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Occurrence of Soil-Transmitted Helminths (STHS) in Some Selected Vegetables Used as Salad Available in Local Markets in Owerri Municipal, Imo State Nigeria

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Abstract

Food safety is a serious concern in every part of the world. The interest of the public on food safety issues is on the ascendancy worldwide. The study determined the occurrence of soil-transmitted helminths (STHs) in some ready-to-eat vegetables used as salad vegetables available in local markets of Owerri Municipal Imo State Nigeria. An experimental study design was adopted, where a total of two hundred and fifty (250) samples were used for this study. Seven (7) different ready-to-eat vegetables used as vegetable salads were used in this study. Existing instrument methods described by Samah et al. and Amoah et al were used for data collection after being validated and its reliability tested for quality assurance, 10% of the samples were randomly selected and re-examined by an experienced Laboratory Technologist. Data analysis was done using SPSS version 21, frequency table were generated to determine the association between soil-transmitted helminths (STHs) and ready-to-eat vegetables used as salad vegetables. A total of 54 soil-transmitted helminths were detected in the vegetables comprising 22 *Ascaris lumbricoides*, 7 *Strongyloides stercolaris*, 4 *Necator americanus*, 7 *Ancylostoma duodenale* and 14 *Trichuris trichuria* were detected. Garden egg seeds, cucumber and cabbage had the highest *Ascaris lumbricoides* occurrence, while lettuce had the highest *Strongyloides stercolaris. Necator americanus* was only isolated from garden egg seeds, cucumber, carrot and cabbage. Findings from this study have shown the presence of soil-transmitted helminthes in some ready to eat vegetables. This shows some poor level of adequate washing of these vegetables by farmers, wholesalers and retailers in different markets sampled. Therefore, vegetable vendors should adopt good hygienic practices to ensure that these vegetables are not contaminated with soil-transmitted helminths.

Keywords: Soil-Transmitted Helminths (Sths), Vegetables, Markets

Introduction

The need for food safety is a serious concern for many scientists in recent years. Also, the interest of the public on food safety issues is on the ascendancy worldwide [18]. Nonetheless, food safety problems continue to persist across the globe and remain a great challenge [11]. The consumption of vegetables and vegetables products are vital for the total health of every individual, however, microbial contamination of these vegetables has become a serious challenge deserving of greater attention [3]. Vegetables can be defined as leafy outgrowth of plants which can be consumed in relatively small quantities as a side dish or a relish with a stable food [2]. They form the largest group of plants consumed by man, and this group is also the most heterogeneous. The vegetable maybe the swollen tap roots (e.g carrot, the lypocoty). It can also be the part below the cotyledon, (e.g water leaf), the fruit e.g tomatoes, the flower bud (e.g. globe artiochoke) and exceptionally the whole plants, as in the case of seedlings of some cereals and pulse [9]. Vegetables are plants which are served with or incorporated into a wide range of dishes to add interest; variety, nutritional value, colour and texture and so contribute much to an enjoyable meal. Most

vegetables are leaves, roots and stems of herbaceous plants, although flowers, calyces, immature seeds or fruits may also be consumed as vegetables [9]. African vegetables are common, cheap and nutritious with health value [12]. The consumption of fruits and raw vegetables are vital component to sound health. Vegetable can be eaten either raw or cooked and play an important role in human nutrition being mostly low in fat and carbohydrates but high in vitamins, mineral and fibre [16].

Vegetables are a good source of antioxidants and phytonutrients. They are low in calories and rich in complex carbohydrates, vitamins and minerals. Among the vegetables popularly consumed in Nigeria, especially, Imo State are; cabbage, carrot, okra and curry leaves. These vegetables serve as salad vegetables (carrot and cabbage), dessert (carrot), spices (curry) as well vegetables for cooking soups and other foods (okra). Globally, salad vegetables are one group of vegetables which are a major component of food vending and mostly implicated in this regard. Salads are fresh vegetables which require minimal washing and processing and cut into desired shapes and sizes with knives or other shredding utensils and usually serve as along with other foods including rice [1, 8]. Worldwide, salad vegetables are considered a major source of nutrients for people and particularly as sources of cancer fighting agents for the skin [14]. Recent studies have established that consumption of salad vegetables can prevent heart diseases and skin cancers [5].

