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Parasitological Assessment of Selected Edible Fruits and Vegetables Sold At Some Markets in Lokoja, North Central Region of Nigeria in the Context of Disease Surveillance

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ABSTRACT

Various species of parasites are capable of contaminating most edible fruits and vegetables consumed in Lokoja with chronic consequences leading to malnutrition, iron-deficiency anaemia, morbidity and sometimes, healthcompromising nutritional status and affective cognitive process (especially in children). This assessment was done to isolate and identify common parasites of public health importance, given their high rates of transmission among members of the community, especially the fruits and vegetable consumers; as well as provide data and evidence for surveillance of some soil-transmitted helminthes and foodborne disease parasites. A total of 128 fruits and vegetables were sampled from the four markets, parasites were isolated from samples by washing with distilled water and normal saline. The isolates were examined for contamination using light microscopy. Concentrations of identified parasites were determined using iodine smear and sedimentation methods. 82 of the 128 samples were contaminated with different parasitic stages including ova, cysts, and larvae of Taenia sp., Ascaris lumbricoides, Schistosoma mansoni, Enterobius vermicularis, Hookworm; Balantidium coli, Entamoeba histolytica, Entamoeba coli; and Strongyloides stercoralis. Fruits and vegetables from different markets showed significant levels of parasitological infestation with Lonkongoma (78%) having the highest level compared to Ganaja (75%), Adankolo (56.25%) and Old Market (53.13%). Among the fruits and vegetables examined, pumpkin (41) had the highest frequency of parasitological contamination, with watermelon (21), garden egg (8) and green peas (8). Implications of these findings for effective surveillance programmes of foodborne and neglected tropical diseases of great public health importance were discussed and further control measures recommended.

Keywords

Helminthes, Soil-transmitted, Surveillance.

Introduction

The nutritional and economic values of edible fruits and vegetables are very much recognized among the human populations. However, significant prevalence of diseases resulting directly from consumption of parasitological contaminated fruits and vegetables make it alarming, worrisome and a scientific cause of concern. Indigenous fruits and vegetables are known to play major role in the nutritional livelihood of Nigerians, especially in the rural areas and cities where there is poor socio-economic condition [1]. Consumption of raw or unhygienically prepared vegetable is considered a risk factor for human parasitic infection

[2]. Some vegetables and most fruits are eaten raw to retain the natural taste and preserve heat labile nutrients but laboratory and epidemiological surveys have implicated this unhygienic practice as well as poor human sanitation as a major risk in the transmission of the parasitic diseases. It has also reported that in certain parts of Nigeria, the consumption of raw vegetable without proper washing is an important route in the transmission of parasitic diseases [3]. In Lokoja, Northcentral Nigeria, and its environs, fresh but raw fruit and vegetables are widely consumed, making it a major route/mode of transmission of intestinal parasites and spread of high burdened foodborne diseases of great public health importance.

Various other factors contribute to increase in diseases associated with raw fruits and vegetables and intensification of the risk. They

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include globalization of food supply, introduction of pathogens into new geographical areas through import, use of untreated waste and manure as fertilizer for crop production, irrigation with contaminated water and various agronomic practices and level of hygiene of food handlers. Continued use of untreated waste water and manure as fertilizer and food handling are major contributing factors to contamination that cause numerous food-borne disease outbreaks.

Fruits and vegetables are contaminated with parasites and, some parasitic infections which have direct life cycle and do not need an intermediate host to infect a new host are spread via these faecal-orally transmitted parasites. Infections acquired through direct ingestion of infective egg or cyst is ultimately linked with level of hygiene and sanitation in the community.

Factors like lack of latrine, improper hand washing during prayers and other religious activities/practices, and adequate sewage disposal facilities have been known to contribute to the spread of the infective stages of the parasites as well, thereby bringing about a wide spread contamination of foods. Most times, infections can be acquired through preventable attitude of keeping contaminated unwashed fingers by food handlers and vendors, insects, circulation of currency and by wind during dry season. The resistance capacity of the eggs and cysts of these parasites has been identified as a feature of profound influence on its epidemiology. It is now known that eggs of *Ascaris* can remain viable for up to six years [4].

Protozoan parasites and intestinal helminthes including geohelminthes/soil transmitted helminthes (a group of nematode) are among the most common infectious agent implicated in this contamination worldwide and particularly in developing countries in recent times [5]. Such specific parasites include *Entamoeba histolytica*, *Giardia duodenale*, *Trichuris trichuria*, *Ascaris lumbriodes* and *Enterobius vermicularis* [6].

