Soil-Transmitted Helminths Contamination of Carrots and Cucumbers, and Molecular Identification of *Ascaris lumbricoides* in Jos Plateau State, Nigeria

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Abstract

Background: Soil-transmitted helminths (STHs) are group of parasitic nematodes transmitted through ingestion of eggs in contaminated food and water or through active skin penetration by an infective larva. **Aim:** This study determined the prevalence of STHs and molecular identification of *Ascaris lumbricoides* in fresh carrots and cucumbers in Jos Plateau State, Nigeria. **Materials and Method:** This was a cross-sectional study of carrots and cucumbers obtained from two vegetable markets and streets of Jos metropolis. About 200 g of each sample was weighed and washed separately in a sterile container using 200 ml of normal saline. This was allowed to sediment overnight, and the sediment was examined microscopically for eggs or larvae of parasites using ×10 and ×40 objectives of the light microscope. The positive samples for eggs of *A. lumbricoides* were subjected to molecular identification. The data obtained were computed using IBM SPSS software version 26. **Results:** The overall prevalence of STHs was 38.3% (138/360). The rate of contamination was higher in carrots with 45.0% (81/180) compared to 31.7% (57/180) of the total cucumbers examined. This was statistically significant ($P = 0.009$). The vegetables from the Farin-Gada market were the most contaminated, having a prevalence of 63 (52.5%), followed by the building Materials Market 50 (41.7%) and street traders 25 (20.8%). *Strongyloides stercoralis* was the most identified parasite totaling 69 (50.0%), followed by hookworm 51 (37.0%), *A. lumbricoides* 10 (7.2%), and *Trichuris trichiura* 8 (5.8%) being the least. **Conclusions:** There was a high contamination of carrots and cucumber studied in this research by STHs. Hence, there is a need for proper washing and possible decontamination before consumption to prevent food-borne infections.

Keywords: *Ascaris lumbricoides,* carrots, cucumbers, soil-transmitted helminths

Introduction

Soil-transmitted helminths (STHs) are a group of parasitic nematodes (worms), also known as intestinal helminths infecting both humans and animals through ingestion of contaminated food, water, or soiled hands.[1] The eggs of these parasites contaminate the environment through indiscriminate defecation, improper sewage disposal, and infected animal dung used as manure, and remain viable in warm, moist soil for several weeks.[2] It is common for individuals living in areas without potable water supply, poor health facilities, and environmental hygiene to be chronically infected with two or more STHs such as *Ascaris lumbricoides* (roundworm)*, T. trichiura* (whipworm)*,* hookworms (*Ancylostoma duodenale* and *Necator americanus*), and *Strongyloides stercoralis*.[3] Aside from the fecal–oral route of transmission, as in the case of roundworms, transmission can also occur through active skin penetration of an infective larva such as hookworms and *S. stercoralis.*[4]

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Contaminated soil is one of the major reservoirs of helminth eggs, as it plays a major role in the distribution of STHs.[5] Black soils normally used as organic manure by farmers to increase plant yield, are sometimes contaminated with eggs of intestinal helminths, which eventually contaminate water or vegetables from which humans get infected.[6] STHs infection affects more than one billion people worldwide,[7] and infection is prevalent in some parts of Nigeria due to the poor socioeconomic condition of the people.[8] In addition, some parts of sub-Saharan Africa, America, China, and East Asia have been reported as areas with high STHs infections.[1]

Fruits and vegetables are an important part of the human dietary requirement providing essential micronutrients to man.[9] These vegetables, such as carrots and cucumbers, are rich in vitamins and dietary minerals.[10] Equally, vegetables contain phytochemicals that may have antioxidant, antibacterial, antifungal, and antiviral properties that help to guard against oxidative stress and boost immunity in humans.[11] Although the benefits of these vegetables cannot be overemphasized, the consumption of raw or improperly washed vegetables may serve as an important source of transmission of STHs infection, and such practice should be discouraged.[12]

Peasant farmers are largely involved in the cultivation of fruits and vegetables in Nigeria and most African countries. Their activities are mostly carried out on the same piece of land, and the continued usage of the land can lead to depletion of soil nutrients. Hence, the need for manure to enrich the land may contaminate the soil with various species of parasites that are detrimental to human health.[13] The indiscriminate, open defecation in bushes, and on cultivated farmlands contribute significantly to parasitic contaminations of vegetables that are instrumental in the transmission of STHs.[14] Hence, this study aimed to determine the prevalence of STHs and molecular identification of *A. lumbricoides* in fresh carrots and cucumbers in Jos Plateau State, Nigeria.

