury, which may result in destruction of body parts; blocking food passages in the host body e.g. *Entamoeba* spp, *Ascaris lumbricoides, Ancyclostoma* spp (intestinal bleeding), [18].

Ascaris lumbricoides causes ascariasis, abdominal discomfort, pain, and anaemia. It also causes secondary infection due to the migrating larva. *Ancyclostoma* spp causes ancylostomiasis, associated with larval penetration of the skin causing itching rash, and fever. *Giardia lamblia*, a flagellate protozoan which causes giardiasis with foul swelling diarrhoea with flatulence, stomach cramps, and dehydration. *Entamoeba* spp causes amoebiasis, amoebic liver abscess often associated with bloody diarrhoea as well as stomach cramps. *Balantidium coli*, causes balantidiasis with persistent diarrhoea, vomiting and weight loss. *Fasciola* spp is a flatworm that causes disease known as fascioliasis, which accompanies abdominal pains, at times leading to liver damage [19,20].

Previous studies carried out in some markets in Port Harcourt metropolis have shown that several fruits and vegetables were contaminated with helminths eggs (such as *Ancyclostoma* spp, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Fasciola* spp); as well as protozoan trophozoites, cyst, oocyst (such as *Giardia lamblia*, *Cryptosporidium* spp, *Entamoeba coli*, *Entamoeba histolytica, Toxoplasma gondii* and *Balantidium coli*), [21,14].

Records indicate that in sub-Saharan Africa, Nigeria had the highest prevalence of soil-transmitted helminths [22]. This is mainly due to

the lack of readily available water resources and proper sanitation. In Nigeria, contamination of vegetables and fruits by soil-transmitted helminths and protozoa have been previously reported [23,24,25,26,9,21,17], but there is lack of reports from Port Harcourt Metropolis Oil mill and Creek Road market. The present study is aimed at evaluating the prevalence of soil-transmitted helminths (STHs) and protozoans on fresh vegetables and fruits sold in selected markets in Port Harcourt Metropolis.

2. MATERIALS AND METHODS

2.1 Study Area

Two major markets within Port Harcourt metropolis were selected for this study, Oil mill (Latitude: N 4°51'13.96839", Longitude: E 7°3'47.90519") located in Obio-Akpor, and Creek Road market (Latitude: N 4°45'32.16887" Longitude: E 7°1'36.899"), located in Port Harcourt Local Government area. The selection of the markets was based on the large crowd, from within and outside Rivers State, attracted for trade in these venues. Port Harcourt metropolis covers substantial parts of two local government areas (Port Harcourt City and Obio/Akpor Local Government Areas) and the climate is similar to other cities within the Niger Delta Region of Nigeria. Thick vegetation, regular and intermittent rainfall throughout the year with a very short dry season characteristic climate of this zone.

2.2 Sample Collection

Four different types of fresh fruits and five vegetables were randomly selected and bought from each of these two markets. The study was conducted between September and October, 2019. The fruits included tomatoes (*Solanum lycopersicum*), cucumber (*Cucumis sativs*), orange (*Citrus sinensis*), pear (*Dacryodes edulis*), and the vegetables were pumpkin leaf (*Telfiria occidentalis*), scent leaf (*Ocimum gratissum*), green leaf (*Amaranthus* spp), bitter leaf (*Vernonia amygdalina*), and waterleaf (*Talinum triangulare*). Equal numbers of samples were selected from each vegetable and fruit types, per market location (12 of each item, total 216 for the two markets). The samples were collected in sterile polythene bags and transported to the research laboratory, Ignatius Ajuru University of Education research laboratory for laboratory analysis.

