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Archives of Applied Science Research, 2013, 5 (6):41-48
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Examination of soil samples for the incidence of geohelminth parasites in Ebonyi north-central area of Ebonyi State, south-east of Nigeria

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ABSTRACT

Examination of soil samples for the incidence of geohelminth parasites was conducted in urban and periurban areas of Ebonyi State between July – December, 2011. Three hundred (300) soil samples were obtained from five different locations (Nwofe-Agbaja, Onu-ebonyi area, Ntezi-abba, Umuogharu and Ezzagu) where vegetables and fruits are grown and accessed for the presence of geohelminthic eggs and larvae using a modified Cobb's decanting and sieving methods. The results showed that 92 (30.7%) of soil samples were positive for different species of the parasites. Six soil transmitted helminthes were implicated for soil samples, namely, Hookworms 19 (6.3%), Ascaris lumbricoides 24 (8.0%), Strongloides stercoralis 17 (5.7%), Trichuris trichiura 14 (4.7%), Enterobius vermicularis 12 (4.0%) and Hymenolepis nana 6 (2.0%). Soil samples from Umuogharu was most contaminated 22 (7.3%), while the lowest was from the Ezzagu 15 (5.0%). The Prevalence by seasons (dry and wet), showed that dry season had higher contamination, 56 (18.7%) while wet season recorded 36 (`12.0%). However, the result was found not statistically significance (P> 0.05). The Incidence by the soil type showed that the proportion of ova recovered were 22 (7.3%) for loamy, 19 (6.3%) for sandy and 13 (4.3%) for clayey soil, while the proportion of larvae were 17 (5.7%) for loamy, 12 (4.0%) for sandy and 9 (3.0%) for clayey soils. The work highlighted the public health importance of consumption of these fruits and vegetables grown on faecally polluted environment, especially when not properly prepared. The results also indicated the presence of these parasites in the soil, hence, improper disposal of human faeces, insufficient supplies of potable water, as well as poor personal hygiene are the key factors for the transmission of these parasites.

Keywords: Geohelminths, contamination, public health importance and soil.

