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Human Enteric Parasitic Pathogens in Fresh Fruits and Vegetables Consumed in Ile-Ife, Osun State

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Abstract
The degree of parasitic contamination of fruits and vegetables consumed in Ile-Ife was assessed and poly parasitic contamination of fruits and vegetables was identified. This was with the view to providing information on contamination of fruits and vegetables consumed in the study area. A total of 350 samples of fruits and vegetables were bought from five markets in Ile-Ife between November 2015 and January 2016. The samples were analyzed using sedimentation and floatation technique for detection of parasites. A total of 244 (69.7%) of the fruits and vegetables sampled were positive for eggs, cysts or oocysts of parasites. Among the fruits, tomatoes had the highest number 18 (50%) of parasites while the lowest 8 (25%) was on apples. Four of the vegetables (water leaf, spinach, white jute and African spinach) showed equal degree of parasitic contamination which was 100% while cabbage had the lowest 22 (64.7%). The overall prevalence of isolated parasites from 350 samples of fruits and vegetables investigated in this study was: ova of *E. vermicularis* 64 (18.29%); ova of *A. lumbricoides* 31 (8.86%); cysts of *E. histolytica* 18 (5.14%); cysts of *G. lambila* 19 (5.42%); ova of hookworms 8 (2.29%); cysts of *E. coli* 27 (7.71%); oocysts of *C. cayetenensis* 17 (4.86%); cysts of *B. coli* 29 (8.29%); ova of *Teaniaspp* 15 (4.29%). All fruits and vegetables sampled from various markets showed the presence of more than one parasite on them. The leafy vegetables showed higher poly-parasitic contamination than the fruits. Majority of vendors (92%) displayed produce on the table uncovered. There was a positive correlation between the number of fruits and vegetables sampled and the number of parasites retrieved ($r = 0.358$, $P = 0.016$). This study concluded that fruits and vegetables sold in the study area were contaminated with parasites that can cause food borne illnesses in consumers, which is of public health importance.
Keywords: Fruits and Vegetables, Parasitic contamination, Sedimentation technique, Floatation technique, Poly parasitic, Public health

1. Introduction

Fruits and vegetables are vital to human diet providing minerals, vitamins, fibre proteins, essential micronutrients and other bio-functional components to man (Abe *et al*., 2016). High intake of fruits and vegetables prevents chronic diseases and help in safe child birth (Loy *et al*., 2011). Fruits have the highest antioxidant value which neutralizes free harmful radicals in human body (Halvorsen *et al.*, 2002). However, fruits and vegetables have been recognized as significant reservoirs of food-borne pathogens (Berger *et al.*, 2010). Report of World Health Organization (WHO, 2011) established that food-borne diseases remain a major source of morbidity and mortality in the general population, mainly in susceptible groups, such as infants, the elderly and the immuno-compromised. In another independent study, WHO (2007) submitted that about 1.5 billion cases of diarrhoea and more than three million deaths that occur in children every year are as a result of food and water contamination. Although these infections have received considerable attention, they are considered emerging because they have recently become common (World Health Organization, 2002).

In the past, the risk of human infection with gastrointestinal parasites was limited to distinct geographic regions because of parasites' adaptations to specific definitive hosts, selective intermediate host and particular environmental conditions (Doaa, 2012). These barriers are slowly being breached, by international travel and rapid refrigerated food transport which became available to an unprecedented level at the end of the 20th century (Orlandi *et al*., 2002). Some of the factors responsible for high prevalence of infections in developing countries are well recognized. They include poor personal hygiene, poor environmental hygiene, poor health system (Tamirat *et al*., 2014), poverty, poor sanitation, ignorance, malnutrition (Adefioye *et al*., 2011), ageing, immune suppression and filth flies' abundance (Ogunniyi *et al*., 2015). The tendency in many countries toward eating raw, or lightly cooked vegetables to preserve taste and not denaturing nutrients may also increase the chances of food-borne parasitic infections (Erdogrul and Şener, 2005). Similarly, Adefioye *et al*., 2011 reiterated that eating habit that involves the consumption of raw vegetables, fish, crustacean and meat can lead to transmission of helminth infections. Additionally, the consumption of raw fruits and vegetables without proper washing is an important route in the transmission of parasitic diseases (Slifko *et al*., 2000). Furthermore, intestinal helminthes and protozoa could infect humans due to consumption of contaminated, uncooked, or improperly washed vegetables and fruits (Maysa, *et al*., 2014).