2.3 Laboratory Analysis

The fruits and vegetables were analysed using both sedimentation and zinc sulphate flotation methods. The method of Bishop and Yohanna [15] was adopted for the detection of parasites by sedimentation, with a slight modification. An analog scale was used to weigh 200 g of sliced vegetables and fruits. Slices were soaked in 500 ml of physiological saline solution (0.85% NaCl) overnight to dislodge the parasites ova, larvae, cysts, and oocysts which were in different developmental stages. After 24 hours, the fruits were separated and the vegetables were vigorously shaken to detach the parasites. The water was left to settle after which it was decanted and strained through a sterile sieve to remove large particles and sand. The filtrate was centrifuged in a 15 ml tube at 3000rpm for 5 minutes to concentrate the sediments. The supernatant was cautiously discarded. Few drops of the sediment were collected for microscopic examination. A drop of Lugol's iodine was added to the drop of sample on a clean slide. A clean coverslip was carefully placed over the sample and studied under a light microscope. Protozoan and helminth ova, larvae, cysts, and oocyst were identified using 10x and 40x objectives. Part of the sediment collected was resuspended in 33% zinc sulphate floatation fluid and recentrifuged. More floatation fluid was added to fill to the brim and a cover slip was super-imposed on it and left undisturbed. The cover slip was lifted after 30 minutes and examined under the microscope. Identification was based on their morphological characteristics such as shapes and size of eggs using outlined by Ochei and Kolhatkar [27].

2.4 Data Analysis

The chi-square ($\chi 2$) and T-test were used to determine the relationship between variables at a statistical difference of $P<0.05$.

3. RESULTS

A total of 120 vegetable and 96 fruit samples were randomly bought from the two selected markets of Port Harcourt Metropolis. Out of the investigated samples, 30 (13.9%) of the fruits and 57 (26.4%) of the vegetables were contaminated with parasites.

The result of the study showed that 87 of 216 samples were identified to be contaminated,

which gave rise to an overall contamination rate of 40.3%. The distributions were as shown in Table 1. Out of all the collected samples, the cucumber had no contamination. Tomatoes had the highest contamination rate of 23(10.6%) followed by waterleaf 20(9.3%). Bitter leaf, green leaf, pumpkin leaf, pear, orange, and scent leaf had the prevalence rate of 8.8%, 4.6%, 3.2%, 1.9%, 1.4%, and 0.5% respectively. There was a statistically significant difference (P< 0.05) in the prevalence of parasitic contamination on fruits and vegetables in the study.

Parasites identified during the study included *Entamoeba coli; Entamoeba histolytica, Giardia lamblia, Balantidium coli, Ascaris lumbricodes*, *Ancyclostoma* spp, and *Fasciola* spp. Most prevalent parasite was *Entamoeba coli* 74(83.15%) compared to the least prevalent *Ascaris lumbricodes* (1.12%), *Ancyclostoma* spp (1.12%), and *Fasciola* spp (1.12%), (Table 2). *Entamoeba coli* was found in all the samples except in cucumber and scent leaf while *Ascaris lumbricodes, Fasciola* spp were only found in waterleaf.

The results indicated that fruits 17(15.74%) and vegetables 34(31.48%) from oil mill market were more contaminated with parasites than those fruits 13(12.04%) and vegetables 23(21.29%) from Creek Road market, Table 3. This variation was statistical significance at p< 0.05.

The various species of parasites identified on the fruits and vegetables from fruit Oil mill market included *Entamoeba coli*; *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Ascaris lumbricodes*, and *Fasciola* spp, while the Creek Road market had only *Entamoeba coli*; *Entamoeba histolytica*, and *Ancyclostoma* spp. The study did not show any statistical difference in the type of parasites genus in the two markets (Table 4).

4. DISCUSSION

Contaminations of fruits and vegetables by parasites have become a global public health and socio-economic issue especially in developing countries [4,6]. This is attributed to the little attention given to food-borne parasitic infections in these countries [24]. The presence of intestinal parasite stages on fruits and vegetables is an indication of faecal contamination from human and animal sources. Open defaecation has also been associated as a contributing factor to unsanitary surroundings with constant faecal pollution of soil and drinking water [28].