INTRODUCTION

In a poor and developing countries of the world, geohelminths (soil-transmitted helminthes) infections represent a major public health problem and have constituted universal burden which does not depend on regional ecological conditions but also on local standard of social and economic development of the people (33, 37 and 7). Geohelminths are group of nematode parasites with an essential phase of their asexual life cycle in the soil and there is a period persistence in the soil during which the infective stages are protected and preserved (6, 3). Geohelminth infections are most prevalent in area, where adequate water and good sanitations are lacking (14 and 3). Recent estimates suggest that *Ascaris lumbricoides* infect over 1 billion people, 770 million with *Trichuris trichuira* and 800 million with hookworms (*Necator americanus* and *Ancylostoma duodenale*) (40 and 20). However, in many species of soil helminthes that infect humans, five nematodes in particular stand out because of their wide spread, prevalence and distribution that result in hundreds of millions of human infections. These include the large roundworms, *Ascaris lumbricoides,* the whipworm, *Trichuris trichiura, Strongyloides stercoralis* and two species of hookworms, (*Necator americanus* and *Ancylostoma duodenale*) and *Enterobius vermicularis.* Three of these (*Ascaris lumbricoides, Trichuris trichiura* and *Enterobius vermicularis)* enter the body through the mouth in the form of ova, which will later hatch into larvae; while hookworms and *Strongyloides stercoralis* access the body by larval penetrationof the skin (38 and 18).The public health importance of soil transmitted helminthic infections ranked highest in morbidity rate among school aged children who often present much heavy worm infections because of their vulnerability to nutritional deficiency and close contact with soil (7). These infections have been shown to impact negatively on the physical fitness and cognitive performance of the pupils (3). Intestinal obstruction or rectal prolapse, granuloma, intense malnutrition, iron-deficiency, anaemia, morbidity, mortality, dysentery syndrome, fever, dehydration, vomiting and colitis are the major complications associated with soil transmitted helminthic infections (10). It is well established that indiscriminate disposal of human and animals' faeces, poor personal hygiene and inadequate water supply contribute to high levels of soil transmitted helminthic infections (8 and 32). Soil-transmitted helminthes are some of the most common and infective agents of mankind and are responsible for high morbidity throughout the developing world. This is of great importance in health of many populations in third world countries where illiteracy, poverty and associated poor environmental sanitation practices have been implicated in the heavy burden of helminthiasis among children (34 and 39). Nigeria like other developing countries is faced with the dilemma of inadequate disposal of excreta-related human waste discharged into the environment. Thus, the area of this study in rural farming communities of Ebonyi state, it has been a routine practice defecating on open fields and farmlands thereby giving a stable soil contamination with *Ascaris* eggs throughout the year as it concerns open and indiscriminate defecation. (16) reported high rate of helminthic infections among people without toilet facilities in Lagos State, Nigeria. Contaminations of soil with parasite eggs, thus, constitute a most important risk factor for soil transmitted helminthes as well as for zoonotic helminthic infections. Other researchers demonstrated that, there is consistent intestinal helminthiasis caused by hookworms which are common diseases among rural populations in Nigeria (13). High prevalence was also recorded among pupils whose parents' occupations are farming as in accordance with the report of (28 and 13), who stated a very high incidence rate in females (91.6%) than in males (83.0%). These children follow their parents to the farm. According to (15), who carried out sample studies on some fruits and vegetables in Enugu State, the ova of *Ascaris lumbricoides* was 76.4% and *Trichuris trichuria* 8.30% while the larvae were those of hookworms 10.40% and *Strongyloides stercoralis* 4.90%. (42), in Lagos State, incidence of *Ascaris* was 35.75%, *Trichuris*, 33.30%, Hookworms, 29.15%, *Strongyloides*, 5.90% and *Enterobius vermicularis* 0.40%. The infective *Ascaris* eggs, in endemic areas, have reportedly been found adhering to cooking and eating utensils, fruits, vegetables, furniture, door handles, money and fingers (21). This study was undertaken to identify, assess, evaluate the prevalence and the seasonal variation of geohelminths contamination and suggest ways by which the level of infection can be reduced in Ebobyi North-Central area of Ebonyi State, South East of Nigeria.

MATERIALS AND METHODS

Study area

The survey was conducted in Eboyi North-Central Arae of Ebonyi State, Umuogharu in Ezza North Local Government Area, Ezzagu in Ishielu L.G.A., Ntezi-Abba and Rice-mill in Abakaliki Local Government Area, Nwofe-Agbaja in Izzi Local Government Area. Farmers in these locations live sparsely except Abakaliki Local Government Area and cultivate their vegetables and fruits around their houses. The rainfall pattern is between 150mm-300mm. The annual rainfall has its peak in July; a short break occurs during the month of September, the rain comes again, which finally declines between the months of October and November each year. The average temperature of the area is 29^0C during the rainy season and 32^0 C in the months of dry seasons (December- April). The main occupation of people of the areas is farming at subsistence level and trading. Sanitation facilities are either non-existent or grossly inadequate in the study areas. Thus, defecation on open farm lands is a popular practice. These conditions furnish predisposing factors to many parasitic and other diseases.

Sample collections and analysis

Collection of soils from contaminated foci:

A pin quadrat was thrown at random on the vegetable gardens and shovel was used to collect 2cm deep top soils at 10 sampling sites in one field each month, in the amount of about 200g each from each quadrant area. The sample collected were kept in black polythene bags and taken to laboratory. A total of 300 samples were collected in different locations. The average temperature of the soils when tested was 28^0C for all the areas. The collection was done in the morning hours from 6.00 am — 11am, when the larvae and eggs of geohelminths are still active and fresh. These soils are mostly loamy soil, rich in organic manure or nutrients. It is predominantly a pre-urban setting with mainly banana trees, grassy areas and houses. The humidity is always favourable with the soil full of moisture.

