



PARASITIC AND MICROBIAL INFESTATIONS OF FRUITS SOLD AT OTUOKE, OGBIA LOCAL GOVERNMENT AREA, BAYELSA STATE, NIGERIA

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ABSTRACT: *Fruits and vegetables have numerous health importance but can act as vehicles in the transmission of foodborne diseases of public health importance. This research examined the presence of parasites and microbial organisms on fruits sold at Otuoke community, Ogbia Local Government Area, Bayelsa State, Nigeria using six fruits types, including pineapple (*Ananas comosus*), cucumber (*Cucumis sativus*), lime (*Citrus aurantiifolia*), garden egg (*Solanum aethiopicum*), guava (*Psidium guajava*) and orange (*Citrus sinensis*). The parasites were concentrated by sedimentation and were examined using a light microscope. The result of the study showed the presence of cysts of *Cryptosporidium parvum*, *Entamoeba histolytica*, eggs of *Fasciola hepatica*, *Ascaris lumbricoides* and larva of *Strongyloides stercoralis*. Five out of the 6 fruits types examined were infested with at least one type of parasites. *Ascaris lumbricoides* (33.33%) was the most frequently detected parasite and was found on the pineapple, guava and oranges fruits. Fruit types were not significantly associated with parasitic contamination ($p > 0.005$). Bacteria isolated from the fruits included *Lactobacillus* sp., *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas* sp., *Bacillus cereus*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Staphylococcus aureus*. *E. coli* was isolated in all the sampled fruits types. The total count was determined by pour plate method using MacConkey agar. Total viable bacteria count (TBC) ranged from 21.9×10^5 cfu/ml to 7.27×10^5 cfu/ml. *Aspergillus niger*, *A. flavus*, *Mucor* spp., and *Fusarium* spp were the isolated fungi species. A high number of these microorganisms in fruits and vegetables can lead to public health emergencies. Risk reduction can be achieved through personal and food hygiene by the fruit sellers and consumers. Further studies should be conducted to address the effect of seasonal variation on the infestation of the fruits sold in this area.*

KEYWORDS: Parasites, Bacteria, Fruits, Fungi, Microbial, Public Health, Bayelsa.



INTRODUCTION

The importance of fruits and vegetables in nutrition and health is well known. In recent years many countries have undertaken numerous approaches to encourage people to increase their quantity of fruit intake (Tetens and Alini, 2009). Fruits are very important sources of essential vitamins and are vital for the well-being of humans. They boost host immunity and also contain antioxidants and fibre (Eni *et al.*, 2010; Whitney-Chanex, 2011). A balanced diet rich in fruits has been used to reduce the risk of several diseases (Kalia and Gupta, 2006) such as cardiovascular diseases, cancers, strokes as well as functional retardation associated with ageing (Rui, 2003).

Despite the numerous health benefits derived from fruits and vegetables, they sometimes act as a vehicle in the transmission of infectious diseases, causing foodborne diseases of public health importance (Hannan *et al.*, 2014), resulting in morbidity and mortality (Hanson *et al.*, 2012). Studies (Wedajo and Kadire, 2019; Adamu *et al.*, 2012) have shown that the fact that fruits and vegetables can transmit infectious organisms and pose a great health threat. Pathogenic organisms can enter fruits through damaged surfaces such as punctures, wounds, cuts and splits that occur during growth, harvesting or handling (Durgesh *et al.*, 2008). Infective stages of helminth parasites most commonly contaminate fruits and vegetables, as they are abundant in the environment (Luka *et al.*, 2016). Conditions that predispose humans to contaminated fruits and vegetables are predominantly found in Africa where there is generally poor hygiene and sanitation. Pieces of evidence abound of the increased risk of infection with parasitic organisms in areas, including Nigeria, where untreated wastewater is being used for the irrigation of fruits and vegetable (Ekwunife and Akolisa, 2009, Adamu *et al.*, 2012, Amawulu *et al.*, 2019). Animal dung is used as manure and the poor sanitary habit of eating fruits without washing contribute to the spread of these diseases. Most fruit sellers use poor quality water to wash the fruits before sales to the consumers, thereby exposing them to the risk of both microbial and parasitic infections. The prevalence and magnitude of parasitic and microbial contamination of food products vary from one area to another (Dawet *et al.*, 2019).

Presently, there is no published information about the level of parasitic and microbial infestation of fruits sold in Otuoke Community in Ogbia Local Government Area of Bayelsa State. Therefore, this study was undertaken to determine the level of parasitic and microbial infestations of selected fruits in this area and make available baseline data from this study in the area to create awareness to the public and for the appropriate agencies to work with.