Statement of the Problem

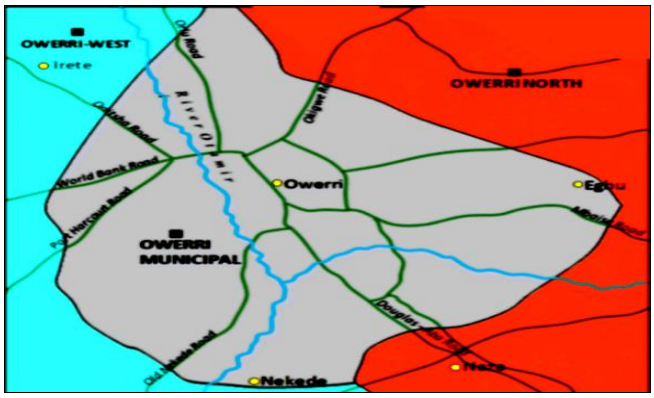
Salad vegetables are becoming more and more popular for their beneficial and nutritional values. The excellent nutritional value is not limited to consumers but to bacteria and other pathogens that predominate the vegetables. Contamination of salad vegetables have been considered as a public health concern. Because it is usually not heat treated, contaminated raw materials would introduce pathogens that may grow to a substantial levels and poses health risks to consumers. Several studies all over the world linked salads containing fresh vegetables or fruits to food-borne illnesses. Though, a number of studies have been carried out on microbial quality of vegetables and vegetable salads, there is a paucity of information on the occurrence of soil-transmitted helminths (STHs) in some vegetables used as salad available in local markets in Owerri Municipal. Therefore these objectives where formulated to guide the study: to determine the helminth load, detect the presence and distribution of soil-transmitted helminths (STHs) in some ready-to-eat vegetables used as salad vegetables available in local markets and ascertain the ready-to-eat vegetables with the highest occurrence of soil-transmitted helminths (STHs).

Materials And Methods

Population of the Study: The target population for this study consists of vegetable sellers in the three (3) different selected markets in Owerri Municipal, Imo State. Since the vegetable sellers are not registered and are not in any association or group, the above population is selected.

Study Area:

Owerri Municipal is a Local Government Area in Imo State, Nigeria. Its headquarters is in the city of Owerri. It has an area of 58 km² and a population of 127,213 according to the 2006 census. The postal code of the area is 460. Owerri city sits at the intersection of roads from Port Harcourt, Onitsha, Aba, Orlu, Okigwe and Umuahia. It is also the trade center for palm products, corn [maize], yams and cassava . Eke Ukwu Owere market is the main market in Owerri Municipal.



Source: Owerri Municipal Council

Fig 1: Map of Owerri Municipal

Sample size calculation: Using Taro Yamane's method:

$n = N / (1+N (e)^2)$

Where:

n signifies the sample size
N signifies the population under study
e signifies the margin error (it could be 0.10, 0.05 or 0.01)

To determine sample size,

$n = N / (1+N (e)^2)$
$n = 495 / (1+490 (0.05)^2)$
$n = 495 / (1+490 (0.0025)$
$n = 495 / 1+ 0.96$
$n = 495/1.96$
$n = 252$

Sampling Technique:

Simple random sampling was adopted in the selection of the vegetable sellers that is used in this study. The sample size for the study consists of two hundred and fifty two (252) vegetables from the three (3) different selected markets in Owerri Municipal, Imo State. Seven (7) different ready-to-eat vegetables used as vegetable salads from the three (3) different markets which include; garden egg seeds, garden egg leaves (akwukwo anara), cucumber, carrot, cabbage, lettuce and utazi leaf. From each market, a total of eighty four (84) samples comprising twelve (12) each of the vegetables samples were collected from different vegetable sellers within the market.