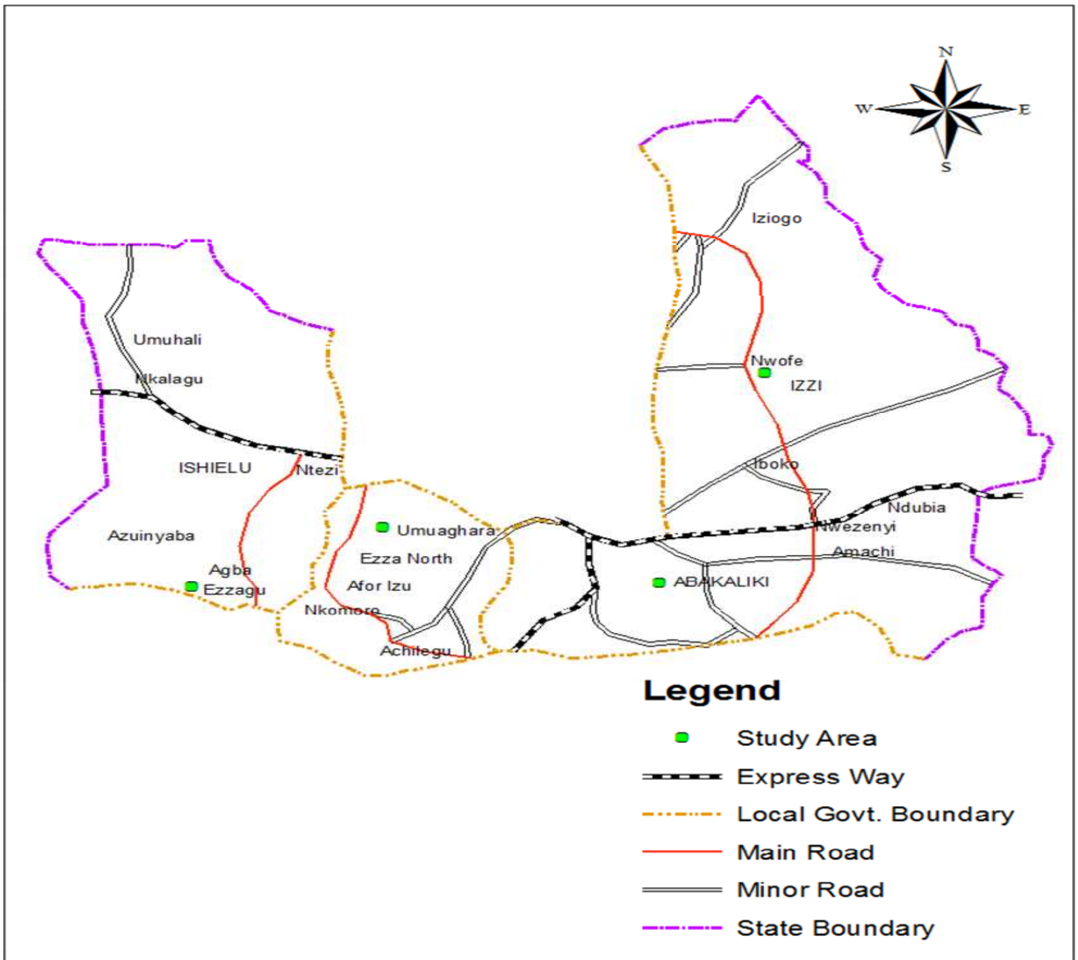

Fig :6 Map of Ebonyi North- Central showing the study area.

