Research Article

Incidence of *Strongyloides stercoralis* in Edible Vegetables of Port Harcourt Metropolis, Nigeria

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Abstract | Background: *Strongyloides stercoralis* is a nematode parasite causing the disease condition referred to as strongyloidiasis in man. Part of its life cycle is spent on the soil qualifying it as a geo-helminth. This research examined the geo-helminth species associated with selected edible vegetables from markets and farms in Port Harcourt metropolis, Nigeria. Methodology: Vegetables (fluted pumpkin leaves, waterleaves, bitter leaves and scent leaves) were purchased and harvested from selected markets (Creek Road, Mile 1, Mile 3, Timber, Rumuokoro, and Rumuokuta Markets) and farms (Rivers State University Agricultural Demonstration Farm, a farm at Nkpolu-Oroworukwo and another at Rumuolumeni) in Port Harcourt metropolis in November and December, 2021. Samples were taken biweekly and in two replicates. They were transported in clean waterproof bags to the Entomology and Parasitology Laboratory, Rivers State University, Port Harcourt, Nigeria. 200g of each sample was washed in 200ml of 0.85% normal saline solution and taken through standard sedimentation procedures. The sediment recovered after centrifugation was applied on a grease-free microscope slide; a drop or two of Lugol's iodine was added to it. This was then covered with a cover slip and viewed under the light microscope at x4 and x10 objective lens. Results: Only *S. stercoralis* was isolated from the samples examined. Water leaves (50%) and pumpkin leaves (11%) were infected. Bitter leaves and scent leaves were uninfected. The parasite was encountered in all locations except Creek Road market and the farm at Rumuolumeni. Statistical analysis: Prevalence of infection was computed following standard formula. Recent finding and conclusion: It is concluded that waterleaves sold in and harvested from the selected locations are commonly infected with *S. stercoralis* larvae. It is hence recommended that special care be taken in the cleaning and washing of this vegetable, possibly with saline water, to limit the chances of human infection.

Keywords | Strongyloidiasis, Geo-helminths, Edible vegetables, Immunocompromised.

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INTRODUCTION

Strongyloides stercoralis is a geohelminth of public health importance especially in tropical and subtropical regions. It undergoes a complex lifecycle alternating free-living and parasitic stages, and causes the intestinal disease, strongyloidiasis (Mansfield et al., 1995; Greaves et al., 2013). The infection is often asymptomatic but it can progress in immunocompromised individuals to a fatal hyper-infection syndrome. Autoinfection is also common with *S. stercoralis*

(Keiser and Nutman, 2020) as a result of which the infection can persist indefinitely in the host if left untreated. The nematode is often encountered in parasitological examinations of fruits and vegetables in Nigeria (Uneke and Udegbulam, 2015; Ejike et al., 2018; Agbalaka et al., 2019). In fact, Agbalaka et al. (2019) reported *S. stercoralis* to be of the highest prevalence amongst the geohelminths isolated from vegetables purchased from locations in Jos, Plateau State, Nigeria. The following geohelminths are also frequently encountered in such surveys: *Ascaris lumbricoides*, hookworm species (*Ancylostoma duodenale* and *Necator americanus*), *Enterobius vermicularis* and *Trichuris trichiura* (Akoma et al., 2017; Ejike et al., 2018; Agbalaka et al., 2019). However, a report by Karshima (2018) revealed *Ancylostoma duodenale* and *Ascaris lumbricoides* to have the widest distribution on vegetables sold in Nigeria markets.
Vegetables are regular components of food, especially for their nutritional benefits. They provide the body with fiber, vitamins, minerals and phytochemicals which play beneficial roles such as detoxicants in man (Slavin and Lloyd, 2012). It is recognized that regular consumption of vegetables reduces the risk of cancer in several organs (Steinmetz and Potter, 1991). However, some research has shown that vegetables can be contaminated with heavy metals and become a health risk (Otitoju et al., 2012; Ogunkunle et al., 2014; Edogbo et al., 2020). As already stated, they could also harbor infective stages of helminth parasites and pathogenic microorganisms that could be injurious to man (Aboh et al., 2011).

This research examined readily available vegetables such as fluted pumpkin leaves (*Telfairia occidentalis*), waterleaves (*Talinum fruticosum* previously known as *Talinum triangulare*), bitter leaves (*Vernonia amygdalina*) and scent leaves (*Ocimum gratissimum*) for geo-helminth infections. It was aimed at recovering and identifying geo-helminth parasite species associated with the vegetables and quantifying their prevalence of infection.

