Prevalence and species diversity of soil-transmitted helminths (STH) from selected soil samples in Penang Island, Malaysia

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Abstract. A total of 90 soil samples were collected from playground, beach, and residential areas in Penang Island, Malaysia. STH parasites eggs were recovered by using McMaster method and sedimentation method which were then examined using the light microscope. It was found that 17.8% (16/90) soil samples examined were identified to be infected with STH parasites in this study. Out of 40 soil samples collected from residential areas, *Ascaris* sp. eggs (22.5%) were identified most frequently, followed by hookworm eggs (7.5%) and *Toxocara* sp. eggs (5%). In playground areas, only two soil samples (6.67%) were found positive for *Ascaris* sp. eggs. Meanwhile, all samples collected from beach areas were negative for STH parasites. It was also found that sandy soil type (85.71%) had the most occurrence of STH parasites compared to loamy soil type (14.29%) whereas none was found in clay soil. In this study, the overall prevalence of STH parasites was consider as moderate in all the three different sampling sites. Environmental contamination with *Ascaris* sp. and *Toxocara* sp. could possibly be due to defecation by stray animals and owned animals (pets) at the playground and the residential areas. Nevertheless, appropriate measures need to be taken in order to improve the environment and basic hygiene through a comprehensive community-oriented health education program along with periodic deworming of the companion animals.

1. Introduction

Soil-transmitted helminths (STH) infection is common among developing and tropical countries. Globally, more than billion people were estimated at risk of being infected with soil-transmitted helminths (STHs) namely, the whipworm (*Trichuris trichiura*), roundworm (*Ascaris lumbricoides*), and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1]-[2]. Not only in humans, diseases cause by STHs may cause deadly diseases in animals too [3]. Most of the STHs eggs that are found in the soils may grow into an infective stage for several months or even years before ingestion occurs [3]-[4]. Commonly infection of STH occur via fecal-oral route [5] while hookworm infection may occur via the penetration of larvae into the skin especially walking barefoot on the contaminated soils which could lead to cutaneous larva migrans (CLM) cause inflammation [6].

Previously, studies on STHs in Malaysia have been carried out by [7], [8] and [9]. A study by [7] reported on soil-transmitted helminth eggs from 60 playgrounds in urban and suburban areas from five different states in Peninsular Malaysia which include Kuala Lumpur, Selangor, Pahang, Penang and Malacca. The author reported that all playgrounds were found to be contaminated with eggs from four different nematode genera with *Toxocara* being the most prevalent (95.7%), followed by *Ascaris* (93.3%), *Ancylostoma* (88.3%) and *Trichuris* (77%). Higher infection rate of *Toxocara* sp. eggs were most likely to be linked with playgrounds which normally exposed to stray animals; cats and dogs due
to the open access of playgrounds with no proper fencing that protect the areas. Meanwhile, [8] reported on the prevalence of *A. lumbricoides* and *T. trichiura* obtained from soil samples in Orang Asli settlements in Ulu Semenyih, Selangor. Out of 40 samples examined, *A. lumbricoides* recorded the high number of prevalence of with 90% and *T. trichiura* with 15%. The most recent study on STH was conducted by [10] who particularly examined particularly on Toxocara eggs contaminating the four public playgrounds and six neighbourhood playgrounds in Ipoh, Perak with 32.5% prevalence. Therefore, this study was undertaken to determine the level of soil contamination with soil-transmitted parasites in soil samples from selected playgrounds, beaches and residential areas in Penang Island, Malaysia as very limited data is available on STH in the northern region of Peninsular Malaysia. In addition, this study also evaluates the prevalence of STH in different types of soil texture.

2. Materials and Methods

2.1. Study Sites

This study was conducted at three different sampling sites from Penang Island, Malaysia which was located on the North West coast of Peninsular Malaysia in which the soil samples were collected from the playgrounds, beaches and residential areas. For the beach areas, soil samples were obtained from: (i) Batu Ferringhi beach (5°28'22.8"N 100°14'44.7"E) and (ii) Teluk Bahang beach (5°27'35.7"N 100°12'42.7"E) whereas soil samples for the playground areas were obtained from: (i) Taman Pekaka Field, Gelugor (5°21'01.8"N 100°17'35.4"E), (ii) Raffel Park, Gelugor (5°22'10.1"N 100°18'00.9"E) and (iii) Taman Pantai Jerjak, Bayan Lepas (5°20'24.1"N 100°18'14.2"E). As for the residential areas, soil samples were collected from: (i) Kampung Nelayan Tanjung Bungah (5°27'28.2"N 100°12'37.7"E), (ii) Kampung Dua Bukit, Gelugor (5°20'43.2"N 100°17'55.7"E), (iii) Taman Tun Sardon, Gelugor (5°22'10.5"N 100°18'26.7"E) and (iv) Desa Permai Indah, Gelugor (5°20'54.5"N 100°17'54.2"E).