Validity of Instruments

The validity of the instruments was done following existing methods described by Samah *et al*. and Amoah *et al* [15, 4].

Reliability of the Instrument

For quality assurance, 10% of the samples were randomly selected and re-examined by an experienced Laboratory Technologist. The completeness of the required data was regularly checked by the supervisors. Sample collection,

transportation, and accurate results registration were done according to the operational procedure standards.

Sample Collection and Preparation:

The collection of vegetables was done between the hours of 7 a.m. and 11 a.m. Sound fresh samples were placed separately into labeled sterile plastic bags without preservation and then transported to the closest laboratory as fast as possible. Sample preparation for parasitological examinations described by Samah et al. was adopted in the preparation of the vegetables for parasitological examination [15]. For each of the vegetable samples, 200 g was soaked for fifteen minutes (15 minutes) in 1 L of physiological saline, and then vigorously shaken for 5 minutes with the help of a mechanical shaker. After removing the vegetable sample, the washing water was filtered through a sterile 32–36 m hole-size sieve to eliminate undesired debris while allowing different sizes of parasite forms to pass. The residual wash solution was allowed to settle for 8 hours. The filtrate was then emptied into clean centrifuge tubes and centrifuged for 10 minutes at 2000 rpm. The supernatant was removed, and the sediment yielded parasitological examination to find various parasite eggs, larvae, cysts, and oocysts.

Parasitological examination of the ready-to-eat vegetables: The wet mount microscopic method of the edible vegetable samples described by Amoah et al. [4] was adopted in the parasitological examination of the ready-to-eat vegetables. A drop of each of the residue was placed on a clean grease-free glass slide with the addition of tincture of iodine and was examined for parasites stages under the microscope using the x10 and x40 objective lenses. Parasites were identified using parasite atlas.

Formal ether sedimentation concentration technique described by Amoah et al. [4] was adopted in the parasitological examination of the stained and unstained sediment smears of the ready-to-eat vegetables. For the unstained smear, a drop of the sediment was transferred on a newly cleaned slide, and a cover slip was delicately placed to minimize air bubbles and flooding. After that, the preparation was examined under a light microscope with 10×, 40×, and 100× objectives. For stained smears, a drop of the sediment was mixed with a drop of Lugol's iodine solution and inspected microscopically as in a simple smear. To identify cryptosporidium oocysts, modified Ziehl– Neelsen staining was used. It is a stain used to identify acid-fast organisms as *Cryptosporidium* spp. that stain red, whereas the background of debris stains blue. The procedure was repeated until the mixture in each test tube was depleted.

Data analysis

The data obtained from completed questionnaires was collated, analyzed, using statistical package for social sciences, (SPSS) using frequency analysis to determine the prevalence of soil-transmitted helminths in the vegetables. The data obtained was used to determine the association between soil-transmitted helminths (STHs) and ready-to-eat vegetables used as salad vegetables. It also determined the association between type of vegetable and the prevalence of soil-transmitted helminths (STHs) in ready-to-eat vegetables used as salad vegetables.

Ethical Considerations

Ethical approval to undertake the study was obtained from Research and Ethics Committee of School of Health Technology Federal University of Technology Owerri Imo State and local ethics committee of the 3 selected markets also a verbal informed consent was obtained from all the vegetable sellers before being allowed to participate in the study.

Results:

Helminth load in the garden egg seeds from the different markets: Helminth load recorded in the garden egg seeds. Helminth load was detected in six (6) out of the twelve (12) garden egg seeds gotten from Eke-Ukwu market. For Relief market, helminth load was detected in nine (9) out of the twelve (12) garden egg seeds used. For the samples gotten from World Bank market, helminth load was detected in four (4) out of twelve (12) samples used.

