Evaluation of the Diagnostic Utility of geohelminths from environmental and stool samples in Nnewi metropolis

ABSTRACT

Background: Geohelminths are groups of four intestinal soil transmitted parasites.They are of public health concern due to thei serious morbidity they cause in children which are the major groups affected. **Aim/Objective**: This aim of this study was to determine the prevalence and risk factors promoting the transmission of geohelminths among primary School children aged 6-13 in Nnewi North Local Government Area Anambra state. **Methodology**: Eighty stool samples were collected from four primary schools, while forty soil samples were collected from the important sites (playground, and classroom areas) in each of the school accessed. The stool samples were examined with the wet preparation and formol -ether concentration technique, while the soil samples were examined with the formol-ether concentration technique. Ethical approval was sought from the Faculty of Health Sciences and Technology. **Results**: The results of geohelminths showed an overall prevalence of 6.25% (5/80) from the stool samples and 27.5%(11/40) obtained from the soil. Recovery rates from the stool showed the presence of only two geohelminth;Hookworm (5%) and *Strongyloides stercoralis* (1.25%). Out of the four schools examined only three showed positive results with geohelminth infections, while the remaining school showed positive results with only *Giardia.lamblia*. The recovery rates from the soil also showed the same pattern of distribution as with the stool but with a higher prevalence of Hookworm (20.7%) and *Strongyloides stercoralis* (7.5%) with the toilet areas showing the highest prevalence (50%). The dominant specie of geohelminth observed in both stool and soil was *Hookworm*. The results also showed that both male (6%) and female (6.7%) are susceptible to the infection with no significant difference between their prevaleances (p>0.05). **Conclusion**: The prevalence observed in this present study is very low which was attributed to several factors such as; previous deworming of the children, urbanization of the area, improved environmental sanitation, small sample size, cost of the study insecurities of both the headteachers and parents. The present study recommended more efforts should be directed in maintaining adequate environmental sanitation and continuous sensitization about the knowledge of geohelminths should be carried out especially in government public schools, until there's zero prevalence of this soil- transmitted helminth.

INTRODUCTION

Geohelminths also known as soil transmitted helminths or intestinal nematodes are group of major intestinal parasites which belong to the phylum Nematoda that are transmitted primarily through contaminated soil either with the embryonated eggs or larvae.Geohelminths are soil transmitted parasites in which their immature stages (eggs) require a period of development or incubation in the soil before they become infective [1]. Thephylum Nematoda are dioecious(meaning that the male and female sex organs are separate), have a direct life cycle and no intermediate host or vector involved in their transmission which implies that man is the only definitive host. The adult forms are essentially parasites of humans, causing soil-transmitted helminthiasis.

The infective forms (embryonated eggs or filariform larva) undergo tissue migratory stages during which they invade vital organs such as the lungs and liver. The soil transmitted helminths of major concern in the phylum Nematoda which causes Soil-transmitted helminthiasis are; *Ascaris lumbricoides*,*Trichuris trichuria* and Hookworm(species involved; *Ancylostoma duodenale*, *Necator americanus*),[2;3].

Humans get infected by ingestion of food (raw fruits and unwashed vegetables),water, soil, contaminated with the infective forms of these intestinal parasites (either the embryonated eggs or larva forms) which serves as the main pathways for human infection, also through penetration of the filiariform larva into skins of humans who walk bare footed in contaminated soils which is seen in hookworm infections [4]. Usually, the infective stages (viable ova or larvae) are acquired from the contaminated environment. In this regard, associated infections are referred to as Intestinal Geohelminthiases or Soil-Transmitted Helminthiasis. Thus, it seems that soil is the main resource for human infection and can be a direct indicator for the risk of infection among human populations, especially children.

However, the study is to identify and determine the prevalence of geohelminths in soil and stool samples of primary school children in Nnewi.

MATERIALS AND METHODS

Duration of Study

The research was carried out for a period of three month spanning from March-June 2021.

Study Area

The study was conducted in Nnewi North Local Government Area, Anambra State. Nnewi is the second largest commercial city in Anambra State. It is located between the latitudes 5 30E, 605N and longitudes 655E and 700E. Nnewi has population of over 900,000. The major occupations of the people are production, farming and trading.Nnewi North comprises of four villages which are: Otolo, Nnewichi, Uruagu, Umudim.

Study Design

This study was a cross-sectional study designed to access the prevalence of geohelminth in both stool and soil and risk factors promoting the transmission in students aged 6-13 years among the four schools.