2.2. Soil Sampling

The soil sampling method used was based on [10]. A total of 90 samples of soil were collected from October 2019 until January 2020. Approximately 250 g of soil specifically the surface layer of the ground was collected by using a hand shovel. The sampling soil was selected randomly per 5 m² of each sandpit which was not exposed to direct sunlight. Each sample were then placed in a zip lock bag with ID number and general descriptions. The samples were brought back to the laboratory for screening process.

2.3. Determination of Soil Texture

The soil texture was classified according to the USDA soil texture triangle by Steinbaum et al. [11]. In this study, three different categories of soil texture were used namely, clay, loam and sandy soil in which the smoothness and grittiness of the soil can be determined by rubbing the soil between the fingers. Soil that has high sand content was considered as gritty whereas soil with high silt content was considered very smooth while grittiness was the abrasive action felt by the thumb and fore finger or palm.

2.4. Detection of Eggs/Cysts in Soil

2.4.1 Mc Masters’s Flotation Method. Approximately 1 g of the soil samples were weighted and air-dried in room temperature. Then, it was thoroughly ground with a small amount of distilled water using a pestle and mortar. The suspension was washed, filtered through a strainer and centrifuged at 1500 rpm for 2 minutes. After centrifugation, the sediment was re-suspended and 15 ml of saturated sodium chloride solution was added and thoroughly mixed until all particles are distributed evenly. These procedures were repeated for three times until the solution appeared clear. After the final centrifugation, the sediment was re-suspended and the suspension was placed into a McMaster’s counting chamber. After 5 minutes, the chamber was examined under a compound microscope.
2.4.2 Sedimentation Method. Approximately 2 g of soil was mixed in 10 ml distilled water and filtered through a strainer. The filtrated solution was placed into 15 ml centrifuged tube for centrifugation at 1000 rpm for 7 minutes. The supernatant was discarded whereas the sediment was re-suspended in 7 ml of 10% formalin and allowed to stand for 10 minutes followed by adding 3 ml of ethanol into the suspension, vortexed and centrifuged again at 1000 rpm for another 7 minutes. After about 5 minutes, four layers were formed with soil sediment containing helminth eggs at the bottom layer. The sediment was poured on a clean petri dish and examined under the stereo microscope after mixing with a drop of formalin [10].

2.5. Identification of STH Eggs
Identification of the STH eggs was based on the key identification form [12]. The observation as well as the measurement of helminth eggs was performed using a compound microscope with ocular micrometric.

2.6 Data analysis
The prevalence of STH species identified was calculated by using the formula suggested from [13]. Kruskal-Wallis test was used to compare the contamination rate of soil-transmitted helminths in three different types of soil with the significance level of \( p<0.05 \) using the IBM Statistical Package for Social Science® (SPSS) v24.

3. Results and Discussion
3.1. Prevalence of STH
A total of 90 soil samples were collected which consists of 30 samples from playgrounds, 20 samples from beaches and 40 from residential areas. It was found that 17.8% (16/90) soil samples examined were positive for STH parasites out of the three different sampling sites (Table 1). Out of 40 soil samples collected from residential areas, nine soil samples were positive for Ascaris sp. eggs (22.5%), followed by three samples for hookworm eggs (7.5%) and two positive soil samples for Toxocara sp. eggs (5%). In playground areas, only two soil samples (6.67%) were found positive for Ascaris sp. eggs whereas none of the samples from beach areas were positive for STH parasites.

According to [14], the high occurrence of Ascaris sp. egg in the soil samples could be due to the thickness of the egg wall which makes them more resistant to harsh environmental conditions and airborne compared to the eggs of other nematodes. Another possible reason could be due to the over dispersion of Ascaris sp. eggs in the environment as a single female Ascaris can lay relatively large number of eggs. For instance, the daily average egg produced by female A. lumbricoides was estimated to be 23,872 [15].

