PARASITES CONTAMINATION OF SALAD VEGETABLES SOLD IN ABAKALIKI, EBONYI STATE, NIGERIA

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ABSTRACT

Parasitic infections are of great public health importance because of their high prevalence and effects on both nutritional health and immune status of people living in tropical and sub-tropical countries. Five types of vegetable samples such as tomatoes, (Solanium iycapersicum), cucumber (Cucumis sativus), carrot (Daucus carota), cabbage (Brassica olerecea), lettuce (Lactuca sativa), were investigated for parasites using sedimentation and floatation methods. Out of 430 samples of the vegetables screened, 258(60%) were found to be contaminated with helminth ova/larvae and protozoan cysts. Ova/larvae of Ascaris lumbricoides 23(20.4%), Strongyloides stercoralis 32(28.3%, Trichuris trichuria 21(18.6%), hookworm 28(24.8%) and Fasciola species 6(5.3%) were identified. Strongyloides stercoralis and hookworm were the most encountered with prevalent rates of 28.3% and 24.8%, respectively, while the protozoan cysts recovered were those of Entamoeba histolytica 3(2.7%). Among the vegetables, lettuce 36(28.6%) was the most contaminated while cucumber 14(11.1%) had the least contamination. The results of this study implicated salad vegetables in the transmission of intestinal parasites. It is therefore imperative that the sanitary condition of these vegetables be improved from the producers down to the consumers.

Keywords: Parasites contamination, *Strongyloides stercoralis,* Hookworm, *Ascaris lumbricoides, Trichuris trichuria, Fasciola* species, *Entamoeba histolytica* Salad vegetables, Tomatoes, Carrot, Cucumber, Cabbage, Lettuce

INTRODUCTION

Salad vegetables are green vegetables which are rich in vitamins and minerals and are consumed without any heat treatment. They are essential for human health and well-being and form a major component of a healthy diet in every family (Sunil *et al*., 2014). They contain valuable nutritional factors that are used in the build up and repair of the body. According to WHO (2003) a minimum of 400g of vegetables and fruits per day is needed for prevention of chronic diseases such as cancer, diabetes and obesity and alleviation of several micronutrient deficiencies especially in less developed countries. They substantially improve food quality and have high water content. Many vegetables are good sources of vitamin C, carotene, iron, thiamin, niacin and riboflavin (Frazier and Westhoff, 1998). Consumption of salad vegetables is encouraged because they retain natural flavour and preserve heat labile nutrients which could be destroyed by cooking.

Despite all these benefits, green vegetables, often act as potential sources and route for the spread of various parasitic infections. In view of this, the 54th World Health Assembly passed a resolution in May, 2001 affirming that the control of vegetable transmitted helminths should be considered a public health priority (Horton, 2003). Throughout the processes of planting to

consumption, vegetables can be contaminated with different pathogens. The extent of such contamination depends on several factors such as the use of untreated wastewater for irrigation, unsanitary post-harvest handling and unhygienic conditions of vegetables preparation in restaurants and homes. Others are low level of education and lack of adequate health education. Often, vegetables are cultivated on the same piece of land every year and as a result of this continuous land usage, there is depletion of soil nutrients, hence, the need for fertilizer or manure in order to improve yield. Most local farmers who usually cannot afford artificial fertilizers therefore use untreated animal and human faeces which are known to contain various species of parasites that are of medical and veterinary importance (Hajjami *et al.,* 2013). This practice favours the transmission of intestinal parasitic infections.

Consumption of unhygienically prepared salad vegetables is therefore considered a risk factor for human parasitic infections. Immune-suppression may also occur in the face of the parasitic infections as they lower the resistance of such population to other infections and render active immunization procedures less effective. The major ways of transmitting these parasites include; ingestion of infective eggs, active penetration of infective larvae and post-harvest contamination of vegetables with their ova and larvae.

Nigeria, like other developing countries is faced with the dilemma of inadequate disposal of excreta-related human wastes. Thus, in rural communities, defecation on open fields (farm land) is still a common practice. This practice enhances parasite populations on farm lands. The infection can also be a household affair where infected children or persons provide the chief source of soil contamination by their promiscuous defecation in the soils.

Epidemiological studies have indicated that in areas of the world where helminth diseases are endemic in the population and where raw untreated waste water is used to irrigate vegetables generally eaten uncooked, the consumption of such vegetables may lead to parasitic infection (WHO, 1980). In Nigeria, the cultivation of salad vegetables for commercial and domestic purposes is mostly carried out in the northern part of the country and transported to other parts of the country. Usually, cultivation is done throughout the year using rain during wet season and irrigation during dry season (Damen *et al.,* 2007). During the dry season, the sources of irrigation water are usually polluted with human and animal faeces and the use of such polluted or wastewater for irrigation poses health risks to both the local farmers and the consumers of the crops produced and enhance recycling of infections.