Significant and alarming rates of diseases resulting from parasitological contamination of fruits and vegetables have been reported as well. Studies have shown that high prevalence of parasitic contamination of fruits and vegetables are most common in the tropics and sub-tropics. Geohelminthic infections are most prevalent in tropical and sub-tropical regions of the developing world, where adequate water and sanitation are lacking [7]. Recent estimate suggests that *Ascaris lumbriodes* infects over one billion people, *Trichuris trichuria* infects 79 million and hookworm (*Ancyclostoma duodenale and Necator americanus*) infects 740 million people [6].

Furthermore, amoebiasis is known to cause about 450 million infections per annum in developing countries with an incidence of about 50 million and 100,000 deaths. Giardiasis, which is more common in children has a worldwide prevalence of about 1-30%. Ascaris, one of the geohelminthes is the commonest nematode of man especially in tropical Africa, with a prevalence of about 40% in Enugu State and may be as high as 96-100% in the rural community in Enugu state. More recent studies in

rural villages of Ebonyi state, southeast Nigeria and other part of Nigeria have corroborated these high prevalence of parasites isolated from edible fruits and vegetables. 40% contamination of a total of 250 samples of edible fruits and vegetables sold in markets in rural villages of Ebonyi state has also been reported. Some of the parasites identified with their stages were ova of *Ascaris lumbriodes* (54.5%); *Strongyloides stercoralis* (6.9%); ova of hookworm (23.8%); *Trichuris trichuria* (8.9%); *Enterobius vermicularis* (5.9%) [8].

In a recent study done in Kwara State, it was reported that 41% of a total of 250 samples were contaminated of the selected edible fruits and vegetables. Some of the parasites identified were ova and cysts of; Ascaris lumbroides (48%), Entamoeba histolytica (15%), E. coli (13%), Hookworm (10%), Balantidium coli (8%), Trichomonas hominis (3%), Trichuris trichiura (2%), Giardia lambia (2%) [9].

Public health impact of these statistics is notable. Chronic and intense geohelminth infections can contribute to malnutrition, irondeficiency anaemia, morbidity and sometimes, health-comprising nutritional status, affecting cognitive process (especially in children), which include tissue reaction such as granuloma and provoking intestinal obstruction or rectal prolapsed [4,10,11] Though studies in different parts of Nigeria have implicated ingestion of parasitologically contaminated fruits and vegetables

ingestion of parasitologically contaminated fruits and vegetables as a major health risk factors for the alarming rates of intestinal and foodborne diseases, this study was designed to conduct a first parasitological assessment of samples of edible fruits and vegetables sold in Lokoja markets to further ascertain the risk status of the situation, and determine the prevalence of parasites on these food samples using effective laboratory examination methods. The study also examined the effectiveness and relevance of a new and locally-adapted technology for control of parasitological contamination of edible fruits and vegetables.

Methods Study area

The study was conducted in four major markets in Lokoja, the capital of Kogi State, with three major ethnic groups and diversities of languages. It is located in the Northcentral region of Nigeria and surrounded by mountain of about 1,500 meter above sea level. There are two major rivers (River Niger and River Benue) meeting each other to form confluence in this city.

Sample collection

A total of 128 samples consisting of fresh edible fruits and vegetables which included pineapple (Ananas comosus), apple (Malus domestica), watermelon (Citrullus), orange (Citrus aurantium), garden eggs (Solanum melongena), pumpkin leaf (Telfairia occidentalis), carrot (Daucus carota) and green peas (Pisum sativum) were collected from four different markets in Lokoja including Ganaja, Adankolo, Lokongoma and Old markets. In each of the markets, four samples of each of the fruits and vegetables were collected. All the samples were collected in sterile universal plastic bags and transported to Salem

University Advanced Microbiology laboratory for processing and examination.

Isolation and identification of parasites on selected edible fruits and vegetables

Parasitological examination of the fruits and vegetables using different solvents [distilled water and normal saline solution (0.09% NaCl)] to assay the samples. In the laboratory, 100g of each of the samples were weighed and put into a clean beaker containing 100ml of the normal saline solution (0.9% Nacl) for washing out the parasites. After removing the fragments of the sample from the washing solution using clean forceps, it was kept for 24 hours to allow sedimentation to take place. The same experiment was carried out using 100 ml of distilled water for each sample. After 24 hours of sedimentation, the top layer of the washing solvents was carefully discarded leaving 5ml of the sediments. This was finally centrifuge at 3000 revolution/minute for 15 minutes [12].

After discarding the supernatant, the residue was mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope for the presence of intestinal helminth parasites or their ova, cysts of *E. histolytica* and *G. intestinalis* in the samples [13].