Helminth load in the African eggplant leaves (akwukwo anara) in the different markets: Helminth load recorded in the African eggplant leaves (akwukwo anara) was not detected in nine (9) out of the twelve (12) African eggplant leaves (akwukwo anara) gotten from Eke-Ukwu market. For Relief market, helminth load was detected in seven (7) out of the twelve (12) garden egg seeds used. For the samples gotten from World Bank market, helminth load was not detected in four (4) out of twelve (12) samples used.

Helminth load in carrot from the different markets: Helminth load was detected in five (5) out of the twelve (12) carrot samples gotten from Eke-Ukwu while helminth load was detected in six (6) out of the twelve (12) carrot samples gotten from Relief market. For samples gotten from World Bank, helminth load was detected in six (6) out of the twelve (12) samples used.

Helminth load in cabbage from the different markets: Helminth load was detected in ten (10) out of the twelve (12) cabbage samples gotten from Eke-Ukwu while helminth load was detected in nine (9) out of the twelve (12) cabbage samples gotten from Relief market. For samples gotten from World Bank, helminth load was detected in five (5) out of the twelve (12) samples used.

Helminth load in cucumber from the different markets: Helminth load was detected in six (6) out of the twelve (12) cabbage samples gotten from Eke-Ukwu while helminth load was detected in seven (7) out of the twelve (12) cucumber samples gotten from Relief market. For samples gotten from World Bank, helminth load was detected in six (6) out of the twelve (12) samples used.

Helminth load in utazi leaf from the different markets : Helminth load was detected in four (4) out of the twelve (12) utazi leaf samples gotten from Eke-Ukwu while helminth load was detected in three (3) out of the twelve (12) utazi leaf samples gotten from Relief market. For samples gotten from World Bank, helminth load was detected in three (3) out of the twelve (12) samples used.

Table 1 shows the occurrence of soil-transmitted helminths in the vegetables from relief market. A total of 54 soil-transmitted helminths were detected in the vegetables comprising 22 *Ascaris lumbricoides*, 7 *Strongyloides stercolaris*, 4 *Necator americanus*, 7 *Ancylostoma duodenale* and 14 *Trichuris trichuria* were detected. Garden egg seeds, cucumber and cabbage had the highest *Ascaris lumbricoides* occurrence (18.2%), while lettuce had the highest *Strongyloides stercolaris* (57.1%). *Necator americanus* was only isolated from garden egg seeds (25.0%), cucumber (25.0%), carrot (25.0%) and cabbage (25.0%). Garden egg seeds (28.6%), lettuce (28.6%) and cabbage (28.6%) had the highest *Ancylostoma duodenale*

occurrence while garden egg seeds (21.4%) had the highest *Trichuris trichuria* occurrence.

Table 1: Occurrence of soil-transmitted helminths in the vegetables from Relief market

Helminths detected	Vegetables/number examined (%)							
	GES	LT	GEL	CC	CR	CB	UL	Total
Ascaris lumbricoides	4(18.2)	1(4.5)	4(18.2)	4(18.2)	3(13.6)	4(18.2)	2(9.1)	22(100.0)
Strongyloides stercolaris	0(0.0)	4(57.1)	2(28.6)	0(0.0)	0(0.0)	0(0.0)	1(14.3)	7(100.0)
Necator americanus	1(25.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	1(25.0)	0(0.0)	4(100.0)
Ancylostoma duodenale	2(28.6)	2(28.6)	0(0.0)	1(14.3)	0(0.0)	2(28.6)	0(0.0)	7(100.0)
Trichuris trichuria	3(21.4)	1(7.1)	2(14.3)	2(14.3)	2(14.3)	2(14.3)	2(14.3)	14(100.0)
Total	10(18.5)	8(14.8)	8(14.8)	8(14.8)	6(11.1)	10(18.5)	5(9.3)	54(100.0)