Table 1. Prevalence of parasitic contamination in fruits and vegetables samples

Fruit/Vegetables	Number Examined	No positive (%) N=216	Percentage positive in individual fruits/ vegetables (n=24)	χ2	*(p-value)*
Fruits					
Tomatoes	24	23 (10.6)	95.8		
Cucumber	24	0 (0)	0		
Orange	24	3 (1.4)	12.5		
Pear	24	4 (1.9)	16.7		
Sub total	**96**	**30(13.9)**			
Vegetables					
Pumpkin leaf	24	7 (3.2)	29.2		
Scent leaf	24	1 (0,5)	4.2		
Green leaf	24	10 (4.6)	41.7		
Bitter leaf	24	19 (8.8)	79.2		
Water leaf	24	20 (9.3)	83.3		
Sub total	**120**	**57 (26.4)**		**108.087**	**0000**
Total	**216**	**87 (40.3)**			

Negative 129 (59.7%)

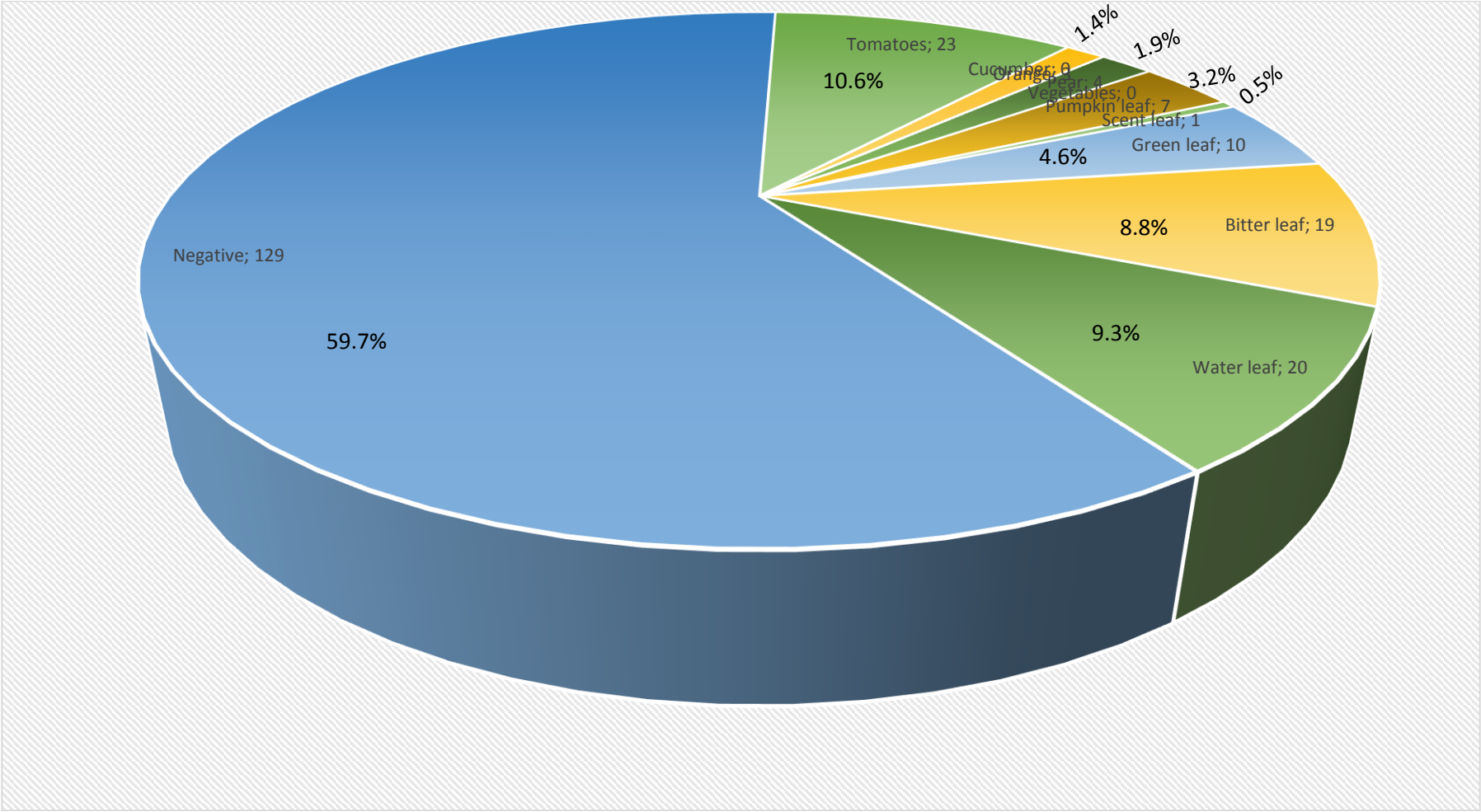

Fig. 1. Percentage frequency of parasite contamination in fruits and vegetables

Table 2. Percentage survey of parasite genus in contaminated fruits and vegetables in the study

