***Original Research Article***

**Comparative Recovery of Soil-Transmitted Helminths Using Different Diagnostic Techniques in Akure South Local Government, Ondo State**

**Abstract**

This study assessed the prevalence of soil-transmitted parasites in Akure South Local Government, Ondo State, using three diagnostic techniques (salt flotation, zinc sulphate flotation, and sedimentation) on 50 soil samples from five different sites (house, gutter, dumpsites, vegetable farm, and hospital vicinity). Of the samples, 84% were positive for parasites and three parasites were recovered *Ascaris lumbricoides*, *Toxocara* spp., and *Strongyloides stercoralis* with *Toxocara spp*. being the most prevalent, particularly in gutter (75.33%) and hospital vicinities (55.56%). Diagnostic recovery varied as *Toxocara spp*. was detected only by flotation methods and there was no significant difference when comparing the prevalence of Toxocara spp. across the collection sites for both salt flotation (p = 0.79) and zinc sulphate flotation (p = 0.76). *Ascaris lumbricoides* and *Strongyloides stercoralis* were isolated via sedimentation and the prevalence of the parasite were not significantly different (p = 0.59) across the study sites. These findings highlight widespread soil contamination and underscore the importance of improved sanitation and animal management especially stray dogs and cats to reduce zoonotic disease risks. The study also demonstrates the need to select appropriate diagnostic techniques for accurate surveillance of soil-transmitted helminths.

Keywords: Soil-Transmitted, Prevalence, Diagnostic, Geohelminths, Soil Sample and Parasites

**INTRODUCTION**

“Soil-transmitted (geohelminth) parasites are parasites found in soil; this helminth group spends some of their life cycle or developmental stages in soil” (Mandarino-Pereira et al., 2010). “Contamination of soil with parasite eggs, infective larvae, cysts, and oocysts constitutes the most important risk factor for zoonotic parasite infection” (Waenlor and Wiwanitkit, 2007). “Parasites such as Toxocara spp. Ascaris spp., Trichuris trichiura, and Strongyloides stercoralis are the main parasites that could be transmitted by soil” (Waenlor and Wiwanitkit, 2007). “More than 1.5 billion people, or 24% of the world’s population, are infected with soil-transmitted helminth infections worldwide” (WHO, 2020). “Helminth infections are widely distributed in tropical and subtropical areas, with a significant number occurring in sub-Saharan Africa, the Americas, China, and East Asia. Over 267 million preschool-age children and over 568 million school-age children live in areas where these parasites are intensively transmitted and require treatment and preventive interventions.Soil-transmitted parasite infections are common chronic human infections worldwide, affecting the poorest and most deprived communities. They are transmitted by helminthic eggs in human faeces, contaminating soil where sanitation is poor. The main helminth that infects humans belongs to the group of helminths known as nematodes; these include roundworm (Ascaris lumbricoides), the whipworm (Trichuris trichiura), and hookworms (Necator americanus and Ancylostoma duodenale). These soil-transmitted helminths (STHs) are generally addressed as a group because they are diagnosed using similar procedures and respond to the same helminthic medications or drugs. According to prior reports, soil-transmitted helminths (STHs) were the second cause of mortality in children under six years of age in Africa” (Ogbe et al., 2002).

“Most soil-transmitted helminth parasitic diseases cannot be diagnosed by physical examination; thus, laboratory investigations are necessary. Unfortunately, these infections mostly remain undiagnosed due to a lack of trained personnel and appropriate technologies. Intermittent shedding of eggs or larvae further makes the diagnosis difficult. Thus, there is a dire need for rapid and accurate tests for the diagnosis of STHs. As there is no adequate gold standard for STHs detection, this further makes the comparison and standardisation of any new technique difficult” (Tarafder *et al*., 2010). The diagnostic methods include conventional and molecular methods. Moreover, quantitation of worm burden is also important to assess the intensity of infection and prognosis.

“Most diagnoses are made by identifying the appearance of the worm or eggs in faeces. Due to the large quantity of eggs laid, diagnose can generally be made using only one or two faecal smears” (Al-Soud *et al.,* 2005). The diagnosis is usually incidental when the host passes a worm in the stool or vomit. The eggs can be seen in a smear of fresh faeces examined on a glass slide under a microscope and there are various techniques to concentrate them first or increase their visibility. There is need to study different diagnosis techniques used in soil helminths. Thus, this research aimed to study the population of soil helminth parasites from soil collected from five different location using different techniques.