Determination of concentration level of eggs, larva and cysts of intestinal parasites on selected fruits and vegetables

The concentration levels of eggs, larva or cysts of intestinal parasites on the selected fruits and vegetables were determined by the methods of sedimentation.

100g samples of each fruits and vegetables were washed in 100ml of distilled water and normal saline solution in a sterile beaker for the removal of the parasitic ova, larva or cysts. The suspension was strained through a sterile sieve to remove undesirable materials [14]. The filtrate was centrifuged at 3000 rpm for 15 minutes [15] and the supernatant was discard into the disinfectant jar. The sediment was mixed up and a drop was apply on the centre of a clean grease-free microscope slide and a clean cover slip was placed gently to avoid air bubbles and over-flooding. The preparation was examined under microscope for parasites using X10 and X40 objectives.

Results

Four different fruits and four different vegetables were sampled from four different markets in Lokoja, Kogi State. Four samples of each fruit and vegetable respectively; a total of 128 samples were collected for both fruits and vegetables altogether from the four markets, and examined for parasitological contamination. The outcomes of the laboratory examinations were analyzed and presented in tables.

Out of the 128 samples of the edible fruits and vegetables examined, 82 samples were contaminated with various protozoan and helminthes parasites such as cysts of *Entamoeba histolytica*, *E.coli*, *Balandtidium coli*; eggs of *Ascaris lumbricoides*, *Taenia*

saginata; and larvae of Strongyloides stercoralis, E. vermicularis, Schistosoma mansoni, Hookworm (Table 1).

| Fruits & vegetables | Ganaja Market | Adankolo | Longokma Market | Old Market | Number examined | Number of identified parasites |
|---------------------|------------------|----------|--------------------|------------|--------------------|--------------------------------|
| Market | 4 | 4 | 4 | 4 | 16 | 8 |
| Apple | 4 | 4 | 4 | 4 | 16 | 11 |
| Watermelon | 4 | 4 | 4 | 4 | 16 | 12 |
| Pineapple | 4 | 4 | 4 | 4 | 16 | 8 |
| Pumpkin | 4 | 4 | 4 | 4 | 16 | 14 |
| Carrot | 4 | 4 | 4 | 4 | 16 | 12 |
| Green pea | 4 | 4 | 4 | 4 | 16 | 8 |
| Garden egg | 4 | 4 | 4 | 4 | 16 | 9 |
| Total | 32 | 32 | 32 | 32 | 128 | 82 |

Table 1: Number of fruits and vegetables contaminated with parasites in each market.

Table 2 shows the prevalence of various parasites on edible fruits and vegetables in selected market in Lokoja. This laboratory examination showed that Lonkongoma market had the highest prevalence of parasite-infected edible fruits and vegetables (25 or 78%). Indicating the hyper endemic levels of diseases associated with these parasites in Lokoja, especially the Lonkongoma area. Ganaja village a peri-urban area as well as other urban areas, like Adankolo and old market areas probably had significant measures of parasites transmitted via edible fruits and vegetables in the amounts of 24 or 75.00%, 18 or 56.26% and 17 or 53.13% respectively.

| Fruits& Vegetables | Ganaja | Market | Adankolo | Market | Lokongoma | Market | Old | Market |
|--------------------|----------|----------|----------|----------|-----------|----------|----------|----------|
| 3 | Examined | Infected | Examined | Infected | Examined | Infected | Examined | Infected |
| Orange | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 1 |
| Apple | 4 | 2 | 4 | 4 | 4 | 2 | 4 | 3 |
| Watermelon | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 2 |
| Pineapple | 4 | 2 | 4 | 0 | 4 | 4 | 4 | 2 |
| Green pea | 4 | 2 | 4 | 2 | 4 | 1 | 4 | 3 |
| Carrot | 4 | 4 | 4 | 2 | 4 | 3 | 4 | 3 |
| Pumpkin | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 |
| Garden egg | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 0 |
| Total | 32 | 24 | 32 | 18 | 32 | 25 | 32 | 17 |

Table 2: Prevalence of parasites on fruits and vegetables from each market.