Ethical Considerations

Ethical approval for this research was obtained from the Ethics Committee of Nnamdi Azikwe University Teaching Hospital Nnewi and the College of Health Science Nnamdi Azikwe University Okofia Nnewi. Informed consent was also obtained from the school authorities, parents and guardians of pupils involved with adequate enlightenment on the purpose theresearch.

Feacal Collection [5]

A clean leak-proof wide-mouthed transparent universal container and applicator stick was given to the 80 consented pupils in the selected schools. The students were educated on how to collect the feacal samples into the stool containers and appropriate disposal of the contaminated materials. The pupils were asked to take the containers home and return with it the freshly collected stool next morning. The stool specimens collected were transported to the Nnamdi Azikwe University Parasitological Laboratory immediately for processing.

Collection of Soil Sample

40 Soil samples were collected from the important sites of the four selected primary schools which were areas of the playground, toilet, and near classrooms. About 20g of the top soil (down to a depth of not more than 2cm) samples were collected with a clean spoon and kept in clean transparent polythene bags which were carried to the laboratory for parasitic examination [5].

Macroscopic Examination: The stool wereanalyzed macroscopically for the presence of blood, consistency, colour, mucus, and presence of adult intestinal parasites or their segments.

Wet preparation examination: A drop of normal saline and a drop of 1% lugol's iodine placed at both ends on a clean grease free slide. A clean applicator stick was used to collect 1g of the properly mixed stool and emulsified in normal saline and lugol's iodine. The preparation was carefully cover slipped and examined with the x10 and x40 microscope objective lens. The geohelminths were identified with the Atlas of parasitology by [5].

Formol Ether Concentration Technique: A clean applicator stick was used to emulsify 1g of the stool sample in 4ml of 10% formol ether contained in clean test tube. An additional 3ml of 10% formol ether was added to the tube for homogenization. The emulsified feaces was sieved through a tea strainer into a clean centrifuge tube. 3ml of diethyl ether was added to the filtrate, stoppered and mixed vigorouslyfor one minute. This was centrifuged at 1000g for one minute. After centrifugation, there was a 3 layered separation seen in the tube which comprised of the ether, fecal debris, and the formol water. The contents of the tube were decanted leaving the sediment at the bottom of the tube. The sediment was resuspended and placed on a clean- grease freeslide and covered with a cover slip. The preparation was examined with the x10 and x40 microscope objective. The presence of geohelminth ova or larvae was identified with the Atlas of Parasitology by [5].

Laboratory Analysis of Soil Sample [6].

5g of the soil sample was placed into a clean tube which contained 10% formol water and homogenized for one minute. The suspension was strained through a wet cheese cloth placed over a funnel to remove coarse particles. Ether was added to the filtrate in a centrifuge tube, which was centrifuged at 2,300 rpm for 3 minutes. The supernatant was decanted with the sediment placed on a clean-grease free slide. This was examined with the x10 and x40 objective lens of the microscope for any ova or larvae of any of the three geohelminth mentioned above. The ova or larvae was identified with the Atlas of Parasitology by [5].

.**Statistical Analysis**

The data was statistically analysed with Statistical Package for social science (SPSS) version 21.Chi square (x^2) test was used to determine the differences in prevalence of geohelminth infection among different explanatory variables. Pearson correlation test was used to test the relationshipbetween geohelminth contamination of soils and pattern of infection among the students. Statistical significance was set at $p<0.05$.

RESULTS

Table 1: shows the overall prevalence of the geohelminth observed from the study population. 5 out of 80 stool samples were positive for geohelminth ova or larvae, which gave an overall prevalence of 6.25%. The two geohelminth egg or larvae observed in the stool samples were those of Hookworm (4/80) and *Strongyloides.stercoralis* (1/80) which gave an overall prevalence of 6.25%. Hookworm had the highest prevalence (5%) and the most dominant specie, while the least was Strongyloides.stercoralis (1.25%). Only single infections were observed with no cases of mixed infection. However, *Giardia.lamblia* was observed in some of stool samples.

Table 2: shows the pattern and distribution of geohelminth infection among the four schools. Out of the 4 schools examined, only were 3 schools had pupils with positive results of geohelminth infections. In relation to the prevalence observed among the schools, Umuezeanam Central School had the highest prevalence of geohelminth infections (2/20(10%), followed by Odida Central School (2/23(8.7%) and Akwudo Central School which had the least prevalence (1/20(5%). There was no case of geohelminth infection observed among the pupils in Okpunoeze Community School, however *Giardia lamblia* was observed in this school. Correlation between the stool samples and schools showed a significant association ($p< 0.05$).