MATERIALS AND METHODS

Study Area

Otuoke town in Ogbia Local Government Area, Bayelsa State, Nigeria, is located approximately at Latitude 4°49' North and Longitude 6°20' East. It is bounded to the east by Emeyal 1 and Kolo; to the west by Onuebum and Otuogori and to the south by Otuaba and Ewoi communities, all in Ogbia Local Government Area of Bayelsa State, Nigeria. It has a wet terrain with a fairly good road network. The prevalent climatic condition in the area is marked by rainy and dry seasons. The rainy (wet) season is from April to October while the dry season starts in November to March. The average monthly temperatures are high throughout the year. Farming and fishing are the main occupations of the indigenes while a majority of the non-



indigenes are traders. Both indigenous and non-indigenous residents in Otuoke eat fresh fruits. Fruit sellers in Otuoke mostly get their fruits supplies directly from the local farmers while some sellers often go to some other markets to purchase, ensuring all-round supply. People from other nearby communities buy fruits from these sellers when on a visit to family and friends.

Sample Collection

Six different types of fruits were examined for infestation with parasites and pathogenic microorganisms. The fruit sampled included garden egg (*Solanum aethiopicum*), orange (*Citrus sinensis*), cucumbers (*Cucumis sativus* L.), lime (*Citrus aurantiifolia*), guava (*Psidium guajava* L.) and pineapple (*Ananas comosus*) which were randomly bought from the sellers in the study area. All the samples were collected in sterile universal plastic bags and transported to the Laboratory in the Department of Microbiology, Federal University, Otuoke, Bayelsa State for analysis.

Parasitological Examination

As soon as the samples were brought into the laboratory, 10g of each fruit was washed in a sterile beaker containing 250ml of sterile distilled water and saline solution for the removal of parasitic ova, cysts and larva as described by Awe and Madueke (2015). The water use in washing each of the fruits was allowed to stand for 20 minutes. The suspension was sieved using a sterile sieve to remove any unwanted materials. The filtrate was centrifuged at 3000rpm for five minutes in plastic test tubes and the supernatant, discarded. The supernatant was decanted, leaving the sediment which was mixed and examined under a light microscope using $\times 10$ and $\times 40$ objectives. The parasites detected were identified using an identification key (Cheesbrough, 2006),

Bacterial and Fungal Examination

The samples were aseptically chopped into smaller pieces using a sterile stainless steel knife prior to weighing. A 10g of a subsample of each vegetable was aseptically weighed and vigorously shaken in 90ml of sterile 0.1 % (w/v) buffered peptone water for 3min separately to homogenize the samples (Shalini, 2010).

For enumeration of microorganisms present in each sample, 10-fold serial dilutions of the resultant homogenates were made. Sterile test tubes were labelled as (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5}) for each sample. One milliliter (1ml) of the sample was added into 9ml of sterile normal saline (10^1) and mixed properly in order to get equal distribution of microorganisms. The procedure was repeated to complete the serial dilution up to 10^{-5} . Nutrient agar, MacConkey agar, Salmonella-Shigella agar and Potato Dextrose agar were prepared according to manufacturers' instruction. The freshly prepared and cooled media were poured into sterile flat-bottomed Petri dishes on a levelled, horizontal surface to give a uniform depth of approximately 4 mm. This was achieved by pouring 20 ml of the medium for plates with diameters of 100 mm.

Total Coliform Count

The total count was determined by pour plate method using MacConkey agar as the primary choice of medium. 1ml of 10^{-4} dilution was pipetted into the centre of the petri-dish, using a



fresh pipette for each dilution. The agar was poured into the plates aseptically, enough to cover the 1ml solution in them. The plate was rocked and allowed to solidify. It was inverted and incubated at 37°C for 24 hours. The colonies on the plates were then counted with a colony counter.

Biochemical tests such as Catalase test, Citrate utilization test, Indole test, Coagulase test, Oxidase test and Motility test were performed for the identification of the bacteria isolated from the fruits (Cheesebrough, 2006).

Identification of Fungi Isolate

After incubation, different types of fungi species and mucor growths were formed on the surface of the potato dextrose agar. The fungal isolates were identified by direct observation with unaided eyes and identification of microscopic features with the microscope.

Statistical Analysis

Results were presented as means \pm standard error (SE). The percentage frequency was also determined. Data differences were determined using one-way ANOVA. The minimum significance level was $p < 0.05$. All analysis was carried out using SPSS version 20.