Materials and Methods

Study area

This study was conducted in two vegetable markets (Farin-Gada market and Building Materials Market) and the major streets within Jos metropolis, the capital of Plateau State, Nigeria. Jos lies on an average of 1300 m above sea level with a mean annual rainfall of 1400 mm, making the weather favorable for fruits and vegetable cultivation.[15] Farin-Gada market is a major vegetables market on the northern part of the Jos metropolis along the Farin-Gada road linking the northern states. The Building Materials Market, on the other hand, is located in the southern part of the city along a major road linking the state with Nasarawa State. These markets receive the supply of fruits and vegetables from different parts of the state and the neighboring states in North Central Nigeria and also serve as a major supplier of vegetables to the southern parts of the country.

Study design and sample size determination

The study was a descriptive cross-sectional study of 360 samples of carrots and cucumbers selected from the traders using a nonprobability sampling technique due to how the markets are structured. The sample size was determined using the formula provided by Kirby and Gebski, 2002, at a 95% confidence interval and a 5% expected error of margin. A local prevalence of 36% obtained from a previous study by Damen *et al*., 2007, was used.[14]

Sample collection and processing

Samples of fresh carrots and cucumbers were collected from the two markets and streets of the Jos metropolis. The samples were collected in a different well-labelled sterile polythene bag and taken to the Medical Microbiology Laboratory of the University of Jos for parasitological analysis within 30 min of collection. This process was repeated on each market day until 360 samples were collected from the three selected areas while avoiding multiple sampling from the same trader. The samples were processed by weighing 200 g of each sample and washed separately in a well-labelled, sterile wide open-mouthed container using 200 ml of normal saline to separate the ova or larvae of STHs as described by Bekele *et al*.[16] After overnight sedimentation of the washed solution, the supernatant was decanted. The sediment was remixed, and 15 ml was transferred to a centrifuge tube using a sterile sieve to remove undesirable matters. The preparation was centrifuged at 5000 × *g* rpm for 5 min. The supernatant was decanted without shaking, and the sediment was agitated gently by tapping the centrifuge tube to redistribute the parasitic stages of the helminths. A drop of the sediment was placed on a clean, grease-free microscopic slide and stained with Lugol's iodine, after which a clean coverslip was placed gently over it to avoid air bubbles. The preparation was examined using × 10 and × 40 objective lenses of the light microscope. The ova and larvae of STHs were identified by comparing their morphological features with that of a standard Color Atlas of Parasitology, and the results were recorded.

Deoxyribonucleic acid extraction

Deoxyribonucleic acid (DNA) extraction from samples positive for ova of *Ascaris* was carried out using Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research Corporation, South Africa), following the manufacturer's recommendations with a little modification.[17] The eluted DNA was transferred to a prepared Zymo-Spin™ III-HRC filter in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 16,000 g for 3 min. The filtered DNA was now suitable for polymerase chain reaction (PCR) and other downstream applications.[18] The eluted DNA was stored at − 20°C until it was used for PCR.

Amplification reactions

The molecular target for *Ascaris* PCR was done by amplifying a 142 bp fragment of the cytochrome b (*Cyt* b) mitochondrial gene. This was done using the following primers set,

Asc1 (5'GTTAGGTTACCGTCTAGTAAGG-3')-forward and

Asc2 (5' CACTCAAAAAGGCCAAAGCACC-3')-reversed.