Previous research had laid adequate foundation for various sources of human enteric parasitic pathogens. For instance, Christine and Christopher (2000) detected eggs of *Ascaris* species on fresh vegetables in

industrialized countries**.** Furness *et al*., (2001) found out that giardiasis infection is frequently associated with ingestion of infective stage of the organism in water, vegetables and uncooked meat. In a study of herbs in Vietnam, Tram *et al*., (2010) observed that 10.4% of basil (n=96) samples were positive for *Cyclospora* spp. oocysts. Food Standards Austrailia New Zealand (FSANZ, 2013) discovered that the outbreak of *C. cayetanensis* have largely been associated with fresh produce and has been identified in international surveys of fresh produce. *E. histolytica* is the second leading parasitic cause of death worldwide and it is its cysts have found fresh fruits and vegetables from agricultural fields (Tamirat *et al*., 2014). Beuchat (2002) reported that contamination of soil with animal wastes and increased application of improperly composted manures to soil in which vegetables are grown also play a role in parasite contamination to green vegetables. Orlandi *et al*., (2002) stated that the risk of spreading these contaminations to other countries cannot be under estimated considering exportation of cultivated fruits and vegetables in the developing countries to developed ones. WHO (2002) asserted that changes in lifestyle, food consumption patterns, such as the increase in the number of people eating meals prepared in restaurants, canteens and fast food outlets as well as from street food vendors who do not always respect food safety increase the risk of exposure to foodborne infections. Mahvi and Kia (2006) reported that in many developing countries, use of insufficiently treated waste water to irrigate vegetable farms is a source of contamination with pathogenic parasites. Therefore, Amoah *et al*. (2009) emphasized that the continued use of untreated waste water and manure as fertilizers to produce fruits and vegetables is a major contributing factor to fresh food contamination. Gupta, *et al.*, (2010) discovered that bad hygienic practice during harvesting, transporting, processing and preparation by handlers including consumers also contribute to parasitic contaminations of fruits and vegetable.

In summary, the existing studies had established the possibility of transmission of foodborne illnesses. However, there is dearth of research on the level of parasitic contamination on fruits and vegetables in the Nigerian context. As such, this current study is necessary because Nigeria is one of the countries accounting for the highest population infected with food borne pathogens (Federal Ministry of Health, 2013). In Nigeria, due to inadequate and non-existing system for routine diagnosis, monitoring and reporting for many of the food-borne pathogens, most outbreaks caused by contaminated fruits and vegetables go undetected and the incidence of their occurrence in food is undermined (Dorny *et al*., 2009). From the foregoing, it is therefore imperative to assess the degree of parasitic contamination on non-seasonal fruits and vegetables and the predisposing factors will help in proper evaluation of the prevalence and control of enteric parasitic load among indigenous population of Ile-Ife, Southwest, Nigeria.

2. Methodology

2.1 Study Area

Ile-Ife is an ancient Yoruba city in south-western Nigeria. It is located in the present Osun State. It is considered a semi-urban town with human population of about 355,281 (National Census, 2006). Ile-Ife is located within the Tropical savanna climate zone of West Africa. It has average rainfall of 1000-1250mm usually from March to October and a mean relative humidity of 75% to 100%. There is Opa river and reservoir that serves as a water treatment facility for Obafemi Awolowo University.

2.2 Sample collection

The fruits and vegetables used in this study were selected from five markets in the study area. They include apple (*Malus domestica*), tomatoes (*Lycopersicon esculentum*), carrot (*Daucus carota*), Cucumber (*Cucumis sativus*) Waterleaf (*Talinum trangulare*), Spinach (*Amaranthus spinosus*), White jute (*Corchorus olitorius*), African spinanch (*Solanecio biafrae*), Fluted pumpkin (*Telfairia occidentalis*), Cabbage (*Brassica oleracea*). The markets sampled were fresh food markets which were chosen at random. They include: New market, Central market of Obafemi Awolowo University, *Ilode* market, Ife market and Better life market.