Fruits/vegetable	Tomatoes	Cucumber	Orange	Pear	Pumpkin leaf	Scent leaf	Green leaf	Bitter leaf	Water leaf	Number of Parasites (%)
E. coli	21	0	3	4	6	0	8	15	17	74 (83.15)
E. histolytica	2	0	0	0	1	0	2	2	0	7 (7.87)
G.lamblia	1	0	0	0	0	0	0	1	0	2 (2.25)
B.coli	0	0	0	0	0	0	1	1	1	3 (3.37)
A.lumbricodes	0	0	0	0	0	0	0	0	1	1 (1.12)
Ancyclostoma spp	0	0	0	0	0	1	0	0	0	1(1.12)
Fasciola spp	0	0	0	0	0	0	0	0	1	1 (1.12)
					N= 216	*89 (100)*				

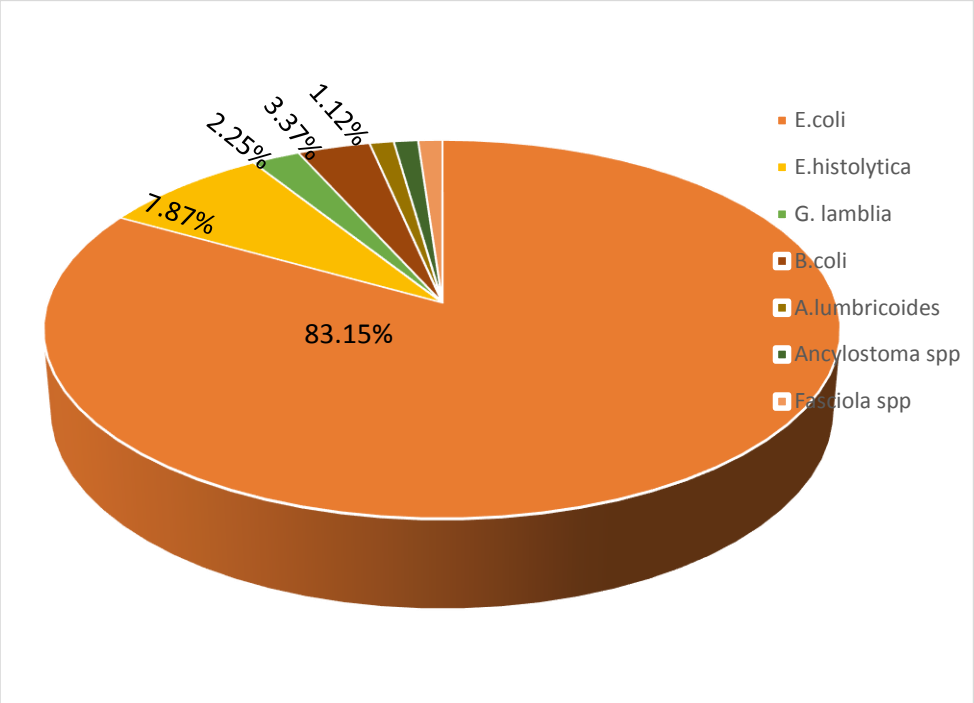

Fig. 2. Distribution of parasites in contaminated fruits and vegetables

Table 3. Comparison of parasitic contamination from the two local markets in Rivers State