Detection of helminthic eggs in the soil

The parasite eggs and larvae were detected using modified Cobb's decanting and sieving method (9). This method requires five buckets (white plastic buckets preferably) and three series of sieves of 1000µm, 215µm and 65µm. The contaminated soil samples (100g) of top soil were suspended with 1-litre of water in white plastic bucket and when heavy particles have settled, the nematode suspension is poured off (decanted). Stir the remaining sediment again with water and decant the supernatant in the same plastic bowl. Repeat a third time. The sediment in the beaker can be discarded. A proper stirred mixture of suspension (supernatant) was first filtered with 1000µm aperture into the second bucket, leaving behind heavy soil particles on the top of 1000µm sieve. Shake the sieve, which is submerged in the suspension, to help nematodes to pass through. The debris remaining on the 1000µm sieve can be discarded.The filtrate was then turned into another bucket through the 215µm sieve, to obtain the residue. The residue was stained with eosin (8.0ml) solution and filtrate of the second sieve was then turned into another bucket with the third sieve of 65µm. The filtrate of the last sieve was left overnight until, so that the water could settle down, excess water was decanted out and centrifuged. The preparations were examined or viewed under microscope for the parasites using, X10 and X40 objectives.

RESULTS

The results of the study revealed an overall prevalence of soil transmitted helminth infections of 92 (30.7%) out of the 300 soil samples examined for the parasites (Table 1). The reult in table 1, shows the true parasitic profile of contamination of soil in different locations. The screened soil samples from each location showed that Umuogharu was most contaminated 22 (7.3%), followed by Onu-Ebonyi area with occurrence of 20 (6.7%), while the lowest was from the Ezzagu, 15 (5.0%). However, statistical analysis did not show a significant difference among the results obtained on the five locations ($P > 0.05$).

Table 2, shows the frequency of isolated intestinal parasites from different soil samples. *Ascaris lumbricoides* had the highest occurrence in the contaminated foci, 24 (8.13%), followed by Hookworms 19 (6.3%) and *Strongyloides stercoralis* species had 17 (5.7%). While the least contamination was *Hymenolepis nana* of 6 (2.0%). The results of presented study also showed that the contamination of soil with parasite eggs was considerably higher on loamy soil 39 (13.0%), followed clayey soil 31 (10.3%), while sandy soil was the least contaminated 22 (7.3%)

Table 3: shows the comparison between the two seasons, dry and wet seasons; dry season recovered the highesr number of parasites 56 (18.7%), while wet season recovered only 36 (12.0%).

Table 4; showed the frequency distribution of geohelminth ova and larvae on the different soil types (loamy, clayey and sandy) samples. The proportions of ova recovered were, for clayey soil 13 (4.3%), sandy soil 19 (6.3%) and loamy soil 22 (7.3%), while the proportions of larvae were, for clayey soil 9 (3.0%), sandy soil 12 (4.0%) and loamy soil 17 (5.7%).

Table 1: Intensity of geohelminths contamination of soil in different locations

Locations	Number of soil samples screened	Number contaminated	Percentage contamination
Nwofe-Agbaja	60	18	30.0%
Onu-Ebonyi area	60	20	33.3%
Ntezi-abba area	60	17	28.3%
Umuogharu	60	22	36.7%
Ezzagu	60	15	25.0%
Total	300	92	30.7%

Table 2: The Distribution of isolated helminthic parasites from sand, loamy and clay soil samples

Parasites	sandy (n= 100)	Loamy (n=100)	clayey soil (n= 100)	Total (n= 300)	Percentage
Hookworms	5	8	6	19	6.3
Ascaris lumbricoides	6	11	7	24	8.0
Strongloides stercoralis	4	7	6	17	5.7
Trichuris trichiura	3	6	5	14	4.7
Enterobius vermicularis	3	4	5	12	4.0
Hymenolepis nana	1	3	2	6	2.0
Total	22 (7.3%)	39 (13.0%)	31(10.3%)	92	30.7

Table 3: Type and frequency of helminthic egg recovered in two different seasons

Parasites	Wet season (n=150) Frequency(%)	Dry season (n=150) Frequency(%)	Total (n=300) Frequency (%)
Hookworms	7 (4.7%)	12 (8.0%)	19 (6.3%)
Ascaris lumbricoides	9 (6.0%)	15 (10.0%)	24 (8.0%)
Strongloides stercoralis	6 (4.0%)	11 (7.4%)	17 (5.7%)
Trichuris trichiura	6 (4.0%)	8 (5.4%)	14 (4.7%)
Enterobius vermicularis	5 (3.4%)	7 (4.6%)	12 (4.0%)
Hymenolepis nana	3 (2.0%)	3 (2.0%)	6 (2.0%)
Total	36 (12.0%)	56 (18.7%)	92 (30.7%)