MATERIALS AND METHODS

Sample collection

Bi-weekly collections of edible vegetables were made from nine locations in Port Harcourt metropolis, Nigeria, from November to December, 2021. The vegetables included fluted pumpkin leaves (*Telfairia occidentalis*), waterleaves (*Talinum fruticosum* previously known as *Talinum triangulare*), bitter leaves (*Vernonia amygdalina*), and scent leaves (*Ocimum gratissimum*).

The locations included six markets (Rumuokuta [4°46'38.71" N and 7°00'48.24" E], Timber- Diobu [4°79'94.36" N and 7°00'39.51" E], Creek Road- Town [N 04° 45'29", E007° 01'15"], Mile 3 [4°46'12.796"N and 6°58'24.4866"E], Mile 1 [4°79'19.334"N and 6°99'79.326"E] and Rumuokoro [4.8664° N and 6.9991° E] markets) and three farms (Rivers State University Agricultural Demonstration Farm [6°58'37.74 E and 4°48'1.03212 N], a farm at Nkpolu-Oroworukwo [6°58'37.04 E and 4°48'1.03210 N], and another at Rumuolumeni [6° 56' 31. 938" E and 4° 49'7.84224" N]). Three vegetables were examined from each location: pumpkin leaves, waterleaves and bitter leaves were collected from each of the six markets; while pumpkin leaves, waterleaves and scent leaves were collected from each of the three farms investigated.

Sample Preparation (Sedimentation Technique)

Samples were transported in sealed polythene bags to the Entomology and Parasitology Laboratory, Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria. They were weighed using an electronic weighing balance (Denver instrument, model TP-512A) to a mass of 200g for each sample. Samples were prepared in duplicates and results were pooled for both replicates of each sample.

Each sample was then washed in 200ml of 0.85% normal saline solution. Fragments of the vegetables were removed from the solution using clean forceps after which the solution was left to stand for 24 hours in properly labelled containers to allow for sedimentation (Damen et al., 2007; Arora and Arora, 2010). The following day, the supernatant was decanted from each sample; sediments were sieved using a net gauge of 0.5mm mesh size and 2ml of this filtrate was filled into a labeled test tube. When this had been accomplished for each sample, they were centrifuged at 3000rpm for 15minutes in a bucket centrifuge (Cheesebrough, 2006). Afterwards, excess fluid in each test tube was decanted, one to two drops of the deposit, after re-agitation, were placed on a clean grease free microscope slide; two drops of Lugol's iodine were added, and covered with a cover slip avoiding air bubbles. The slides were examined under a light microscope at magnifications x10 and x40. This was repeated twice for each sample. Identification was done after Cheesebrough (2006). Photomicrographs of parasites were taken with a Nikon digital camera attached to the objective lens of the microscope.

Statistical Analysis

Prevalence was computed as the result from the division of the number of samples infected by the total number of samples examined expressed as a percentage (Bush et al., 1997).

RESULTS

Four edible vegetables obtained from six markets and three farms within Port Harcourt metropolis, Nigeria, were

Table 1: Total number of *Strongyloides stercoralis* isolated from the edible vegetables, Port Harcourt Metropolis, Nigeria

Vegetables	Parasites Isolated	Number of Parasites	Number of samples examine	Number of samples infected	Prevalence (%)
Water leaves	*Strongyloides stercoralis*	18	18	9	50.0
Bitter leaves	-	-	18	0	0
Fluted pumpkin leaves	*Strongyloides stercoralis*	2	18	2	11.1
Scent leaves	-	-	18	0	0

examined in the course of this research. Throughout the course of the research, only the larvae of *Strongyloides stercoralis* (Figure 1) was isolated from these vegetables.

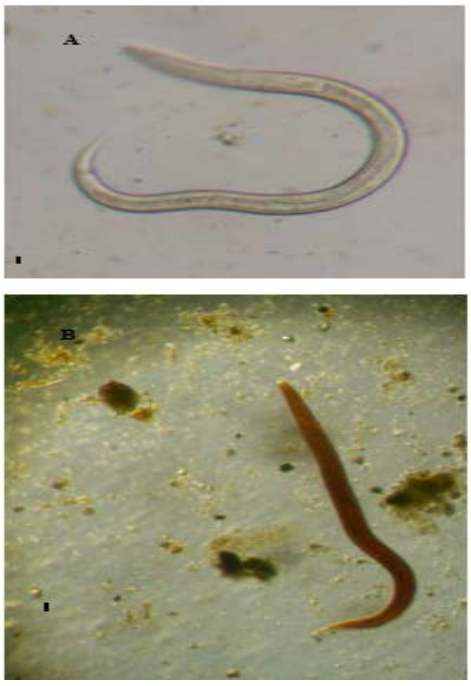

Figure 1: Unstained (A) and Stained (B) *Strongyloides stercoralis* isolated from waterleaves, Mile 3 Market, Port Harcourt, Nigeria (x10 objective lens; scale: 0.5mm).