All PCR reactions were performed in a 50 μL mixture containing 25 μL One Taq® Quick-Load® 2X Master Mix with Standard Buffer (20 mMTris-HCL (pH 8.9), 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCL, 0.2Mm dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween®20, Xylene cyanol FF, Tartrazine, 25 units/ml one *Taq* DNA Polymerase. 16 μL molecular grade water, 2 μL each of forward and reverse primer, and 5 μL of extracted DNA was added to the Master Mix. The PCR thermal cycling conditions were as follows: denaturing step 1 at 94°C for 10 min, followed by 50 cycles of denaturation at 94°C for 1 min, annealing at 55C for 45s. an elongation at 72°C for 45s, while the final extension was performed at 72°C for 5 min according to the method described by Odile *et al*.[19] The amplicon was held at 4°C until analyzed by electrophoresis on 2% agarose gel.

Agarose gel electrophoresis

Approximately 2 g of agarose gel was weighed and mixed with 100 ml of Tris-acetate-EDTA buffer in a conical flask and was heated in a microwave for 5 min at 15°C and allowed to cool. About 1.5 μl of ethidium bromide solution was added. The mixture was poured into a gel casting glass, and combs were carefully removed, and the cast was then placed into the electrophoretic tank. Loading buffer of 1 μl was mixed with 8 μl of amplicon in a loading tray and was dispensed into the various wells in the gel. Eight (8 μl) of 100 bp ladder was placed in the 1st well. The gel was then run at 120 volts for 40 min, and the result was visualized under an ultraviolet transilluminator, and amplicons of 142 bp were considered positive[17] [Figure 1].

Data analysis

Data obtained from this study were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0. (Armonk, NY: IBM Corp) and presented in tables, charts, and figures.

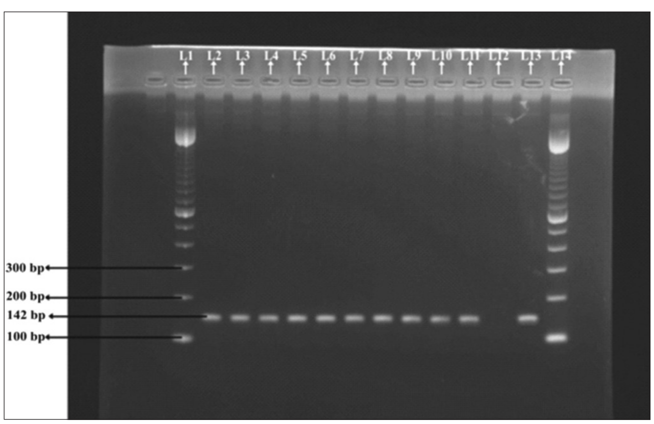

Figure 1: The gel electrophoresis of the PCR product after amplification of cytochrome b gene fragment from DNA of *Ascaris lumbricoides* eggs. PCR: Polymerase chain reaction, DNA: Deoxyribonucleic acid
Key: Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 = DNA bands from test samples. Lanes 12 and 13 = Negative and positive controls, respectively. Lanes 1 and 14 = 100bp DNA molecular ladder, Cytochrome b gene = 142bp size DNA.

Results

The overall rate of STHs detected was 138 (38.3%). The highest rate of contamination was found in carrots, with 81 (45.0%), while cucumbers were contaminated in 57 (31.7%) of the samples. The difference in parasitic contamination of carrots and cucumbers was statistically significant at $P = 0.009$ [Table 1].

There was a significant difference in the rate of parasitic contamination of vegetables sold at the various markets at $P < 0.001$ [Table 2]. The highest parasitic contamination occurred in vegetables from the Farin-Gada market with a prevalence of 63 (52.5%), followed by the Building Materials Market with 50 (41.7%), while street markets had the least contamination of 25 (20.8%). The unwashed vegetables demonstrated more parasitic contamination than the washed vegetables. The prevalence was 117 (65.0%) in unwashed vegetables compared to 21 (11.7%) in the washed vegetables. This was statistically significant at $P < 0.001$ [Table 3].