Table 4: Frequency distribution of geohelminth ova and larvae on the different types, loamy, clayey and sandy soil samples

Samples (n=300)	Ova (%)	Larvae (%)
Clayey	13 (4.3%)	9 (3.0%)
Sandy	19 (6.3%)	12 (4.0%)
Loamy	22 (7.3%)	17 (5.7%)
Total	54 (18.0%)	38 (12.7%)

DISCUSSION

A total number of 300 samples of soil obtained from five different locations were examined geohelminthic parasites. Out of the 300 samples, 92 (30.7%) were positive for geohelminths. Six different types of parasites were isolated from samples collected from these locations. These include Hookworms, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Enterobius vermicularis* and *Hymenolepis nana*.

The reults in table 1, showed parasitic profile of contamination of soil in different locations. Umuogharu was most contaminated 22 (7.3%), followed by Onu-Ebonyi area with occurrence of 20 (6.7%), while the lowest was from the Ezzagu, 15 (5.0%). The results showed that statistical analysis did not show a significant difference among the results obtained on the five locations ($P > 0.05$).

The results obtained in the present studies has also shown that geohelminth ova and larvae are commonly found on farmlands in some parts of Ebonyi State, South-East of Nigeria. The detection of helminthic ova/ larvae on the five studied areas has a significant public health implication to many who have close contact with the soil (7). This is of great importance in health of many populations in third world countries where illiteracy, poverty and associated poor environmental sanitation practices have been implicated in the heavy burden of helminthiasis, (34 and 39). Nigeria like other developing countries is faced with the dilemma of inadequate disposal of excreta-related human waste discharged into the environment. Thus, the area of this study in rural farming communities of Ebonyi State, it has been a routine practice defecating on open fields and farmlands thereby giving a stable soil contamination with parasite eggs and larvae throughout the year as it concerns open and indiscriminate defecation.

This study has shown the potential risk of contracting helminthic ova and larvae through ingestion of unwashed, raw/uncooked fruits and vegetables obtained from these farmlands (10). Specific occupations and behaviours influence the prevalence and intensity of soil transmitted helminth infections. Engagement in agricultural pursuits remains a common denominator for human hookworm infection. Heavy infections in Sichuan Province, China and in Vietnam, for instance, are attributed to widespread use of faeces as night-soil fertilizer (19 and 18), whereas in other parts of Asia (e.g., Hainan) 15 and other parts of the world, high rates of hookworm occur despite the absence of night-soil use. Hookworm has been noted to target families who are involved with agricultural pursuits, (17). It may not be wrong to say that the hygienic status of individuals influence the infection rate in area where toilet facilities are inadequate. The contamination might have resulted from rain splashes, irrigation or river flush of contaminated soil during heavy rain-fall which deposit contaminated soil on the surface of leaves of vegetables, (41 and 2). It is also possible that the soil moisture must have favoured contamination of the areas and the survival of the parasites. In each of these areas, the soil ecology was very suitable with a lot of organic matter that ensure the survival of geohelminth eggs and larvae. As long as ecological conditions are favourable in the contaminated foci, the larvae of hookworm and *Strongyloides stercoralis* remain quiescent in the moisture films of contaminated soils until contact with suitable host is made where it penetrate through the skin or remain viable on leaf surface of low-growing vegetation which are common features of the study areas, (38, 18 and 3). These environmental conditions which were observed during this study included inadequate sanitation, poor hygiene, untreated farmlands which previous workers (1 and 22) had observed in their studies. The presence of economic trees such as plantains, bananas, raffia palms, paw-paw and local pears provide cover for defaecating humans and developing larvae and eggs of geohelminths, therefore, making such environments conducive for the transmission of geohelminths (3). Plantation-style agriculture is a particular set-up for endemic hookworm infection (35).