Table 3: shows the overall prevalence of geohelminth egg or larvae observed in soil samples of the four schools examined. A total of 40 soil samples were collected from important sites of the

four schools such as; playground, toilet areas and near classroom areas. An overall prevalence of 27.5% (11/40) was obtained which showed the same pattern of geohelminth (Hookworm and *Strongyloides stercoralis*) observed in the stool samples. Hookworm was found to be the most dominant specie with the highest prevalence (7/40(17.5%), while *Strongyloide stercoralis* had the least prevalence (4/40(10%). The distribution of geohelminths in the soil varied among the four schools with Odida having the highest prevalence, 60% (6/10), followed by Umuezeanam, 30% (3/10) Akwudo 10% (1/10) and Okpunoeze 10% (1/10) which had the least prevalence. The correlation between the isolates from the soil and schools showed no significant association (p> 0.05).

Table 4: shows the pattern of geohelminth infection in soil samples in relation to sample site. The prevalence of geohelminth eggs/larvae was found to be the highest in the toilet areas 50% (8/16), followed by the playgrounds 12.5% (2/16), near the classrooms 12.5% (1/8).

From the study population examined, the pattern of geohelminth infections in relation to gender was assessed (Figure 1). It was observed that both males and females were infected with either of the two species of geohelminth recorded. Out of 50 males examined, 3 (6.0%) were infected, while 2 (6.7%) out of 30 females were infected. The result also showed that males had a higher prevalence of hookworm infection, (3/50 (6.0%) than the females (1/30(3.3%). The presence of *Strongyloides stercoralis* was only seen in females, (1/30(3.3%). Although the prevalence obtained from the females (6.7%) was slightly higher than the males (6.0%), the difference was not statistically significant (p> 0.05).

Table 1: showing the overall prevalence of geohelminths in the stool samples examined

Geohelminths Observed	Frequency	Percentage (%)
Hookworm	4	5
Strongyloides.stercoralis	1	1.25
Total	5	6.25

Table 2: Showing the prevalence of geohelminth infections of pupils among the four schools

Schools	No of stool samples examined	No (%) of positive samples	Hookworm no (%)	*Strongyloides.stercoralis* no (%)	Pearson-r	p-value
Odida central	23	2 (8.7)	2 (8.7)	0	-.963	.009
Umuezeanam central	20	2(8.7)	2(10)	0		
Akwudo central	20	1(5)	0	1(1.25)		
Okpunoeze	17	0	0	0		

community				
Total	80	5 (6.25)	4 (5)	1 (1.25)

P<0.05

Table 3: showing the prevalence of geohelminth egg/larvae in soil samples of the four schools

Schools	No of samples examined	No (%) of samples positive	Hookworm no (%)	Strongyloides.stercoralis no (%)	Pearson -r	p-value
Odida	10	6 (60)	5 (50)	1 (10)	-.585	.059
Umuezeanam	10	3 (30)	2 (20)	1 (10)		
Akwudo	10	1 (10)	0	1 (10)		
Okpunoeze	10	1 (10)	0	1 (10)		
Total	40	11 (27.5)	7 (17.5)	4 (10)		

P> 0.05

Table 4: Showing the prevalence of geohelminth egg/larvae in soil samples with respect to sample sites.

Schools	Sample sites	No of samples examined	No (%) of positive samples	Hookworm no (%)	*Strongyloides.stercoralis* no (%)
Odida	Playground	4	2 (50)	2 (20)	0
	Near classroom	2	1 (50)	0	1(10)
	Toilet	4	3 (75)	3 (30)	0
Umuezeanam	Playground	4	0	0	0
	Near classroom	2	0	0	0

	Toilet	4	3 (30)	2 (20)	1 (10)
Akwudo	Playground	4	0	0	0
	Near classroom	2	0	0	0
	Toilet	4	1	0	1 (10)
Okpunoeze	Playground	4	0	0	0
	Near classroom	2	0	0	0
	Toilet	4	1	1 (10)	1 (10)
Total		40	11(27.5)	7 (17.5)	4 (10)