2.3 Questionnaire survey

Purposive sampling methods were adopted to select vendors of fresh fruits and vegetables from five markets. Structured questionnaire, written in English and translated into indigenous *Yoruba* language, was employed for the survey. The participants were interviewed on the participants' demographics (i.e. age, gender, education attainment, family size) socio-economic background (i.e. occupation, household income), behavioural risks (personal hygiene practices such as, hand washing, defecation habits and garbage disposal), and environmental sanitation conditions (i.e. latrine system, source of water supply).

2.4 Laboratory analysis

Fruits and vegetables bought were put in a sterile bowl, covered, labelled and transported to the Department of Medical Microbiology and Parasitology laboratory, Obafemi Awolowo University, Nigeria for laboratory analysis.

Sedimentation and floatation methods were adopted for microscopic examinations. 200-250 g of each fruit and vegetable sample was lapped in distilled water for 10-20 minutes. The resulting wash formed was strained through a sterile sieve (pore size) to remove undesirable materials. The filtrate was centrifuged at 3000 rpm for 15 minutes, and decanted. The supernatant was discarded into the disinfectant jar. The sediment was placed on a clean grease-free slide on which a clean cover slip was gently placed to avoid air bubbles and over flooding. The preparation was examined under light microscope using X10 objective lens. Consequently, the remnant of the sediment was re-suspended in zinc sulphate fluid filled up to the brim with a superimposed cover slip. The cover slip was picked from the edges by sterile forceps re-examined under light microscope to investigate presence of parasites. Developmental stages of parasites were identified following the descriptions of Cheesbrough (2009).

2.5 Statistical Analysis

Data analyses were performed using Statistical Package for Social Science version 20. Pearson's chi-square test was used to investigate the association between number of parasites recovered as the dependent variable, and personal hygiene practices as the independent variables. Descriptive analysis was used for the demographic characteristics of the population. Prevalence of enteric human parasites and other parameters were expressed in percentages.

3. Results

A total of 350 batches of fruits and vegetables were examined for parasitic contamination. A total of 244 (69.7%) of the fruits and vegetables sampled were positive for eggs, cysts or oocysts of parasites. Table 1 shows the level of parasitic contamination among the fruits and vegetables examined; tomatoes had the highest number 18 (50%) of parasites while the lowest 8 (25%) was on Apples. Four of the vegetables (Water leaf, Spinach, White jute and African spinach) showed equal degree of parasitic contamination which is 100% while Cabbage had the lowest number 22 (64.7%) of parasites retrieved from it.

Table 1. Degree of Parasitic contamination of fruits and vegetables from various markets

Fruits and Vegetables	New Market		Ilode Market		Ife Market		Better life Market		O. A. U. Central market		Total number positive (%)
	Number examined	Number (%) +ve	Number examined	Number (%) +ve	Number examined	Number (%) +ve	Number examined	Number (%) +ve	Number examined	Number (%) +ve	
Apples	4	2 (50)	6	4 (66.6)	6	2 (33.3)	8	0 (0)	8	0 (0)	8 (25)
Tomatoes	8	2 (25)	6	0 (0)	6	2 (33.3)	8	6 (75)	8	8 (100)	18 (50)
Carrots	8	2 (25)	6	2 (33.3)	6	2 (33.3)	8	4 (50)	8	0 (0)	10 (27.7)
Cucumbers	8	0 (0)	6	4 (66.6)	6	2 (33.3)	8	2 (25)	8	4 (50)	12 (33.3)
Waterleaf	8	8 (100)	4	4 (100)	6	6 (100)	8	8 (100)	8	8 (100)	34 (100)
Spinach	8	8 (100)	6	6 (100)	6	6 (100)	8	8 (100)	8	8 (100)	36 (100)
White jute	8	8(100)	6	6 (100)	6	6 (100)	8	8 (100)	8	8 (100)	36 (100)
African spinach	6	6 (100)	6	6 (100)	6	6 (100)	8	8 (100)	8	8 (100)	34 (100)
Fluted pumpkin	8	8 (100)	6	6 (100)	6	6 (100)	8	6 (75)	8	8 (100)	34 (94.4)
Cabbage	8	6 (75)	6	4 (66.6)	6	4 (66.6)	8	2 (25)	6	6 (100)	22 (64.7)
Total	74	50(67.5)	58	42(72.4)	60	42 (70)	80	52 (65)	78	58 (74)	244 (69.7)