The frequencies of stages of various isolated parasites are shown in table 3. The different stages of the parasites identified include eggs of Ascaris lumbricoides. Larvae of Strongyloides stercoralis; eggs

of hookworm, Taenia saginata, Schistosoma mansoni, Enterobius vermicularis and cysts of B. coli, E. coli and E. histolytica. Table 3 shows the larvae of Ascaris lumbricoides isolated from pumpkin sold at old market had the highest frequency of (10). Other stages of parasites isolated with significant frequencies were eggs of Ascaris lumbricoides on pumpkin sold at Longkongoma market (8); and cysts of E. coli on watermelon sold at Lonkongoma market (10).

| Markets | Parasite | Apple Sop(no.) | Pumpkin Sop(no.) | Carrot Sop(no.) | Pineapple Sop(no.) | Garden egg Sop(no.) | Green pea Sop(no.) | Watermelon Sop(no.) | Orange Sop(no.) |
|---------------|-----------------|-------------------|---------------------|--------------------|-----------------------|------------------------|-----------------------|------------------------|--------------------|
| aja | B.coli | - | C(7) | - | - | - | - | - | - |
| Ganaja | E.coli | - | - | - | - | - | - | C(6) | - |
| olo | E. vermicularis | - | E(5) | - | - | - | - | - | - |
| Adankolo | Hookworm | - | E(5) | - | - | - | - | - | - |
| PΑ | S. mansoni | - | | - | - | - | - | E(5) | - |
| | A. lumbricodes | - | E(10) | - | - | - | - | - | - |
| na | T. saginata | - | C(3) | - | - | - | - | - | - |
| Lonkongoma | B.coli | - | C(3) | - | - | - | - | - | - |
| onko | E.coli | - | - | - | - | C(8) | - | - | - |
| Ľ | E.histolytica | - | - | - | - | - | C(8) | C(10) | - |
| | S. stercoralis | - | - | E(5) | - | - | - | - | - |
| Old Market | S. stercoralis | L(5) | - | - | - | - | - | - | - |
| O Ma | A. lumbricoides | - | E(8) | - | - | - | - | - | - |

Table 3: Frequency of stages of isolated parasites on fruits and vegetables from each market. SOP= stages of parasites, E =egg/ova, L=larvae, C=cyst.

From the table 4, *Ascaris lumbriocoides* had the highest frequency (18 or 21.95%) in the contamination of 128 sampled edible fruits and vegetables. Frequencies of various other parasites contributed to the overall parasitic contamination of commonly consumed fruits and vegetables. *E. coli* (12 or 14.63%); *B. coli*, *Strongyloides stercoralis*; *E. histolytica* (10 or 12.2%); *T. saginata* (7 or 8.84%); *S. mansoni*; hookworm and *E. vermicularis* (5 or 6.10%) respectively.

| Parasites | Frequency | Percentage (%) |
|---------------------------|-----------|----------------|
| Ascaris lumbricoides | 18 | 21.95 |
| Strongyloides stercoralis | 10 | 12.20 |
| Schistosoma mansoni | 5 | 6.10 |
| Hookworm | 5 | 6.10 |
| Enterobius vermicularis | 5 | 6.10 |
| Taenia saginata | 7 | 8.54 |
| Balantidium coli | 10 | 12.20 |
| Entamoeba coli | 12 | 14.63 |
| Entamoeba histolytica | 10 | 12.20 |
| Total | 82 | 100 |

Table 4: Frequency of isolated parasites.

In overall, table 5 shows that distilled water dislodged more parasites (42) than normal saline (40). However, the effect is

inversely varied on specific fruits and vegetables sampled. On watermelon, there is wide margin shown in dislodging *Entamoeba histolytica* using normal saline (9) against distilled water (1). Similarly, the big margin was observed in the number of *B. coli* (8) dislodged when distilled water was used in washing pumpkin against the distilled water that dislodged 2 parasites. The number of *E. coli* (5) dislodged when distilled water was used on garden egg varied from the number of parasites dislodged when normal saline was used (2). The two agents applied in washing and dislodging parasites like *Ascaris lumbricoides* (4) from pumpkin and *Strongyloides stercoralis* (4) from carrots equally varied.

| Fruits and | D 4 11 1 1 | No. of parasites dislodged using | | | |
|------------|---------------------------|----------------------------------|---------------|--|--|
| vegetables | Parasites dislodged | Distilled water | Normal saline | | |
| Apple | Strongyloides stercoralis | 1 | 2 | | |
| Carrot | Ascaris lumbricoides | 4 | 2 | | |
| Carrot | Strongyloides stercoralis | 3 | 4 | | |
| Candon ogg | Ascaris lumbricoides | 4 | - | | |
| Garden egg | E. coli | 5 | 2 | | |
| Green peas | E. coli | 3 | 4 | | |
| Orange | - | - | - | | |
| Pineapple | - | - | - | | |
| | Ascaris lumbricoides | 4 | 4 | | |
| | Balantidium coli | 8 | 2 | | |
| Pumpkin | Taenia saginata | 4 | 3 | | |
| | Enterobius vermicularis | 3 | 2 | | |
| | Hookworm | - | 5 | | |
| Watermelon | E. histolytica | 1 | 9 | | |
| | Entamoeba coli | 1 | - | | |
| | Schistosoma mansoni | 4 | 1 | | |
| Total | | 42 | 40 | | |

Table 5: Parasitological dislodging effects of distilled water and normal saline on edible fruits and vegetables.