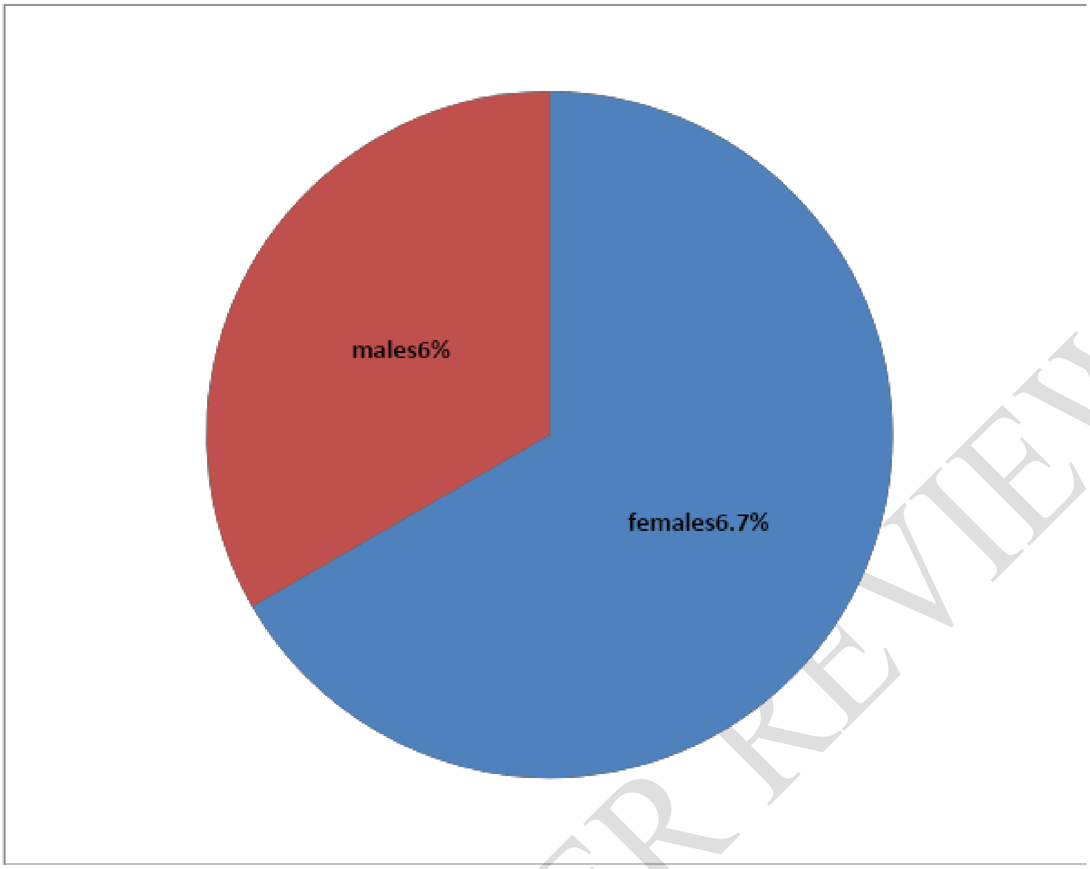

Figure 1: Showing the prevalence of infection in relation to gender

Discussion

Geohelminth infection is a major public health challenge in children as they cause severe morbidity such as; intestinal obstruction, malnutrition, growth retardation, anaemia, impairment of cognitive abilities, heavily infected children living in rural areas of tropical and sub-tropical areas which leads to school abseentism and hinders economic development, particularly in the tropical and sub-tropical regions of the world where sanitary conditions are poor .

This study recorded an overall prevalence rate of 6.25% of geohelminths isolated from the stool samples of the four selected primary schools with two types of geohelminth identified. Contray to this result a much lower prevalence have been recorded in studies done by Egbe-Sarah *et al.*, [7], who recorded a prevalence of 1% among primary school children in Cameroon, A much higher prevalence have been reported by Chukwuma *et al.*, [6] who recorded a prevalence of 87.7% among primary school children in Ebenebe town, Anambra state.