Nine genera of parasites were observed from fruits and vegetables examined in the study. Parasites identified include helminths; *Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia spp* and hookworm. While the protozoan parasites identified include *Entamoeba histolytica*, *Entamoeba coli*, *Balantidium coli*, *Giardia lamblia* and *Cyclospora cayetenensis*. Overall, Table 2 shows the prevalence of isolated parasites from fruits and vegetables investigated in this study; ova of *E. vermicularis* (18.29%), *A. lumbricoides* (8.86%), cysts of *E. histolytica* (5.14%), cysts of *G. lambila* (5.43%), ova of hookworms (2.29%), cysts of *E. coli* (7.71%). Oocysts of *C. cayetenensis were* 4.86%, cysts of *B. coli* (8.29%), ova of *Teania spp* (4.29%). The overall prevalence of parasites was 65.16%.

There was a positive correlation between the number of fruits and vegetables sampled and the number of parasites retrieved from them. ($r = 0.358$, $P = 0.016$). In Table 3, it was observed that all fruits and vegetables sampled from various markets had more than one parasite on them. The leafy vegetables showed higher poly parasitic contamination than the fruits. White jute and water leaf had the highest poly parasitic contamination, followed by spinach, African spinach and fluted pumpkin while the lowest poly parasitic contamination was found on carrot with the presence of two parasites.

Table 2. Prevalence of isolated parasites from fruits and vegetables in various markets

Isolated Parasites	New market Nos (%)	Ilode market Nos (%)	Ife market Nos (%)	Better life market Nos (%)	O.A.U Central market Nos (%)	Total Number (%)
E. vermicularis	21 (6)	7 (2)	12 (3.42)	13 (3.71)	11 (3.14)	64 (18.29)
A. lumbricoides	14 (4)	7 (2)	3 (0.85)	6 (1.7)	1 (0.3)	31 (8.86)
E. histolytica	7 (2)	1 (0.3)	1 (0.3)	5 (1.42)	4 (1.14)	18 (5.14)
G. lambila	4 (1.14)	1 (0.3)	2 (0.57)	3 (0.85)	9 (2.57)	19 (5.43)
Hookworm	2 (0.57)	1 (0.3)	1 (0.3)	2 (0.57)	2 (0.57)	08 (2.29)
E. coli	7 (2)	0 (0)	6(1.7)	6 (1.7)	8 (2.28)	27 (7.71)
C. cayetenensis	1 (0.3)	1 (0.3)	7 (2)	5 (1.42)	3 (0.85)	17 (4.86)
B. coli	1(0.28)	6 (1.7)	6 (1.7)	8 (2.28)	8 (2.28)	29 (8.29)
Teania spp	2 (0.57)	3 (0.85)	3 (0.85)	4 (1.14)	3 (0.85)	15 (4.29)
Total	59 (16.85)	27 (7.71)	41 (11.71)	52 (14.86)	49 (14)	228 (65.16)

Table 3. Poly parasitic infection of fruits and vegetables

Parasites	Apple	Tomatoes	Carrot	Cucumber	Waterleaf	Spinach	White jute	African spinach	Fluted pumpkin	Cabbage
E. vermicularis	+	-	+	-	+	+	+	+	+	-
A. lumbricoides	-	-	-	+	+	+	+	+	+	-
E. histolytica	-	+	-	+	+	+	+	+	-	+
G. lambila	+	-	-	+	+	+	+	+	+	-
Hookworm	-	-	-	-	+	+	+	-	+	-
E. coli	-	+	-	+	+	+	+	+	+	-
C. cayetenensis	-	-	-	-	+	+	+	+	+	-
B. coli	+	+	+	+	+	-	+	+	-	+
Teania spp	-	-	-	-	+	+	+	+	+	-

In Table 4, it was discovered that food vendors played important roles in the transmission of intestinal parasites. Examining factors associated with parasitic contamination of fruits and vegetable, two hundred vendors comprising 172 female and 28 male respondents were interviewed. 52% rinsed hands with only water after using the toilet, 45% washed with soap and 3% did not wash hands at all. 47% of respondents seldom washed hands before handling produce while 12% always washed hands. Well water (55%) was mostly used to wash produce by vendors followed by water from stream (24%). Majority of vendors (92%) displayed produce on the table uncovered where they were exposed to dusts and flies. Most of the vendors (86%) asserted that produce was gotten from middle men as 14% had their produce supplied by farmers directly. 53% used the store room, 43% of the vendors stored in open air baskets while 4% used the floor.