These larvae were isolated from all locations except for Creek Road market and the farm at Rumuolumeni. In November, waterleaf samples from all locations were infected and in December, both waterleaves and fluted pumpkin leaves obtained from Mile 3 and Rumuokoro markets were infected while the other vegetables were free of geohelminths. Parasites were not encountered in bitter leaves and scent leaves.

Among samples examined from all locations, waterleaves were found to be most infected throughout the period of investigation. Generally, eighteen specimens of *S. stercoralis* were isolated from waterleaves, two from fluted pumpkin and none from either scent leaves or bitter leaves (Table 1). Of the eighteen samples of waterleaves examined, nine were infected accounting for a prevalence of 50.0%. On the other hand, only two of the eighteen samples of fluted pumpkin leaves were infected accounting for a prevalence of 11.1%.

DISCUSSION

Strongyloides stercoralis has been reported from several researches on geohelminths of edible vegetables in Nigeria (Uneke and Udegbulam, 2015; Karshima, 2018; Agbalaka et al., 2019). In this research, waterleaves, pumpkin leaves, bitter leaves and scent leaves were examined but the waterleaves were most infected. Some authors also reported very high prevalence of geo-helminths on waterleaves. For instance, Ejike et al. (2018) observed these vegetables to have the highest helminth contamination among the fruits and vegetables they examined which included Pumpkin leaf (*Telfairia occidentalis*), water leaf (*Talinum fruticosum*), scent leaf (*Ocimum gratissimum*), carrot (*Daucus carota*), cabbage (*Brassica oleracea*) etc. These authors isolated the following helminths from waterleaves: *Ascaris lumbricoides*, *Trichuris trichiura*, *S. stercoralis* and hookworm. In their research, however, *A. lumbricoides* (with a prevalence of 28.9%) had the highest rate of occurrence. Other researchers however, reported higher occurrence of parasites on other vegetables such as green leaf (*Amaranthus spinosus*) (Uneke and Udegbulam, 2015) and pumpkin leaves (Akoma et al., 2017).

The prevalence of *S. stercoralis* and pronounced susceptibility of *Talinum fruticosum* in this study is made possible by the stage of infection, and surface texture and high moisture content of the *T. fructicosum* leaves. Eggs of these parasites could easily be washed off by the traders but the larval stages could still be anchored to the vegetables because of their adhering structures.

Though strongyloidiasis can be asymptomatic, the number of immunocompromised persons in the world has led to worries of this parasitic disease becoming a major public health concern (Keiser and Nutman, 2020). Acute and hyper-infections can be fatal involving several organs of the body (Schär et al., 2013), and are often confused for other diseases. This would be of major concern in several impoverished communities where diagnosis and treatment facilities are unavailable. It is therefore of great public health importance to control transmission of *S. stercoralis* especially among high risk individuals.

Control of the transmission of this helminth parasite would require provision of adequate toilet facilities to discourage disposal of faecal matter on farm lands. Through public enlightenment campaigns, rural farmers should be discouraged from the use of untreated faecal matter as bio-fertilizers (Uneke and Udegbunam, 2015). Regular use of foot wears on farms and markets is also advocated. In addition, medical laboratory scientists should improve on the diagnosis of this infection as its prevalence in the society may be highly underestimated.

This research has shown *S. stercoralis* to be a prevalent geohelminth in waterleaf plants (*Talinum fruticosum*) sold and farmed in the locations examined in Port Harcourt, Nigeria. It is hence recommended that special care be taken in the cleaning and washing of this vegetable, possibly with saline water, to limit the chances of human infection through skin penetration.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

NOVELTY STATEMENT

This research has highlighted the possible emergence of strongyloidiasis as an important public health risk among inhabitants of the study location.

AUTHORS CONTRIBUTION

CCA designed, supervised the research and drafted the initial manuscript; other authors were involved in specimen collection and laboratory work; BR proofread the first draft suggesting corrections that improved on the quality of the manuscript.

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