In recent years, there has been an increase in the number of reported cases of parasitic infections associated with contaminated fresh vegetables. Presently, there is high demand of green vegetables especially salad which component vegetables are not usually cooked. It becomes necessary therefore that the contamination of these vegetables used in salad making be investigated in order to contribute information on the control of these parasites.

The aims of this study therefore were to: (a) investigate the level of parasitic contamination of salad vegetables sold within the study area, (b) determine the parasites responsible for such contamination and (c) provide information on the ways of preventing contamination with these parasites and their associated infections.

Study Area: The study was conducted in Abakaliki, the capital city of Ebonyi State, south eastern Nigeria, between June and July, 2013. Abakaliki is the capital city of Ebonyi State. The people of the town and its environs are of Igbo speaking ethnic group, with different dialects. Their major occupation is farming and mining activities. It is a common practice that majority of the farmers use human and animal manures to argument commercially processed fertilizer in order to reduce the cost of farming.

Vegetables Sampling: The salad vegetables were purchased from Abakpa market in Abakaliki in the early hours of 8 – 10 am, into sterile polyethene bags, labeled accordingly and taken to Applied Biology Laboratory, Ebonyi

State University for screening for parasites within six hours of collection. The samples consisted of five vegetables which include *Brassica oleracea* (cabbage), *Lactuva sativa* (lettuce), *Daucus carota* (carrot), *Lycopersium esculentum* (tomatoes) and *Cucumis sativus* (cucumber).

Parasitological Screening: Two hundred grams of each type of the fresh salad vegetables was weighed and washed vigorously with normal saline (0.85%NaCl) in 100ml round bottom lean plastic container. The mixture was strained (sieved) through sets of sieve to remove unwanted debris and allowed to stand on the bench for 10 hours to allow time for proper sedimentation. The supernatant was discarded with a Pasteur pipette leaving about 15ml at the bottom. Ten milliliters of the deposit mixture was transferred into a centrifuge tube and spun for five minutes at 1500 rpm. The supernatant was decanted by quickly inverting the tube. After this, the deposit was mixed and examined following the techniques of Arther *et al*. (1981) and Hajjami *et al*. (2013) as presented below.

Direct Smear: A drop of the sediment was applied on the center of a clean grease-free slide and gently covered with clean cover slip avoiding air bubbles and over flooding. The preparation was examined under a light microscope using x10 and x40 objectives. The whole area under the cover slip was systematically screened. This procedure was repeated until the sediment in each test tube was completely exhausted.

Iodine Smear: A drop of the sediment was mixed with a drop of Lugol's iodine solution and examined as in direct smear for detection of parasite eggs, cysts and larva. The parasites found under the light microscope were identified as described by Alhabbal (2015).

RESULTS

The results of this study showed that the vegetables were heavily contaminated. Sixty percent of the samples were contaminated with different intestinal parasites. Out of 113 parasitic occurrences, 104 were nematodes while 6 were trematodes. This work also revealed poly-parasitic contamination of some of the samples which makes them vehicles for multiple parasitic infections. The prevalence of intestinal parasites in the salad vegetables showed that *Strongyloides stercoralis* (Figure 1) had the highest prevalence, 32(28.3%), followed by hookworm 28(24.8%), while *Entamoeba histolytica* had the lowest 3(2.7%) (Table 1).

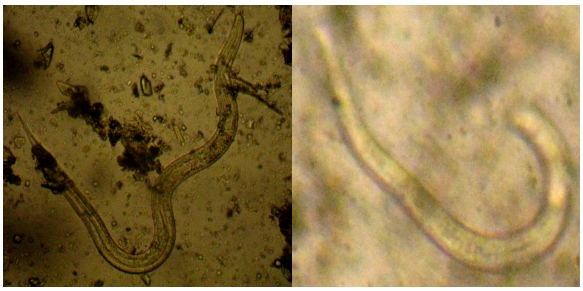

Figure 1: Larval stages of *Strongyloides stercoralis* isolated from salad vegetables sold in Abakaliki, Ebonyi State, Nigeria

The frequency of occurrences of nematodes in each of the salad vegetables indicated that Lettuce had the highest 29(27.9%) contamination while cucumber 10(9.5%) had the least (Table 2). The frequency of isolated parasites in the order of occurrence is *S. stercoralis* > Hookworm > *Ascaris lumbricoides* > *Trichuris trichuria* > *Fasciola* species > *E. histolytica* (Table 3).

DISCUSSION

The presence of intestinal parasites in vegetable samples is suggestive of faecal contamination of these products. This study recorded heavy contamination of salad vegetables and this has significant public health implication. Sixty percent of the samples were contaminated with different intestinal parasites. This corroborated the earlier reports of Abougrain *et al*. (2010), Fallah *et al*. (2011), Klapec and Borecka (2012), Omowaye and Audu (2012) and Hajjami e*t al*. (2013), who in their respective studies recorded high rates of contamination in different countries.