Fruits /Vegetables	Oil Mill		Creek road	
	No examined	No Infected (%)	No examined	No Infected (%)
Fruits				
Tomatoes	12	12 (11.11)	12	11(10.18)
Cucumber	12	0 (0)	12	0 (0)
Orange	12	2 (1.85)	12	1(0.93)
Pear	12	3 (2.78)	12	1(0.93)
Subtotal	**48**	**17 (15.74)**	**48**	**13 (12.04)**
Vegetables				
Pumpkin	12	5(4.63)	12	2 (1.85)
Scent leaves	12	0(0)	12	1(0.93)
Green leaves	12	6(5.56)	12	4 (3.70)
Bitter leaves	12	1211.11)	12	7 (6.48)
Water leaves	12	11(10.19)	12	9 (8.33)
Subtotal	**60**	**34 (31.48)**	**60**	**23 (21.29)**
Grand total	**108**	**51 (47.22)**	**108**	**36 (33.33)**
	Negative: 57 (52.78%)		**Negative: 72 (66.67%)**	

$\chi 2$ = 8.6407; (p-value 0.0032)

The findings from this study have shown that parasites ova, cyst, and larva can be found on fresh fruits and vegetables sold in two of the markets in Port Harcourt, Rivers State. Out of the 216 samples of fruits and vegetables examined, 87(40.3%) were contaminated with parasites. The outcome of this study is similar to the study carried out by Elom [23] in Ebonyi state where they reported 40.4% contamination. Similarly, Auta et al., [9] in Katsina state and Malann and Tim [12] in Gwagwalada, Abuja, reported a contamination rate of 45% and 42% respectively. In 2017, two separate studies reported the screening of parasites in vegetables sold in selected markets in Port Harcourt. In these studies, Mbata et al., [21] reported a contamination rate of 85% compared to 29.5% by Tchounga et al., [14].

Table 4. Types of parasites genus in the two markets

Parasites	Oil Mill market No of parasites (%)	Creek road market No of parasites (%)		
E. coli	40 (75.47)	34 (94.4)		
E. histolytica	6 (11.32)	1 (2.8)		
G.lamblia	2 (3.77)	0 (0)		
B.coli	3 (5.66)	0 (0)		
A.lumbricodes	1(1.89)	0 (0)		
Ancyclostoma spp	0 (0)	1(2.8)		
Fasciola spp	1(1.89)	0 (0)		
Total Positive	**53**	**36**	**89**	**F=0.038, p = 0.848**
Total Negative	**55**	**72**	**127**	
Grand total	**108**	**108**	**216**	

The variation of reports from these researchers in Port Harcourt markets could be due to the hygiene practices of the farmers and sellers. These changes might also be due to the differences in the origin of fruits and vegetables (produce), weather or environmental conditions.

In 2011, a similar study was carried out in Jos by Idahosa et al., [8] who reported a higher contamination rate of 56.25%, while in 2012, another report from Kogi state Omowaye and Audu [11] recorded a lower contamination rate of 11.87%.

In this study tomatoes had the overall highest prevalence rate (10.6%). This result is similar to that of Bekele et al., [10] in Ethiopia and Ajitha et al., (2020) in Tiruchirappalli. South India with the highest prevalence rate from tomatoes 71.1% and 26.6 % respectively. But this result was in contrast to the studies carried out by Mbata et al., [21]in Port Harcourt, Abe et al., 2016 and Tefera et al., 2014 also recorded tomatoes among the lowest contaminated samples. The variation in report of tomatoes prevalence from other researches in Port Harcourt markets may be attributed to the fact that most tomatoes sellers in the two markets studied, sell their produce in bulk and do not wash them before displayed for sale. This also agreed with the findings of Bekele et al., [10] and Ajitha et al., [29], where association of washing and contamination of fruits and vegetables were highly related.

Runoff waste from livestock and poultry farms is a common source of produce contamination. For example, Salmonella bacteria normally inhabit the digestive tract of wild and domestic birds and animals. Salmonella that infects a tomato blossom can flourish inside the growing fruit. If the water in which tomatoes rinsed harbors the pathogen, bacteria can stick on to the skin or seep inside a harvested tomato through the stem scar [30].