Table 4. Factors associated with human enteric parasites transmission among vendors

Risk Factors		Frequency	Percentage
Age (years)	≤ 30	52	26
	31-40	94	47
	41-50	38	19
	>50	16	8
Gender	Male	172	86
	Female	28	14
Educational level of vendor	No education	26	13
	Primary	54	27
	Secondary	114	57
	Tertiary	6	3
Sewage Disposal System	Water closet	8	40
	Latrine	120	60
	Bush	-	-
Hand washing after toilet	Rinse only with water	104	52
	Wash with soap	90	45
	Do not wash	6	3
Hand washing before handling produce	Seldom	94	47
	Frequently	94	47
	Always	12	6
Cleaning platform before display	Seldom	44	22
	Frequently	136	68
	Always	20	10
Platform for display	Floor	4	2
	Shelf in shop	4	2
	Tables	184	92
	Wheel barrow	8	4
Source of Produce	Farmers	28	14
	Middlemen	172	86
	Private Garden	-	-
Produce Storage	Storeroom	106	53
	Basket	86	43
	Floor	8	4
Source of water for washing	Tap	42	21
	Well	110	55
	Stream	48	24
Covering after display	Yes	02	1
	No	198	99
Having pet(s)	Yes	74	37
	No	126	63

4. Discussions

The presence of intestinal parasites on fruits and vegetables sampled in this study is suggestive of faecal contamination either directly or indirectly. The consumption of raw or undercooked vegetables is a means by which the transmission of these parasites is being effected. Study by Shahonazi and Jafari-Sabet (2010) had pointed out that the poorly washed vegetables are a major route for transmitting intestinal parasitic infections. Faecal contamination of water sources used in irrigation could also be an important source of human infection, therefore, contamination of fresh fruits and vegetables are of greatest concern (Orlandi *et al.*, 2002). Isolation of more than one parasite per fruit and vegetable samples in this work reflects the possibility of faecal contamination of vegetables and fruits which most probably results into poly parasitic infection in man. The high occurrence of these parasites reflects a high level of contamination and persistence of human infection. This agrees with the report of Gibson (1994), that the prevalence of intestinal parasites among a particular people is an attribute of environmental pollution by human faeces.

The high level of contamination of the leafy vegetables might be due to their uneven surfaces that made

parasitic eggs, cyst and larvae attach to their surfaces easily, when washed either in the farm or in the market (Amoah *et al*., 2006). Waterleaf was among the leafy vegetables that were most contaminated with parasites as reported by Ojemudia (2011) who reported more than 90 per cent contamination of waterleaf. The least level (25%) of parasites found on apples and cucumbers (33.3%) in this study was due to the fact that they have smooth skin which enhances the removal of parasite eggs when washed.

Among the fruit varieties, tomato was the most contaminated with a rate of 50%, which could be as a result of its low growth height above the soil level that predisposed it to contamination with parasites during flooding as well as heavy rain splashes. More so, tomato grows in uncultivated soil frequently in poor environment where sewage and other human effluents are deposited. However, this result is in accordance with the findings of Simeon-Oke (2014) in which Tomato had 37.5% contamination rates but disagreed with the work of Dauda *et al.* (2011) in which Tomato and Carrot had 10% and 6% contamination rates, respectively. The high prevalence of parasitic contaminations of fruits and vegetables in these open markets are characterized by the presence of poor drainage, lack of modern toilet facilities, improper disposal of faeces from children of traders, refuse dumping sites nearby and poor hygienic practice. The parasites recovered from the samples include, ova of *E. vermicularis* 64 (18.29%), *A. lumbricoides* 31 (8.86%), cyst of *E. histolytica* 18 (5.14%), *G. lamblia* 19 (5.43%), ova of Hookworm 8 (2.29%), cyst of *E. coli* 27 (7.71%), oocyst of *C. cayetenensis* 17 (4.86%), cysts of *B. coli* 29 (8.29%) and ova of *Teania spp* 15 (4.29%). The most frequent parasites recovered were *E. vermicularis* 64 (18.29%), *A. lumbricoides* 31 (8.86%) and cysts of *B. coli* 29 (8.29%). This result contrasts with the findings of Simeon-Oke (2014) who reported a contamination rate of 25.7% in *A. lumbricoides*, 22.8% in *B. coli*, 20.0% in *E. histolytica*, 11.4% in *G. lamblia* and *H. nana*, respectively and 8.5% in *A. duodenale.*