RESULT

Six different types of fruits including pineapple (*Ananas comosus*), cucumber (*Cucumis sativus*), lime (*Citrus aurantiifolia*), garden egg (*Solanum aethiopicum*), guava (*Psidium guajava*) and orange (*Citrus sinensis*) were examined for parasitic and microbial organisms. The result of the parasitological examination showed that cysts, eggs and larva of parasites were recorded. Five out of the 6 fruits types examined were contaminated with at least one type of parasites. Helminths and protozoan parasites were encountered (*Strongyloides stercoralis*, *Cryptosporidium parvum*, *Fasciola hepatica*, *Ascaris lumbricoides*, *Entamoeba histolytica*). *Ascaris lumbricoides* (33.33%) was the most frequently detected parasite and was found on the pineapple, guava and oranges fruits. *Cryptosporidium parvum* (20%) was found only on cucumbers. Garden egg was found to be infested with *Strongyloides stercoralis* (66.67) and *Fasciola hepatica* (33.33%). There was no parasite found on the lime. Fruit types were not significantly associated with parasitic contamination ($p > 0.005$) (Table 1).

Table 1: Frequency of distribution of parasite species on fruits sampled at Otuoke, Bayelsa State, Nigeria.

Parasites	Fruit Types						
	Pineapple (%)	Cucumber (%)	Lime (%)	Garden egg (%)	Guava (%)	Orange (%)	Total (%)
<i>Strongyloides stercoralis</i>	0(0)	0(0)	0(0)	2(66.67)	0(0)	1(50)	3(20)
<i>Cryptosporidium parvum</i>	0(0)	3(75)	0(0)	0(0)	0(0)	0(0)	3(20)
<i>Fasciola hepatica</i>	1(33.33)	0(0)	0(0)	1(33.33)	0(0)	0(0)	2(13.33)



<i>Ascaris lumbricoides</i>	2(66.67)	0(0)	0(0)	0(0)	2(66.67)	1(50)	5(33.33)
<i>Entamoeba histolytica</i>	0(0)	1(25)	0(0)	0(0)	1(33.33)	0(0)	2(13.33)
Total	3(100)	4(100)	0(0)	3(100)	3(100)	2(100)	15 (100)

Nine species of bacteria isolated and identified from the fruits included *Lactobacillus* sp., *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas* sp., *Bacillus cereus*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Staphylococcus aureus*. *E. coli* was seen in all the sampled fruits types while *Lactobacillus* spp was isolated only from guava. The results indicated that *Proteus mirabilis*, *Shigella* spp. and *E. coli* were obtained from the pineapples; *B. subtilis*, *S. typhii*, *Shigella* spp., and *E. coli* were recovered from cucumber; *P. mirabilis*, *Pseudomonas* spp., *S. typhii*, *E. coli* and *S. aureus* were found on the limes. *Bacillus cereus*, *S. typhii*, *Shigella* spp. and *E. coli* were recovered from the garden eggs, while *Pseudomonas* spp., *S. typhi*, *Shigella* spp and *E. coli* were found on the oranges (Table 2).

Table 2: Micro-organisms isolated from fruit types sold at Otuoke, Bayelsa State, Nigeria

Bacteria spp.	Fruit Types.					
	Pineapple	Cucumber	Lime	Garden egg	Guava	Orange
<i>Lactobacillus</i> spp.	-	-	-	-	+	-
<i>Proteus mirabilis</i>	+	-	+	-	-	-
<i>Bacillus subtilis</i>	-	+	-	-	-	-
<i>Pseudomonas</i> spp.	-	-	+	-	-	+
<i>Bacillus cereus</i>	-	-	-	+	-	-
<i>Salmonella typhi</i>	-	+	+	+	+	+
<i>Shigella</i> spp.	+	+	-	+	+	+
<i>Escherichia coli</i>	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	+	-	+	-

Key: (+) = present; (-) = absent

The mean total viable bacteria count (cfu/ml) showed that pineapple ($21.9 \pm 4.9 \times 10^5$ cfu/ml) had the highest mean microbial load followed by guava ($11.5 \pm 4.4 \times 10^5$) and cucumber ($10.9 \pm 4.9 \times 10^5$ cfu/ml) while lime ($7.27 \pm 6.1 \times 10^5$ cfu/ml) had the least mean. The total coliform count (TCC) (cfu/ml) revealed that pineapple ($7.7 \pm 4.0 \times 10^5$ cfu/ml) had the highest mean value followed by garden eggs ($7.37 \pm 3.5 \times 10^5$ cfu/ml) and guava ($7.0 \pm 3.5 \times 10^5$ cfu/ml). Pineapple ($9.0 \pm 3.2 \times 10^5$ cfu/ml) again had the highest mean total *Salmonella-Shigella* count (TSSC) followed by cucumber ($7.16 \pm 4.9 \times 10^5$ cfu/ml) while garden egg ($4.57 \pm 6.6 \times 10^5$ cfu/ml) had the least. Total fungal count (TFC) revealed guava ($5.2 \pm 4.6 \times 10^5$ cfu/ml) had the highest mean TFC followed by lime ($3.36 \pm 6.9 \times 10^5$ cfu/ml) while orange had the least ($1.1 \pm 1.7 \times 10^5$ cfu/ml) (Table 3).