Among the vegetables studied waterleaf and bitter leaf had a prevalence rate of 9.3% and 8.8% respectively, this is similar to the work carried out by [23,8,31]. This result is different from the reports by Mbata et al., [21]; Tchounga et al., [14] where they reported highest vegetable contamination was from pumpkin leaf (21.3%) and green leaf (38.8%) respectively. According to Fumilayo et al., [32] parasites eggs and trophozoites can attached to the surface of leafy vegetables more easily than certain fruits and vegetables.

The overall least prevalent in this study was found in scent leaf (0.5%), this is similar to the study carried out in Ihiala local government area, Anambra by Orji (2016). The variance in contamination among the vegetables may be attributed to the uneven leaf surfaces which aid in the easy attachment of parasites [24,14]. The rough surface and the leaf folds of vegetables may also retain dirt, and pathogens, which may not be easily washed off and which may likely carry some of these contaminated parasites [33].

There was no parasitic contamination found in cucumbers in this study, this also agrees with the works of Akoma et al., [26]; Agbalaka et al., [17]; Akoma et al., [26] in Jos and Lokoja respectively. This can be attributed to the smooth surface that makes it difficult for parasitic attachment [24].

In this study seven parasites were identified. These include *Entamoeba coli*; *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Ascaris lumbricodes*, *Ancyclostoma* spp, and *Fasciola* spp. These parasites are similar to those identified in the work carried out by

Omowaye and Audu, [11]; Malanna and Tim, 2016; Auta et al., [9]; and Bishop and Yohanna, [15]. Compared to their reports of the higher prevalence of *Ascaris lumbricoides,* our studies revealed the presence of highest percentage of *Entamoeba coli*. Mbata et al., [21] reports from Port Harcourt markets had only four parasites were identified *Ascaris lumbricodes, Strongyloides stercoralis,* Hookworm, *Entamoeba histolytica*, *Giardia lamblia,* while Tchounga et al*.,* [14] identified ten parasites namely *Ascaris lumbricodes, Entamoeba coli*; *Entamoeba histolytica*, *Cryptosporidium parvum*, *Ancyclostoma duodenale, Giardia lamblia*, *Taenia* spp, *Toxoplasma gondii*, *Fasciola* spp; and *Strongyloides stercoralis.*

Present study revealed the presence of *Entamoeba coli* in all specimens except in scent leaf and cucumbers. This is similar to Auta et al., [9], where *Entamoeba coli* was found to contaminate all specimens. Their predominance could be attributed to faecal contamination of human and animal origin, during the addition of manures [9,17]. *Entamoeba coli* is one of the non-pathogenic protozoa found in humans, transmitted through faecal contact. Matured *Entamoeba coli* cysts can be found in contaminated water [34].

This study recorded a higher prevalence rate among the fruits and vegetables from Oil mill market with 15.74% and 31.48% compared to Creek Road market with 12.04% and 21.29% contaminated fruits and vegetables respectively. This was significance with ($p = 0.0032$). The variation in the two markets did not show any significant difference in parasite genus detections ($p>0.05$). This also agrees with the work of Mbata et al., [21] carried out in Port Harcourt with no significant difference in parasite detection among the various markets they studied. The parasitic contamination of fresh produce sold in various markets is a serious public health problem globally [16]. Reports from Mexico [35], South India Ajitha et al.,[29], Poland Robertson et al., [36] and Malaysia [37] also revealed similar concerns. Rinsing the produce in salt solution before bringing to market can reduce the parasitic contamination. All the customers should make it a habit to wash the fruits and vegetables before eating or cooking, or even saving in the kitchen.

5. CONCLUSION

In conclusion, this study has shown that fruits and vegetables are still potential sources for the transmission of parasitic contamination in Port Harcourt. Produce contaminated with intestinal parasites pose a serious health challenge to the consumers, if they are not properly washed before consumption. A need for adequate sanitization, improvement of health education and personal hygiene for the farmers and vendors, as well as the general public is made relevant in the present report. Government, Non-governmental Organisations and health authorities play a major role in public health. Government should take necessary actions to provide major amenities needed in the society such as good sources of drinking water, housing, and clean water system. Authorities also should provide adequate resources for building sanitation systems in the community to reduce open defaecation. This will directly and indirectly reduce food-borne contamination through faecal matter.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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