Table 1: Prevalence of intestinal parasites in salad vegetables sold in Abakaliki, Ebonyi State, Nigeria

Salad vegetables	*Ascaris lumbricoides*	Hookworm	*Trichuris trichuria*	*Fasciola species*	*Strongyloides stercoralis*	*Entamoeba histolytica*
Tomatoes	5(21.4)	6(21.4)	7(33.3)	3(50.0)	8(25.0)	0(0)
Carrot	6(26.1)	0(0)	2(9.5)	0(0)	5(15.6)	0(0)
Cucumber	4(17.4)	3(10.7)	2(9.5)	2(33.3)	1(3.1)	0(0)
Cabbage	3(13.0)	11(39.3)	4(19.0)	1(16.7)	8(25.0)	1(33.3)
Lettuce	5(21.7)	8(28.6)	6(28.6)	0(0)	10(31.3)	2(66.7)

Key: *Figure in parenthesis = percentage*

Table 2: Frequency of occurrences of nematodes in salad vegetables sold in Abakaliki, Ebonyi State, Nigeria

Vegetables	*Ascaris lumbricoides*	*Hookworm*	*Trichuris trichuria*	*Strongyloides stercoralis*	Total
Tomatoes	5(21.7)	6(21.4)	7(33.3)	8(25.0)	26(25.0)
Carrot	6(26.1)	0(0.0)	2(9.5)	5(15.6)	13(12.5)
Cucumber	4(17.4)	3(10.7)	2(9.5)	1(3.1)	10(9.6)
Cabbage	3(13.0)	11(39.3)	4(19.0)	8(25.0)	26(25.0)
Lettuce	5(21.7)	8(28.8)	6(28.8)	10(31.3)	29(27.9)
Total	23(22.1)	28(26.9)	21(20.2)	32(30.8)	104(92.0)

Key: *Figure in parenthesis = percentage*

Table 3: Frequency of isolated parasites in salad vegetables sold in Abakaliki, Ebonyi State, Nigeria

Parasites	Number of occurrence
Ascaris lumbricoides	23(20.4)
Hookworm	28(24.8)
Trichuris trichuria	21(18.6)
Strongyloides stercoralis	32(28.3)
***Fasciola* species**	6(5.3)
Entamoeba histolytica	3(2.7)
Total	113(100)

Key: *Figure in parenthesis = percentage*

However, Adamu *et al*. (2012) recorded a lower percentage (3.5%) of helminth parasites in their study in Northern Nigeria. Salad vegetables can be contaminated on the field during growth, harvesting, transportation, distribution and marketing. This is due to the fact that rural farmers who are involved in these processes know little or nothing about personal and environmental hygiene. The application of untreated human and animal dung as manure by producers may also be contributing factor to the high rate of contamination. Most often, retailers of these products in a bid to keep them fresh, wash the vegetables with water that has been contaminated with the feaces of infected animals and human beings.

The consumption of vegetables, raw or undercooked is therefore a way through which transmission of parasites is encouraged and may be a major cause of parasitosis in humans. Therefore, recovery of parasites from vegetables may be helpful in indicating the incidence of intestinal parasites in an area.

The intestinal helminths isolated from this study were *S. stercoralis*, *A. lumbricoides, T. trichuria, E. histolytica, Fasciola* species and hookworm. This agreed with the report of Alhabbal (2015) who also isolated similar parasites from fresh vegetables in his study in Deiratyah, Syria. Helminth parasites were found to be more than those of protozoan parasites on salad vegetables in this study as also reported by (Eneanya and Njom, 2002). This may be attributed to the fact that helminths can withstand a wide variety of adverse environmental conditions. The most frequently isolated parasites in the study were *S. stercoralis* 32(28.3%), followed by hookworm 28(24.8%). These intestinal parasites are known to have high fertility and are able to contaminate the environment with their eggs. Eggs of *A. lumbricoides* for instance may survive in the external environment and maintain their invasiveness for up to 6 years Klapec and Borecka (2012).

In this study, lettuce *(Lactuca sativus*) (66.7%) was found to be the most contaminated of the vegetables. This is in line with the report of Ammar and Omar (2013) who recorded 56.6% contamination on lettuce in their study in Al-Qassim region, Saudi Arabia. This could be attributed to the rough surfaces of this vegetable which harbor the infective forms of parasites by enhancing their adherence as was earlier reported by Hajjami *et al*. (2013) in their study in Casablanca, Morocco.

Conclusion: The results of this study have shown that human parasites can be transmitted through consumption of contaminated salad vegetables with different parasite eggs and cysts and this may pose a health risk to consumers. Health education and awareness on the potential health consequences of consumption of contaminated food products should be created among the public. Improvement in both personal and environmental hygiene and soaking salad vegetables for ten minutes in saturated salt solution will also prevent infection. The use of untreated human and animal wastes as manure should be strongly discouraged.

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