Key: GES = Garden egg seeds LT = Lettuce GEL = Garden egg leaf CC = Cucumber
CR = Carrot CB = Cabbage UL = Utazi leaf 0 = Not detected

Table 2 shows the occurrence of soil-transmitted helminths in the vegetables from Eke-Ukwu market. A total of 49 soil-transmitted helminths were detected in the vegetables comprising 26 *Ascaris lumbricoides*, 3 *Strongyloides stercolaris*, 4 *Necator americanus*, 5 *Ancylostoma duodenale* and 11 *Trichuris trichuria* were detected. Cabbage had the highest *Ascaris lumbricoides* occurrence 6(23.1%), while garden egg seeds 1(33.3%), garden egg leaf 1(33.3%) and cabbage 1(33.3%) had the highest *Strongyloides stercolaris.* Cabbage 2(50.0%) had the highest *Necator americanus*. *Ancylostoma duodenale* occurred in garden egg seeds 1(20.0%), garden egg leaf 1(20.0%), cucumber 1(20.0%), carrot 1(20.0%) and cabbage 1(20.0%) had the highest occurrence while cabbage 4(36.4%) had the highest *Trichuris trichuria* occurrence.

Table 2: Occurrence of soil-transmitted helminths in the vegetables from Eke-Ukwu market

Helminths detected	Vegetables/number examined (%)							
	GES	LT	GEL	CC	CR	CB	UL	Total
Ascaris lumbricoides	3(11.5)	2(7.7)	5(19.2)	3(11.5)	4(15.4)	6(23.1)	3(11.5)	26(100.0)
Strongyloides stercolaris	1(33.3)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	3(100.0)
Necator americanus	1(25.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	2(50.0)	0(0.0)	4(100.0)
Ancylostoma duodenale	1(20.0)	0(0.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)	0(0.0)	5(100.0)
Trichuris trichuria	0(0.0)	1(9.1)	2(18.2)	2(18.2)	1(9.1)	4(36.4)	1(9.1)	11(100.0)
Total	6(12.2)	3(6.1)	10(20.4)	10(20.4)	6(12.2)	14(28.6)	4(8.2)	49(100.0)

Key: GES = Garden egg seeds LT = Lettuce GEL = Garden egg leaf CC = Cucumber
CR = Carrot CB = Cabbage UL = Utazi leaf 0 = Not detecte

Table 3 shows the occurrence of soil-transmitted helminths in the vegetables from World Bank market. A total of 44 soil-transmitted helminths were detected in the vegetables comprising 18 *Ascaris lumbricoides*, 6 *Strongyloides stercolaris*, 3 *Necator americanus*, 6 *Ancylostoma duodenale* and 11 *Trichuris trichuria* were detected. Cabbage 4(22.2%) had the highest *Ascaris lumbricoides* occurrence, while lettuce 5(83.3%) had the highest *Strongyloides stercolaris.* Cabbage 1(33.3%), cucumber 1(33.3%) and carrot 1(33.3%) had the highest *Necator americanus*. *Ancylostoma duodenale* occurred highest in lettuce 2(33.3%) and cabbage 2(33.3%). Garden egg leaf 2(18.2%), cucumber 2(18.2%), carrot 2(18.2%) and cabbage 2(18.2%) had the highest *Trichuris trichuria* occurrence.