Discussion

The isolation of intestinal parasitic protozoa and helminth from some of the fruits tends to suggest that fruits are possible sources of transmission of food borne diseases among Lokoja people their presence in those fruits may be associated with the water used in washing the fruits by their sellers. The presence of these parasites in the fruits may not be surprising when the contaminated water the vendors use in washing and sprinkling on the fruits are considered. It is known that faecal matters of animals and human beings harbour some of the protozoa/parasites isolated and the faecal matters are often used as manure for the cultivation of fruits. It is therefore possible that some of the protozoa and parasite isolates, especially those on the pumpkin leaves are contaminants from the field.

This results support those previous studies which found fruits and vegetables contaminated with geohelminth ova and larvae [4,7,16,17]. They concluded that the sources of fruits and vegetables were contaminated with fecal matter. Given the fact

that the adult stage of worms encountered resides in the intestine is a vivid reflection of poor hygiene and sanitation of our rural areas (sources of these plants). This could be traced to the habit of rural farmers and vendors which is often associated with the use of night-soil as manure, regular moisturizing/freshening of fruits and vegetables with contaminated water and poor animal husbandry. The latter may account for the high prevalence of egg and larvae of *A. lumbricoides*, a zoonotic infection with non-human primates and dogs being naturally infected [18], as it is common practice in rural areas around Asaba to combine subsistence with pastorial (companion animals inclusive) farming [19]. The excreta of these animals are used as manure and, this is a serious factor in continued epidemiology of communicable helminthes diseases in Nigeria and third world countries.

Furthermore, as shown in table 2, the high prevalence of parasitological contamination of fruits and vegetables indicate there are likely hyper endemic levels of diseases associated with these parasites in Lokoja, especially the Lonkongoma axis, Ganaja village as well as Adankolo and urban areas around Old market. These rates are in turn reflections of poor level of hygiene, and low level knowledge, attitude and practice towards factors promoting the high rates of associated intestinal diseases.

The zero frequencies of parasites reported for orange and pineapple sample items is an indication of no contact with contaminated water mentioned earlier and implicated in a similar study [9]. The peels or covers of edible parts of these fruits are removed before the edible part, which is not contaminated prior ingestion. The near-sterile packaged form in which these edible fruits are sold also ensures their zero contamination. In table 3 and 4, high frequencies were recorded against the egg (8) and larvae (6) of *Ascaris lumbricoides* isolated from pumpkin sold in Ganaja market. It is therefore most probable that Ascariasis is the intestinal disease associated with edible fruits and vegetables with highest prevalence. With this high level of endemicity for ascariasis as well as other diseases due to fruits and vegetables implicated; outbreak, epidemic proportions and re-emergence are suspected.

Lastly, the washing power or dislodging effects of distilled water and normal saline were compared in table 5. Although distilled water dislodged more parasites than normal saline on the whole, the effects are inversely varied on specific fruit and vegetable item. This overall variation and specific differences in the number of parasites isolated using normal saline and distilled water may be due to action of normal saline on the parasitic stages on these fruits and vegetables given their harmful/destructive effects on the organisms. Thus dead or killed parasites may be identified as debris. The distilled water on the other hand is usually mild on any parasite stage that contaminated the sample items. However, since there are no significant studies to back up these claims, it is proposed or recommended for further investigation.

Conclusions

A total sample of 128 edible fruits and vegetables sold at some markets in Lokoja were examined in the laboratory for

parasitological examination. It was found that various intestinal parasites contaminated 82 of these sample items. These parasites had various frequencies or concentrations on fruits and vegetables contaminated; this is an indication of high prevalence and reemergence of associated diseases such as ascariasis, strongyloidiasis, hookworm infection, taeniasis and amoebiasis.

It is against this back drop and findings that we infer from the study that some selected edible fruits and vegetables harbor high number of parasites, due to this reason it is advisable that appropriate education and control measure be given to the people about the need for proper washing of fruits and vegetables using distilled water and normal saline and proper packaging under hygienic conditions. The control measures includes treatment of irrigation waters before using for agricultural purposes, provision of goods sanitary system in both the urban and rural areas to prevent contamination of soil and water with parasite from poor disposition of faeces. Covering of foods and water to prevent contamination from flies which act as cysts carriers, use of appropriate disinfectant to wash fruits before eating are equally imperative.

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