The result of table 2 shows the frequency of isolated helminthic ova and larvae from different soil types. *Ascaris lumbricoides* has the highest contamination of 24 (8.0%) followed by Hookworm species of 19 (6.3%); while the least contamination was *Hymenolepis nana* of 6 (2.0%). The prevalence of ascariasis as the most common infection in this and other studies in Southern Nigeria has been observed (30; 5; 25; 29 and 31). *A. lumbricoides* eggs are very resistant to harsh environmental conditions and air-borne. They may account for the ubiquitous nature of egg distributions and hence very high prevalence in all the age groups, (33; 14; 37 and 7). Hookworm contamination was the second most prevalent STH in this study with a value of 19 (6.3%). In some regions where marked rainy and dry seasons occur, hookworm transmission rates are higher during the former (26 and 36).

The larvae of hookworm are capable of vertical migration up and down in the contaminated soil, depending on the moisture suction and temperature. It has been reported that under favourable ecological conditions, larvae of hookworm can survive for a period of three weeks (21 days) and still remain infective (36 and 3).

Changing environmental conditions, specifically deforestation and subsequent silting of local rivers may cause deposition of sandy loam top soils and increased soil moisture that might promote the emergence of endemic hookworm (24). Unlike *Ascaris* and *Trichuris* eggs, hookworm eggs hatch in the soil and give rise to first-stage larvae, which moult to infective larval stages only under precise conditions. Egg development in the soil is dependent upon a number of factors including temperature (optimal development at 20-30 C), and adequate shade and moisture (23).

Table 3: shows the comparison between the two seasons, dry and wet seasons; dry season recovered the highesr number of parasites 56 (18.7%), while wet season recovered only 36 (12.0%). The table 3 indicated that incidence of *Ascaris lumbricoides* had highest occurrence among other species encountered. The result showed that dry season is favourable for development of *Ascaris lumbricoides.* The results corroborated with the findings of previous workers in terms of the species of parasites encountered by rural populations in Nigeria, (13). In some endemic areas, soil-transmitted helminth infections exhibit marked seasonality (12). The infective stages of *Ascaris-Trichuris* are embryonated eggs having enormous capacity for withstanding the environmental extremes of urban environments. Contained within the inner layer of *Ascaris* eggs is an unsaponifiable lipid known as ascaroside, which confers many of its hardy properties (4). A study of the prevalence of helminth infections along the coastal plains of South Africa found transmission of *A. lumbricoides* to correlate with variables based on annual data, particularly rainfall and temperature (4).

The results in table 4 showed the frequency distribution of geohelminth ova and larvae on the different soil types, loamy, clayey and sandy. However, the ova 54 (18.0%) showed wide range with the larvae 38 (12.7%) recovered. This could be that the eggs can survive long period and still remain infective. Viable *Ascaris* eggs have been recovered from soil samples for more than 10 years after having been first deposited (11). *Ascaris* eggs develop best in less permeable clay soils, with survivability increasing with their soil depth (11). Clay soils are believed to prevent egg dispersal by water (27). The vulnerability of Ascaris eggs to direct sunlight may account for part of this observation. Well-aerated, non-adhesive sandy soils (0.5 mm to 2 mm) are particularly conducive to promoting hookworm egg hatching, larval development, and larval migration (6). The additional presence of silt that contains microscopic and barely visible particles that provides optimal conditions. Such soil is sometimes known as 'sandy loam' (6).

The examinations showed that parasitological contaminated soil may be the source of contamination of cultivated vegetables and fruits. Vegetables and fruits contaminated with the eggs and larvae of parasitic roundworms may be the source of infestation of humans and animals, and create the risk of infection, not only of consumers, but also the hosts and holiday-makers visiting these farms. Although, the impact of shoes and other footwear on interrupting hookworm and other geohelminths transmission have probably been overestimated, given that *N. americanus* infective larvae penetrate all aspects of the skin and *A. duodenale* larvae are orally infective, (7). The infection should be reduced by the deworming within period of three-six months. The introduction of stricter sanitary standards on all types of farms may decrease human morbidity due to hooknoses in Ebonyi State.

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