**Materials and Methods**

**Study Area**

The survey was carried out in Akure South Local Government. It lies between the latitudes 7017 N and the longitude 5019 E. The major occupation is agriculture, especially cocoa farming, yam, cassava and palm production. Some of the dwellers also engaged in miniature trading, artisanship and privately owned enterprises. The study was carried out in different locations, where soil samples were collected.

**Sample Collection**

About 20g of soil samples were collected with a hand trowel at five different locations (gutters, waste dumps, houses, vegetable farms, and hospitals) and stored in clean Ziploc bags. Samples were collected and transported to the laboratory and analysed within 24 hours of collection

**Saturated Salt Floatation Technique**

About 2 mL of the sodium chloride solution was taken in a tube, and 1g of soil was emulsified in it. The tube was then filled to the brim with the salt solution, and a slide was placed on the tube so that it could be in contact with the surface of the solution, without any intervening air bubbles. After standing for 20-30 minutes, the slide was removed without jerking, reversed to bring the wet surface on top and examined under the microscope after staining with a drop of iodine.

**Zinc Sulphate Centrifugal Technique**

Two grams (2g) of soil were thoroughly mixed in 10 mL of distilled water. The coarse particles were removed by straining through gauze/sieve. The filtrate was poured into 15ml conical centrifuge tube and centrifuged at 2500 revolutions per minute (rpm) for 1 minute. The supernatant fluid was poured off, and distilled water was added to the sediment. It was shaken well, centrifuged and the process was repeated 2 or 3 times till the supernatant fluid was clear. The clear supernatant was poured off and 4 ml of zinc sulphate was added to the sediment and more zinc sulphate solution was added to fill the tube up to the top and centrifuged again at 2500 (r.p.m) for 1 minute. With a platinum wire loop, the sample was taken from the surface onto a clean microscope slide. A cover slip was put on and examined under the microscope after mixing with a drop of iodine (Arora & Arora, 2009).

**Sedimentation Methods (Formalin-ether sedimentation)**

The formolin–ether concentration method has been the most widely used sedimentation method. 1- 2 g of soil was emulsified in 10 ml of distilled water and strained through two layers of gauze in a funnel. The filtrate was centrifuged at 2500 revolutions per minute (rpm) for 2 minutes. The supernatant was discarded, and the sediment was re-suspended in 7 ml of formol saline (10%). It was allowed to stand for 10 minutes. Then 3 ml of ether was added, the tube was stoppered and shaken vigorously to mix. Then the stopper was removed and centrifuged at 2500 rpm for 2 minutes. The tube was allowed to rest in a stand. Four layers became visible: the top layer consisted of ether, the second was a plug of debris, and the third was a clear layer of formol saline, and the fourth is sediment. The plug of debris was detached from the side of the tube with the aid of a rod and the liquid was poured off, leaving a small amount of formol saline for the suspension of the sediment. It was poured on a clean glass slide, covered with a cover slip and examined under the microscope after mixing with a drop of iodine (Paniker, 2002; Arora & Arora, 2009).

**Identification of Recovered Soil Helminths Parasites**

Helminths recovered were identified with the aid of an atlas, encyclopedia of parasitology, photographs and manual of parasitology (Bowman, 2009).

**Data Analysis**

Data was analysed using Statistical Package for the Social Sciences (SPSS) version 27. Proportions of positive soil samples and negative soil samples were estimated. Chi-square analysis was used to compare the prevalence among the soil samples collected from different sites, and also, with the diagnosis techniques used.

**Results**

**Prevalence of soil helminth parasites found in soil samples collected from different locations**

A total of 50 soil samples were examined for the presence of soil-transmitted parasites. The results of the study revealed that 42 (84%) out of 50 samples examined were positive. Table 1 table shows the parasitic profile of contaminated soil in different locations. Some of the examined soil samples from each location showed slight differences in their level of contamination. Vegetable farm soil had the most contaminated soil samples of 10 (100%), followed by gutter and dumpsite 9 (90%), then house vicinity 8 (80%), and the least contamination found in hospital vicinity 6 (60%). Figure 1 shows all the parasites recovered from the soil samples. The egg of *Toxocara sp.* was the most prevalent across the study sites. The eggs were present in all the sampling locations with a very high prevalence rate, except for the dumpsite, where *Ascaris* sp*.* was more prevalent compared to other parasites recovered in the location. *Strongyloides stercoralis* shows the least prevalence in all the locations except in the gutter location, where it is higher than *Ascaris* sp.