The low prevalence encountered in this study is as a result of many underlying factors such as; provision of adequate sanitary facilities such as water closet toilet, refuse bins in schools by governmental and non-governmental interventions, thus leading to improved environmental sanitation, geographical differences, increased awareness and public sensitization about personal hygiene and knowledge of intestinal worms, previous deworming of the students, The fact that majority (73.75%) of the respondents had taken one preventive treatment (deworming drug) or the other in the previous 6 months, before they were tested, may also explain the low prevalence observed in this study. Several studies have abundantly demonstrated that periodic deworming is effective in reducing the burden of intestinal helminthiasis [8; 9; 10]. Moreover, this study was carried in an urban area (Nnewi North), where expectedly, people are better educated and with better access to clean water and environment compared with their counterpart in rural areas, limited sample size and restricted age group as only one age group (11-13years) was used due to restrictions by the headmistresses of the schools visited, insecurities created by the recent COVID-19 pandemic of the country (Nigeria) resulted in decreased participation. As reported by Gboeloh *et al.*, [10], that younger children are more susceptible to STHs infection than older children. They are known for maintaining poor personal hygiene as this plays an important role in transmission of STHs infections.

The predominant soil transmitted helminth recorded in this study is hookworm which is in agreement with studies such as; Galamji *et al.*, (2017), Hadiza *et al.*, (2019), Atsuwe *et al.*, (2019) which is contrary to studies which observed *Ascaris lumbricoides* as the most dominant soil transmitted helminth,[6;10]. The prevalence rate obtained (5%) is comparable to studies carried by Ojurongbe *et al.*,[11], who recorded a prevalence of 5.6%, as well as Hassan *et al.*, [12] who recorded a prevalence of 4% .

A higher prevalence have been obtained on the studies done by Chukwuma *et al.*, [6], who recorded a prevalence of 5.9%, [11], who recorded a prevalence of 3.7%, Nasiru *et al.*, (2017) recorded a prevalence of 3.97%. The differences in the results obtained could be due to rate of environmental contamination, seasonal differences as prevalence of infection increases more during rainy season than dry season, study population which might contribute to the prevalence being low or high.

In this present study it was observed that both male and female were susceptible to the infection and to the same factors which might influence the infection. This further explains that both sexes are equally susceptible to soil-transmitted helminth infection. The high prevalence of male infected with hook worm infected can be attributed to their activities such as playing football, helping their parents on the farm, wrestling, swimming, etc. most of these activities are carried out bare-footed further predisposing them to soil- transmitted helminthiasis especially, hookworm infections when in contact with contaminated soil [10;13].

The studied population had a commendable sanitation this is in contrary with studies which were carried out where the sanitation was poor especially in rural areas, [6; 14]. Majority of them claimed that they used water closet system, while few used pour flush toilet and pit toilet as a form of sewage disposal. This is important as improved sanitation serve as a protective factor against soil transmitted helminthes especially *Ascaris lumbricoides* and *Trichuris trichuria* which are majorly transmitted through feacal-oral route. The prevalence of infection was higher in students who used pour flush toilet than those with water closet. This may be due to inadequate maintenance such as; limited washing facilities (water and soap).

The study also observed an increased knowledge about geohelminths and deworming of the students in all the four schools examined this. School based deworming is one of the strong three pillars of the WHO in eradicating the prevalence of geohelminthiasis in tropical areas, [14]. However, the prevalence (9.5%) of those not dewormed who were infected was slightly higher than those dewormed who were also infected, (5.1%). This is also of a public concern as those not dewormed can serve as a vehicle of transmission of soil-transmitted helminth infection to others.

In the present study, the rate of soil contamination of the school compounds was found to be 27.5%. The overall result was similar to the findings of [4], who recorded a prevalence of 23.3% in Osun state, Awosolu *et al.,* [16] recorded a prevalence of 61% in Owena.

The two helminth species recovered from the soil sample were Hookworms and *Strongyloides.stercoralis*. Although prevalence of STHs was higher in soil samples than in thefaecalsamples, the pattern of distribution of soil-transmitted helminth infections in the soil was similar to the pattern of infection in study subjects; same species of helminth was implicated in both samples. This suggests that the soil plays a major role in the epidemiology of soil transmitted soil transmitted helminth infections in the study area. The fact that the adult stages of these worms reside in the intestine, the presence of the ova in soil is indicative of environmental faecal pollution which is supported by the findings of feaces around the school environment.

The dominant specie observed was still hookworm as the ecological conditions were favourable to the development of the parasite. Hook worms and *Strongyloides steroralis* larvae remain quiescent in the moisture films of contaminated soils until contact with suitable host is made where it penetrate through the skin. This is supported by the fact that the toilet areas showed the highest prevalence as students defecate around these areas due to dysfunctionality and inappropriate maintenance of their toilet system present in schools.

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