Table 3: Occurrence of soil-transmitted helminths in the vegetables from World Bank market

Helminths detected	Vegetables/number examined (%)							
	GES	LT	GEL	CC	CR	CB	UL	Total
Ascaris lumbricoides	2(11.1)	1(5.5)	3(16.7)	3(16.7)	3(16.7)	4(22.2)	2(11.1)	18(100.0)
Strongyloides stercolaris	1(16.7)	5(83.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100.0)
Necator americanus	0(0.0)	0(0.0)	0(0.0)	1(33.3)	1(33.3)	1(33.3)	0(0.0)	3(100.0)
Ancylostoma duodenale	0(0.0)	2(33.3)	1(16.7)	1(16.7)	0(0.0)	2(33.3)	0(0.0)	6(100.0)
Trichuris trichuria	1(9.1)	1(9.1)	2(18.2)	2(18.2)	2(18.2)	2(18.2)	1(9.1)	11(100.0)
Total	4(9.1)	9(20.4)	6(13.6)	7(15.9)	6(13.6)	9(20.4)	3(6.8)	44(100.0)

Key: GES = Garden egg seeds LT = Lettuce GEL = Garden egg leaf CC = Cucumber
CR = Carrot CB = Cabbage UL = Utazi leaf 0 = Not detected

Discussion

Helminth load recorded ranged from 1 to 4 parasites with cabbage having the highest helminth load while utazi leaf had the least helminth load. 22 *Ascaris lumbricoides*, 7 *Strongyloides stercolaris*, 4 *Necator americanus*, 7 *Ancylostoma duodenale* and 14 *Trichuris trichuria* were detected. Garden egg seeds, cucumber and cabbage had the highest *Ascaris lumbricoides* occurrence, while lettuce had the highest *Strongyloides stercolaris. Necator americanus* was only isolated from garden egg seeds, cucumber, carrot and cabbage. Garden egg seeds, lettuce and cabbage had the highest *Ancylostoma duodenale* occurrence while garden egg seeds had the highest *Trichuris trichuria* occurrence.

This result appears to agree with the work of Omowaye and Falola in which helminth diseases caused by *Ascaris* species and *Trichuris* species were endemic in populations that consume salad vegetables irrigated with raw or untreated sewage [10, 13]. Furthermore, the overall species of helminths identified in this examination is similar with the work of Elom et al in their study on the prevalence of geohelminths on edible fruits and vegetables cultivated in rural villages of Ebonyi State, South East, Nigeria [6]. This suggests a serious public health concern over the recurring prevalence of these helminths and the risk it pose to the health of the general populace.

The high occurrences of *Ascaris lumbricoides* could be as a result of the presence of a thick cell wall which make them resistant to adverse environmental conditions and provide them a long life span in the soil. Temperature, relative humidity and light affect the viability of eggs which, in temperate climates (10-15 ^{0}C) can be retained for two to three years and in tropical climates (20-30 ^{0}C) for ten to twelve months [17]. Elom et al. and Sunil et al. (2014) in their study showed that most ready-to-eat vegetables remain for about two to three days in their shops [6, 17]. During this period, there are chances that the vegetables may be contaminated due to handling by worm carriers or cross contamination from other vegetables. In the wholesale market, vegetables come in close contact with soil, which may also add to parasitic contamination.

Conclusion

Soil transmitted helminths (STHs) identified include *Ascaris lumbricoides*, *Trichuris trichuria*, *Strongyloides stercoralis*, *Necator americanus* and *Ancylostoma duodenale*. This

shows some poor level of adequate washing of these vegetables by farmers, wholesalers and retailers in different markets sampled. *Ascaris lumbricoides* was established to appear most in the samples that showed positive with helminth load. Vegetable vendors and users should adopt good hygienic practices to ensure that these vegetables are not contaminated with soil-transmitted helminthes, through ensuring they are adequately washed before sale and use by consumers in the preparation of vegetable salads. There is need to enlighten the general public on adequate washing of these vegetables before consumption as well as government providing inorganic fertilizers to the vegetable farmers in order to discourage the use of animal faeces as fertilizers.

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Authors' contributions:
OOG: Concept and Software Data Analysis.
UOG: Methodology and Supervision.
UWD: Proof Reading and Supervision.
CCI: Proof Reading and Review of manuscript
ILN: Drafting of Manuscript
UEA: Sample Collection
EAC: Literature review

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