**Prevalence of *Ascaris* sp. in relation to the diagnosis techniques and locations of soil samples**

Table 2 shows the relationship among the sample locations and the methods used in the diagnosis of the presence of *Ascaris lumbricoides*. The statistical analysis indicated that there was no significant difference between results of the locations and the techniques used in the recovery of *Ascaris* eggs in the soil samples. Out of the three techniques used, *Ascaris* eggs were only isolated with sedimentation technique method in all the sampling locations, there was no significant difference (P>0.05) when comparing all the prevalence of *Ascaris* *sp* in all the sampling collection sites. *Ascaris* was not detected with the saturated salt flotation method and the Zinc sulphate centrifugal flotation method; chi-square (X2) was not computed.

Table1: Prevalence of Soil parasites contamination in soil samples of different locations

|  |  |  |  |
| --- | --- | --- | --- |
| Collection Site | Number of samplescollected | Number of contaminated samples | Prevalence(%) |
| Gutter | 10 | 9 | 90 |
| Dumpsite | 10 | 9 | 90 |
| House vicinity | 10 | 8 | 80 |
| Vegetable farm | 10 | 10 | 100 |
| Hospital vicinity | 10 | 6 | 60 |
| Total  | 50 | 42 | 84 |

Figure 1: Prevalence of parasites recovered from soil collected from different locations

Table 2: The prevalence of *Ascaris* eggs affected by the techniques and locations of soil samples

|  |  |
| --- | --- |
| Collection Site | No of Parasites Recovered and Techniques |
| Sedimentation | Saturated saltfloatation | Zinc sulphate centrifugal floatation |
| Gutter | 8 | 0 | 0 |
| Dump site | 34 | 0 | 0 |
| House vicinity | 42 | 0 | 0 |
| Vegetable farm | 46 | 0 | 0 |
| Hospital vicinity | 13 | 0 | 0 |
| χ2 | 36.29 | a | a |
| p value | 0.79 | a | a |

Note: χ2 = Chi -square, p value is based on Chi -square significant, a = Chi -square not computed

**Prevalence of *Toxocara sp*. in relation to the techniques and locations of soil samples**.

In Table 3, the analysis showed that *Toxocara sp*. was not recorded with sedimentation techniques in all the sampling locations. The parasite, *Toxocara* *sp* was recovered by two techniques; saturated salt floatation and zinc sulphate centrifugal floatation techniques. There was no significant difference (P>0.05) in the determination of *Toxocara* eggs, among the locations with saturated salt floatation techniques method (*X*2 = 36.29; P = 0.79). The prevalence of *Toxocara* *sp*. across the locations with zinc sulphate centrifugal floatation techniques was not significantly difference (*X*2 = 29.72; P = 0.76). The recovery of soil-transmitted parasite was affected by variability of different types of techniques used in diagnosis of the parasite from the soil.

**Prevalence of *Strongyloides stercoralis* in Relation to the Techniques and Locations of Soil Samples**

Table 4 shows the methods of diagnosis of *Strongyloides stercoralis* soil samples in respect to the sites of soil collection samples. Only sedimentation techniques gave positive for soil-transmitted parasite test for *S. stercoralis* among the three methods used. There was no significant difference (P>0.05) when comparing the parasites recovered among the collection sites (*X*2 = 14.13; P = 0.59). The variability in the level of prevalence is greatly affected by the type of technique used, since there are specific forms and types organisms that can be determined by each technique.

Table 3: The prevalence of *Toxocara* eggs affected by the techniques and locations of soil samples.

|  |  |
| --- | --- |
| Collection site | Number of parasites recovered and techniques |
| Sedimentation | Saturated saltfloatation | Zinc sulphate centrifugal floatation |
| Gutter | 0 | 40 | 18 |
| Dump site | 0 | 14 | 10 |
| House vicinity | 0 | 21 | 24 |
| Vegetable farm | 0 | 29 | 21 |
| Hospital vicinity | 0 | 9 | 11 |
| χ2 | a | 36.29 | 29.72 |
| *p* value | a | 0.79 | 0.76 |

Note: χ2 = Chi -square, p value is based on Chi -square significant, a = Chi -square not computed