Of the parasites identified, *S. stercoralis*, 69 (50.0%), was the most prevalent parasite observed among the vegetables, followed by hookworm, 51 (37.0%). *A. lumbricoides* were observed in 10 (7.2%), while *T. trichiura* was responsible for 8 (5.8%) of the contamination [Figure 2].

Table 4 displays the association of STHs with potential risk factors and knowledge of STHs among the traders. Parasitic contamination was predominant in vegetables obtained from trades who had no formal education, 97 (60.6%). This was followed by secondary education with 15 (28.9%), while those

Table 1: Prevalence of soil-transmitted helminths on carrots and cucumbers

Vegetables	Number of examined	Number of positive	Percentage prevalence
Carrots	180	81	45.0
Cucumbers	180	57	31.7
Total	360	138	38.3

χ^2=6.7698, P=0.009, df=1

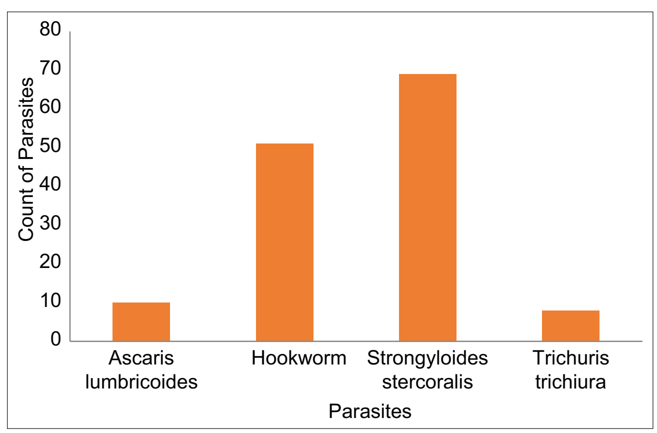

Figure 2: Distribution of soil-transmitted helminths recovered from contaminated carrots and cucumbers sold at various markets in Jos and environs

Table 2: Prevalence of soil-transmitted helminths according to the selected markets

Market	Number of examined	Number of positive	Percentage prevalence
Farin-Gada	120	63	52.5
Building Materials	120	50	41.7
Streets	120	25	20.8
Total	360	138	38.3

χ^2=26.298, P<0.001, df=2

Table 3: Comparison of soil-transmitted helminths contamination rate between washed and unwashed vegetables

Vegetable	Number of examined	Number of positive	Percentage prevalence
Washed	180	21	11.7
Unwashed	180	117	65.0
Total	360	138	38.3

χ^2=108.296, P<0.001, df=1

Table 4: Distribution of soil-transmitted helminths in relation to risk factors

Risks factors	Number of tested	Number of positive, *n* (%)	$\chi 2$	*P*
Educational level				
Nonformal	160	97 (60.6)	62.872	<0.001
Primary	138	25 (18.1)		
Secondary	52	15 (28.9)		
Tertiary	10	1 (10.0)		
Awareness of STH				
Yes	165	44 (26.7)	17.540	<0.001
No	195	94 (48.2)		
MOD				
Fruits and vegetables	60	25 (41.7)	21.568	<0.001
Soiled hands	25	19 (76.0)		
Contaminated water	43	15 (34.9)		
Skin penetration	7	5 (71.4)		
No idea	225	74 (32.9)		
Sources of water				
Stream	120	18 (15.0)	4.556	0.102
Well water	41	3 (7.3)		
Tap water/borehole	19	0 (0.0)		

MOD: Mode of transmission, STH: Soil-transmitted helminth

with primary education had a contamination rate of 25 (18.1%). Those with tertiary education had the least contamination rate of 1 (10.0%). This may be possible because only a few numbers were examined among this group of traders. This was statistically significant at $P < 0.001$. It was observed that 165 of the traders were aware of STHs and 44 (26.7%) had their vegetables contaminated, while 195 of the traders were ignorant of STHs, with 94 (48.2%) of the vegetables sampled from them being positive for STHs. This was statistically significant at $P < 0.001$.