**Table 3: Total Microbial Load Count from Fruit sold at Otuoke, Bayelsa State, Nigeria**

Fruit types	TBC(cfu/ml)	TCC(cfu/ml)	TSSC(cfu/ml)	TFC(cfu/ml)
Pineapple	21.9±4.9 × 10 ⁵	7.7±4.0 × 10 ⁵	9.0±3.2 × 10 ⁵	2.3±3.2 × 10 ⁵
Cucumber	10.9±4.9 × 10 ⁵	6.47±4.7 × 10 ⁵	7.16±4.9 × 10 ⁵	1.16±3.2 × 10 ⁵
Lime	7.27±6.1 × 10 ⁵	4.5±4.9 × 10 ⁵	5.4±4.7 × 10 ⁵	3.36±6.9 × 10 ⁵
Garden egg	8.9±6.1 × 10 ⁵	7.37±3.5 × 10 ⁵	4.57±6.6 × 10 ⁵	1.5±3.2 × 10 ⁵
Guava	11.5±4.4 × 10 ⁵	7.0±3.5 × 10 ⁵	6.07±5.5 × 10 ⁵	5.2±4.6 × 10 ⁵
Orange	10.3±4.9 × 10 ⁵	5.77±5.2 × 10 ⁵	5.7±3.2 × 10 ⁵	1.1±1.7 × 10 ⁵

TBC = Total viable bacterial count, TCC = Total coliform count, TSSC = Total *Salmonella shigella* count, TFC = Total fungal count

Four fungal species were found on the fruits and vegetables, namely, *Aspergillus niger*, *A. flavus*, *Mucor* spp. and *Fusarium* spp. The presence of *A. flavus* and *Fusarium* spp was identified in pineapples; *Fusarium* spp in cucumber; *A. niger* and *Fusarium* spp in lime; *A. niger*, *Mucor*, *Fusarium*; in garden eggs spp. and guava, *Fusarium* spp., while *Fusarium* spp was seen in orange (Table 4).

Table 4: Fungal species isolated from fruits types sampled at Otuoke, Bayelsa State, Nigeria

Fungi	Pineapple	Cucumber	Lime	Garden egg	Guava	Orange
<i>Aspergillus niger</i>	-	-	+	+	-	-
<i>Aspergillus flavus</i>	+	-	-	-	-	-
<i>Mucor</i> spp.	-	-	-	+	-	-
<i>Fusarium</i> spp.	+	+	+	+	+	+

Key: (+) = positive; (-) = negative.

DISCUSSION

Results from this study indicated that the fruits were contaminated by various pathogenic organisms such as parasites (*S. stercoralis*, *C. parvum*, *F. hepatica*, *A. lumbricoides* and *E. histolytica*), bacteria (*Lactobacillus* sp., *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas* sp., *Bacillus cereus*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Staphylococcus aureus* and fungi (*Aspergillus niger*, *A. flavus*, *Mucor* spp. and *Fusarium* spp).

The findings of this study corroborate with those of Asghar *et al.*, (2013), Dada and Olusola-Makinde (2015) and Wedajo and Kadire (2019) who also discovered the presence of both parasites and microbes in fruits and vegetables. Istifanus & Panda (2018) in their study in Bauchi State noted the contamination of vegetables and fresh fruits with various parasitic cysts and ova. Amawulu *et al.*, (2019) confirmed the infestation of fruits (20%) sold in Yenagoa, Bayelsa State Capital, with parasites. Tefera *et al.*, (2014) isolated *Cryptosporidium* species, *Hymenolepis nana*, *Giardia lamblia*, *Ascaris lumbricoides* and *Entamoeba histolytica dispar* from fruits in southwest Ethiopia. *Ascaris lumbricoides* (33.33%) was the most frequently



detected parasite in this study. This is similar to Istifanus & Panda (2018) report of *Ascaris lumbricoides* being the most prevalent parasitic contamination in both vegetables and fruits in his study. Hassan *et al.*, (2013) reported the least infestation in his study for lime fruit. This is not far from no infestation recorded for lime in this study. Hassan *et al.*, (2013) opined that, though lime has a rough surface that may seem to support parasite attachment, other factors apart from skin surface texture might be in play to enhance or inhibit parasite occurrence on fruits and vegetables.