Variations in the parasites recorded in this study may be due to difference in the geographical locations of the study when compared with the reports of earlier investigators. Ova of *A. lumbricoides*, and hookworm, cysts of *E. histolytica* and *E. coli* were common in these studies. In all these studies, ova of *A. lumbricoides* was commonly isolated from specimens in all the markets. This could be due to the ability of the eggs of the parasites, which has a thick shell that is highly resistant to adverse environmental conditions, which could serve as an indication to water pollution as a result of indiscriminate defecation resulting in pollution of water and farmland as observed by Damen *et al*. (2007).

Unhygienic behaviours discovered in the vendors interviewed in this study played important roles in the transmission of intestinal parasites. 52% rinsed hands with only water after using the toilet, 45% washed with soap and 3% did not wash hands at all. 47% of respondents seldom washed hands before handling produce while 12% always washed hands. The rinsing of the hand with water only may pose greater exposure to infection as the source of the water is a determining factor (Sheshu and Amiana, 2014). Majority of vendors (92%) displayed produce on the table uncovered where they were exposed to dusts and flies. It had been well established that flies can act as vectors for a number of pathogenic microorganisms including human intestinal parasites (Ogunniyi *et al*., 2015). Respondents who displayed their produce on the table were 92% as 4%, 2%, 2% displayed on wheel barrows, bare floor and shop shelves, respectively. This is at variance from other African markets where 67.5% of vendors displayed their vegetables on the floor by the road side (Tamirat, 2014).

In this study, there is positive correlation between the number of fruits and vegetables sampled and the number of parasites recovered from them ($r = 0.358$, $P = 0.016$). Hand washing had no significant influence on the number of enteric parasites retrieved. There was no significant difference in the samples washed with different water sources. Also, water source has no significant relationship on the number of parasites harboured by the fruits and vegetables. Therefore, the more the improperly cooked/washed fruits and vegetables are consumed, the more the likelihood of parasites ingested. Thus, preference for eating raw or slightly cooked vegetables to protect heat labile nutrients may increase the risk of foodborne infections (Fallah *et al*., 2012).

All isolated fresh fruit and vegetable samples had more than one species of parasites per sample. This indicates the magnitude of a single faecal contamination of vegetables, which may result in poly parasitic contamination; this is in accordance with the view of Mohamed *et al*. (2016).

5. Conclusion

This paper has investigated human enteric parasitic pathogens in fresh fruits and vegetables consumed in Ile-Ife, Osun State. The findings raised concern of public health being at high risk of infection with *Ascariasis, Amoebiasis, Enterobiasis* and others. This is suggestive that humans are at high risk of intestinal parasitic infections from contaminated fresh fruits and vegetables in the study area. As it is, the combat against diseases transmissible by fresh fruits and vegetables requires familiarity with the ecology of microorganisms and public awareness on the transmission of intestinal parasites. There is need for public enlightenment campaign on the danger of consuming inadequately washed fruits and vegetables. Obviously, another point worthy of buttress is the frank fight by public health workers against unhygienic handling of produce in public place like markets. Consumers should cultivate the habit of thorough washing of fruits and vegetables with clean water before consumption. Such will reduce or completely eliminate the level of parasitic contamination on the produce.

Farmers should be educated on the adverse effects of using untreated or polluted water for irrigation as this can serve as source of contamination. Enlightenment programs for the public on necessity of food sanitation and personal hygiene should be intensified. Other potential routes of transmission, such as post-harvesting procedures, contamination from environment during handling, transport and storage, or direct contamination from individuals involved in the production and processing of products should be investigated as well as treatment of infected individuals should be considered.

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