In terms of the mode of transmission, the traders were asked if they were aware of the different ways parasites can be transmitted, and the result was tabulated. Sixty traders knew that parasites can be transmitted through contaminated fruits and vegetables and had a contamination rate of 25 (41.7%). Of the 25 traders who believed STHs can be transmitted through soiled hands, 19 (76.0%) vegetables examined from this group were positive for STHs. Forty-three vegetable traders believed that STHs are transmitted through contaminated water, and of this number, 15 (34.9%) had their vegetables contaminated with STHs. Only seven traders were aware that active skin penetration is a mode of transmission of STHs, and 5 (71.4%) vegetables examined from this group were positive for STHs. A large proportion of the traders (225) were ignorant of the mode of transmission of parasitic infections and 74 (32.9.1%) of their sampled vegetables were positive for STHs. This relationship between the knowledge of the mode of transmission of STHs and the rate of contamination was statistically significant [Table 4] ($\chi^2 = 21.568$, $P < 0.001$).

Although this was not statistically significant [Table 4] ($X = 4.556$, $P = 0.102$), it was observed that of the 180 samples of washed vegetables, 120 were washed with stream water; of which 18 (15.0%) were positive for STHs. Forty-one were washed with water from the well, and 3 (7.3%) were contaminated with STHs, while there were no parasites observed in the 19 vegetables washed with water from a borehole.

The positive samples for *A. lumbricoides* were subjected to molecular confirmation. DNA extraction was done on all the *Ascaris*-positive samples, and amplification was done using conventional PCR. The positive samples were confirmed to be that of *A. lumbricoides* and not of other species of *Ascaris,* as shown in Figure 1.

Discussion

STHs infections are major public health problems in poor and developing countries where improper sewage disposal and open defecation are still practiced. In this study, the prevalence of STHs contamination of cucumbers and carrots was 38.3%. This result was comparable to a previous study in Jos,[14] where a prevalence rate of 36.0% was reported in 2007 among carrots, tomatoes, lettuce, cabbage, and green leafy vegetables. Similarly, the contamination rate in this study corroborated a study conducted in Iraq that reported a contamination rate of 34.2% and 35% in vegetables and fruits, respectively.[20] In contrast, a study in 2015 reported a higher prevalence of 55.0% among vegetables sold in two selected markets in the southern part of Jos metropolis.

In other parts of the world, various contamination rates have been reported in different countries. While countries such as Sudan and Egypt have reported lower contamination rates of 13.5%, 16.0%, and 29.6%, respectively,[21,22] a higher contamination rate of 75.9% has been reported in Kenya.[23] The variation in contamination rates across different countries maybe because the researchers studied different vegetables and fruits, and the contamination rate could vary from one vegetable and fruit to the other. Another explanation for this could be attributed to variations in climatic conditions, types of soil, sources of water, and manure used for the cultivation of these vegetables and fruits, which could serve as a source of contamination. The handling of the fruits and vegetables by the sellers, as well as the open market space and proximity to the ground/soil, could be a contributing factor to contamination.

The carrot was found to be the most contaminated at 45.0% compared to 31.7% in cucumber. This is corroborated by research conducted in Southwest Nigeria among five selected vegetables in which no parasites were found on 50 samples of cucumber, but cabbage, carrot, lettuce, and leafy green vegetables.[24] The reason given for this was that carrots and other vegetables have uneven surfaces, which make parasitic eggs and larvae attach to them more easily, while cucumbers have smooth surfaces and easy to wash off parasitic contamination.

Farin-Gada market recorded the highest contamination rate of 52.5%, followed by the Building Materials Market (41.7%) and roadside traders (20.8%). This variation in parasitic contamination from different sampling sites could be, in part, to the hygiene of the traders and the environmental condition of the markets. For instance, the Farin-Gada market is open to customers every day of the week with high human traffic. There is a stream just behind the Farin-Gada market with polluted water and a dumping site which is also used for open defecation; this can serve as a source of parasitic contamination of vegetables at the market. The low contamination rate recorded from the street sellers maybe because these are hawkers with fewer products usually well washed and sometimes tied in polythene bags. The streets are also neater and not as crowded as the market environment.