Table 4: The prevalence of *Strongyloides stercoralis* affected by the techniques and locations of soil samples

|  |  |
| --- | --- |
| Collection site | Number of parasites recovered and techniques |
| Sedimentation | Saturated salt floatation | Zinc sulphate centrifugal floatation |
| Gutter | 12 | 0 | 0 |
| Dump site | 11 | 0 | 0 |
| House vicinity | 3 | 0 | 0 |
| Vegetable farm | 6 | 0 | 0 |
| Hospital vicinity | 2 | 0 | 0 |
| χ2 | 14.13 | a | a |
| *p* value | 0.59 | a | a |

Note: χ2 = Chi -square, p value is based on Chi -square significant, a = Chi -square not computed

**Discussion**

Data obtained from this study were consistent with a direct effect of a low level of sanitation. Out of the 50 soil samples, from five different locations (Gutter, dumpsite, house vicinity, vegetable farm, and hospital vicinity), three different types of parasites were observed from the overall samples collected from each location which may indicate soil transmitted helminths infection are highly endemic insub-tropic (Pullan et al., 2014); they are *Ascaris*, *Toxocara* and *Strongyloides*. The result obtained in this study has also shown that soil parasites can be commonly found in any location in the study area (Akure South Local Government). The detection of parasites in the studied areas has a significant implication for public health for many people who have close contact with the soil (Bethony *et al*., 2006).

The high incidence of *Toxocara* egg contamination observed in this study might be due to some factors, like high resistance to environmental conditions, ability to remain transmittable for several years in favourable conditions, poor management and high population of stray and pet dogs and cats, this study was similar to the result of Tavalla *et al.* (2012). The parasite was recovered by two techniques: saturated salt flotation and zinc sulphate centrifugal flotation techniques, because the suspension is in a solution of high specific gravity so that the eggs float up and get concentrated at the surface (Paniker, 2002).

The highest prevalence in *Toxocara* was recorded in the study area, followed by *Ascaris*. *Ascaris* is also one of the most common parasites found almost everywhere *(*Imalele *et al., 2021:* Tadge *et al., 2022)*. *Ascaris eggs* were isolated with only the sedimentation technique, because the suspension is a solution with low specific gravity, so that the eggs settle at the bottom (Chukwuma *et al.,* 2009).

Eggs of *Ascaris* need a period of time, outside the host body to develop and attain infective stage. The prevalence of this parasite in the environment can be a public health indicator (Saathoff *et al.,* 2002). Factors that favour a high prevalence of *Ascaris* include the use of untreated faeces as fertilisers. A health education program promoting improved sanitary facilities and good hygiene is needed to reduce the prevalence of *Ascaris*.

*Strongyloides stercoralis* shows the least prevalence, which is highest in dumpsite, due to having a free-living life cycle in warm, humid soil. *S*. *stercoralis* was observed only by the sedimentation technique because it is suspended in a solution with low specific gravity so that the parasite can get sedimented at the bottom surface (Chukwuma, *et al.,* 2009).

Umar and Bassey (2010) studied “the survival of the free-living form of *Strongyloides stercoralis* which is favoured in damp sandy or friable soil with decaying vegetation and contaminated with human excreta. Moreover, the very low and optimal conditions as warmth, oxygen, light and moisture are required for the survival of its free-living larvae”.

“The existence of viable parasitic eggs in the superficial layer of the soil presents potential public health hazards because these eggs are extremely resistant to adverse weather conditions and chemical agents” (Blaszkowska *et al.,* 2011). “Thus, soil contamination seems to be the most direct indication of soil parasite infection among human populations. For this reason, studies have been carried out in recent years to determine the prevalence of soil-transmitted parasites in different locations” (Blaszkowska *et al.,* 2011). “The non-availability of high-quality epidemiological data on Soil parasites due to the lack of regionally representative sample surveys, as well as differences in study design and sampling strategy, has restricted the ability to understand the true prevalence. The wide heterogeneity in soil transmitted helminths” (Khurana *et al.,* 2021) between studies has restricted the ability to perform in-depth comparisons.

**Conclusion**

This study showed high variation in the prevalence rate between the sample locations and highlighting the importance of the type of techniques used. The results on soil contamination by parasites in different locations indicated that human health is at risk, and soil may play an important role in the transmission of parasitic diseases to humans. This research recommends that the control of the population of animals like stray dogs and cats is necessary to reduce the dispersion of parasites. Implementation of health education is also necessary, encouraging healthy behaviour and proper disposal of human and animal waste. Proper washing of hands before eating, most especially after having contact with soil. This research recommends the discouragement of animal and human faessces as fertilizer.

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**Disclaimer (Artificial intelligence)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models and text-to-image generators have been used during the writing or editing of this manuscript.

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