The parasites detected in this study are of very high public health importance. It had been reported that Nigeria has a very high prevalence of gastrointestinal helminths infection, especially in children (Living-Jamala *et al.*, 2018; Lorina, 2013; Ihesiulor *et al.*, 2013), which have detrimental effects on the growth, physical fitness and cognitive performance of the infected children. The eggs, cysts or larvae of the parasites contaminate the soil or fruits and vegetable through open defecation or the use of infected faeces as night soil. The eggs are ingested by humans through the consumption of contaminated food, soil and drinking water (Kayser *et al.*, 2005). The occurrence of *A. lumbricoides* in some stream, river and other sources of water has been reported by Nwele *et al.*, (2013) and Solomon *et al.*, (2013) which is also a predisposing factor to the contamination of fruits when such water is used for irrigation and washing of the fruits. The presence of the eggs of *F. hepatica* could be an indication of the presence of water or soils polluted by cattle dung. The washing of fruits in already contaminated water gives more room for parasite cysts, larvae and ova of parasites to be transmitted to the fruits. The rate of infection with parasites is generally high among people who have poor personal and environmental hygiene (Naish *et al.*, 2004). Proper washing of fruits and vegetables may reduce microbial and parasitic contaminations. Also, filthy environment and refuge heaps constitute means of contamination for fruits even at the point of sale where these edible fruits are displayed. Flies can mechanically transfer parasites cyst and ova from dirt to already displayed fruits.

The relatively high microbial load of microorganisms gotten from fruits and vegetables could perhaps be linked to the unhygienic condition of point of sale or transport systems. Improper handling of fruits by sellers could also be a source of contamination. Most sellers mix contaminated fruits with good ones during storage leading to the spread of bacteria from contaminated fruits to the good ones. Buck *et al.*, (2003) stated that the presence of many pathogens in the soil is from environmental sources such as faeces or untreated sewage, and microorganisms present in the soil or water can be the source of contamination for fruits and vegetables. The slight variation in the microbial load from other sources could be traced to possible pre-washing of fruits by the fruit sellers before display. Duedu *et al.*, (2014) has shown that washing vegetables with just water was not enough to remove any contaminating parasites.

The isolation of bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas* sp., *Salmonella typhi*, *Shigella* spp, *Lactobacillus* spp and fungi such as *Mucor* spp, *Fusarium* spp, *Aspergillus niger* and *Aspergillus flavus* shows that fruits could act as a medium for the spread of both pathogenic and opportunistic microbes. These isolated organisms have also been reported by Ankita *et al.*, (2014) and Eni *et al.*, (2010). The isolation of *Escherichia coli* (coliforms) and *Proteus mirabilis* (enteric bacteria) from fruits suggested their likelihood of faecal contamination (Issa-Zacharia *et al.*, 2010); they could also arise from the use of human faeces as manure. Also, isolation of environmental isolates such as *Pseudomonas*, *Bacillus* and *Aspergillus* spp, may be indicative of soil contamination. *Pseudomonas* spp. is also associated with spoilage of



vegetable and fruits. *Bacillus spp.* are part of the normal soil flora, occurring in vegetables and fruits that are not handled properly, causing foodborne disease and vegetable spoilage (Michael *et al.*, 2005).

The fungus isolate- *Mucor sp.* has been indicated to cause food spoilage while growing on fruits and vegetables (Michael *et al.*, 2005). *Aspergillus niger* is a common environmental contaminant, forming spore (Michael *et al.*, 2005), and has been implicated in foodborne diseases (Akinleye *et al.*, 2013). Baiyewu *et al.*, (2007) reported that *Aspergillus flavus*, *Aspergillus niger* and *Fusarium spp* etc, were responsible for post-harvest losses in pawpaw in Nigeria.

Variations in geographical locations, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the organisms, type of water used for washing before display by the sellers and contamination extent of irrigation water might bring any noted variation between the result of this study and previous studies. Consequently, the variation of the results would be expected so long as these factors differ.

CONCLUSION

This study has revealed various parasites of public health and agricultural importance. A high number of these microorganisms in fruits can lead to public health emergencies resulting in infections with singular or combined symptoms reflecting the pathogens involved. Risk reduction can be achieved through personal and food hygiene. Relevant agencies should make every effort to educate the local populace on the importance of attitudinal change to reduce the rate of parasitic contamination of these commodities and the environment. Further studies should be conducted to address the effect of seasonal variation on the infestation of the fruits sold in this area.

Conflict of Interests

The authors declare no conflict of interest

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