It was observed that unwashed vegetables had higher contamination rates (65.0%) than washed vegetables (11.7%). This is not surprising as the unwashed vegetables were still stained with the farm soil and may either have been contaminated right on the farm, on transit, or at the market during handling.

The consumption of raw carrots and cucumbers is a common human practice, but in locations where parasitic contamination of these vegetables is prevalent, many people are at risk of human infections. This buttresses the need for thorough washing of these nutritious vegetables before consumption. The interest in vegetable quality concerning helminth contamination has always been the target of many research studies to create awareness about the role of contaminated vegetables in parasitic infections.[14,25]

Apart from *S. stercoralis,* which was reported as the highest parasitic contaminant in this study (50.0%), hookworm (37.0%), *A. lumbricoides* (7.2%), and *T. trichiura* were also detected [Figure 2]. This is quite different from other studies where *A. lumbricoides* were reported as the most common parasite contaminating vegetables.[26,27] *A. lumbricoides have* also been reported as the most prevalent STHs in human infections in Jos.[28] Consequently, one should probably expect that *A. lumbricoides* should be the most prevalent in vegetables. An explanation for this could be attributable to the complex lifecycle of *S. stercoralis*. Its ability to exhibit both parasitic and free-living states could enhance the proliferation of larvae in the soil. These variations could also be as a result of research methodology, geographical location of the study, and the expertise of the researcher in differentiating the different parasites on microscopy. The high rate of *S. stercoralis* contamination might be attributed to its complex life cycle with a free-living stage in the soil that does not require a host for its proliferation.

The risk factors analyzed in this study were significantly associated with STHs contamination of carrots and cucumbers [Table 4]. The highest contamination rate was found in vegetables obtained from traders with no formal education, and the least among traders with tertiary education. Similarly, on awareness of STHs, those with no knowledge of STHs and their mode of transmission had the highest rate of contamination. Although only a few of the traders had tertiary education, this probably suggests that the educated traders were more conscious about their hygiene and probably more careful in handling their vegetables. This buttresses the need for education in controlling the rate of contamination of these vegetables.

In this study, majority of the traders used stream water to wash their vegetables due to its easy accessibility, and their vegetables were the most contaminated. Those that used tap/borehole water had the least rate of parasitic contamination. Although this was not statistically significant, it emphasizes the importance of using clean water in washing vegetables before consumption or selling to end users. This was corroborated by Campbell *et al*. when they analyzed the significance of water, sanitation, and hygiene in the control of parasitic infections.[29]

The absence of any morphological characteristic to distinguish *A. lumbricoides* eggs (parasites of humans) from *Ascaris suum* eggs (parasite of swine) makes it difficult to microscopically differentiate these parasites in the contaminated vegetables. Since farmers used manure from cows, pigs, and chicken droppings to enrich their soil, contamination with *A. suum* from pigs becomes inevitable. To rule out that possibility and confirm the eggs of *Ascaris*, the positive samples for *Ascaris* were subjected to molecular studies using specific primers for *A. lumbricoides,* and all the *Ascaris* eggs were confirmed to be that of *A. lumbricoides* [Figure 1]. This is essential to provide information on whether pig manure was a source of contamination of the vegetables in this study to suggest effective control measures.

Conclusions

There is high contamination of carrots and cucumber studied in this research by STHs. Hence, there is a need for proper washing and possible decontamination before consumption to prevent food-borne infections.

Consent for publication

All the authors reviewed and gave their approval for this article to be submitted for publication.

Limitations to the study

1. The microscopy could be affected by an observer error. To overcome this, a Standard Atlas was used to identify the parasites. Furthermore, the microscopy was reviewed by three independent microscopists
2. Sequencing of the parasites was not done due to financial constraints.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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