Statistical analysis found that there was a significant association between the occurrence of STH parasites with the sampling sites (playground, beach and residential area) \( (\chi^2 = 11.842, df = 2, p = 0.003) \). Higher infection rate of this zoonotic nematodes was likely to be linked with playgrounds and residential areas being exposed to stray and owned companion animals (i.e cats and dogs) scavenging and defecating in those areas.

There were three genera of nematodes were identified in this study namely, Ascaris sp. with the prevalence of 12.22% followed by hookworm (3.33%) and Toxocara (2.22%). As compared to [7] who reported four genera of nematodes namely, Toxocara, Ascaris, Ancylostoma and Trichuris. In this present study, Ascaris sp. dominated the infections whereas [7] that Toxocara being the most common nematodes with 95.7% from playgrounds in five states of Peninsular Malaysia similarly reported [16] who examined soil samples collected from different parks in Bucharest areas, Romania with the highest prevalence soil-transmitted species was reported on Toxocara spp. (17.17 %).

Such high levels of environmental contamination, especially with Toxocara, were mainly due to defecation by stray animals and owned animals (pets), or the public in the case of Ascaris. According to [17], dogs can mechanically transmit human parasites such as Ascaris sp. The relatively high incidence of Ascaris found in this present study was more likely to originate from dog faeces either
stray or owned dogs. A study by [18] shown that children were found to be infected with *Ascaris lumbricoides*. In future, molecular approach is also necessary to confirm whether the sampling sites were contaminated with Ascaris are of human or animal origin. The egg counts in this present study was considered low ranging from 50 to 150 epg in which out of 90 samples collected, 14 samples (15.6%) were found to be positive for soil-transmitted helminths eggs by using McMaster method in which nine (10.0%) soil samples having 50 epg, followed by two samples (2.22%) with 100 epg and one (1.11%) samples with 150 epg.

| STH               | Playground n = 30 (%) | Beach n = 20 (%) | Residential area n = 40 (%) | Total n = 90 (%) |
|-------------------|------------------------|------------------|-----------------------------|------------------|
| *Ascaris* sp.     | 2 (6.67)               | -                | 9 (22.50)                   | 11 (12.22)       |
| *Toxocara* sp.    | -                      | -                | 2 (5.00)                    | 2 (2.22)         |
| Hookworms         | -                      | -                | 3 (7.50)                    | 3 (3.33)         |

### Table 1. The prevalence (%) of soil infected with STH in different sampling sites.

#### 3.2. Soil Texture Associated with STH

In this study, 62 soil samples were categorized as sandy soil, 26 of soil samples were loamy type and two were clayey soil type. It was found that sandy soil type had the highest occurrence of STH with 85.71% compared to loamy soil type with 14.29% prevalence whereas no occurrence of STH parasite found in the clayey soil (Figure 1). However, there was no significant difference between prevalence of STH parasite and the types of soil texture.

![Figure 1](image1.png)

**Figure 1.** The prevalence (%) of soil-transmitted helminths found from different types of soil texture.

These findings were supported by [19] who showed that sandy soils had the highest prevalence of helminth eggs with 38%, followed by loamy (32%), and clay soil (18%). Meanwhile, [10] reported highest prevalence of *Toxocara* infection in sandy soils with 61.53%, followed by silty (23.07%), loamy (15.38%) whereas none from clayey soil (0.00%). It was also found that the increase of moisture and pH of the soils does contributed to the increase of contamination of *Toxocara* spp. eggs.

According to [20], clayey soils do not provide enough aeration for STH egg development. Besides, sandy and loamy soils are preferred by animals for defecation as they have loose texture for animals.
such as cats and dogs to burrow their faeces deeper into the soil. Furthermore, dogs, exhibit behavioral patterns by selecting previously used defecation sites [21]. STH eggs survive more in sandy soils, with high silt content, since they provide aeration and moisture for the egg development.

4. Conclusions

STH are transmitted due to poor hygiene practices via accidental ingestion of contaminated food, soil, and fomites. Parasites from soils may infect humans and other animals through accidental ingestion of contaminated soil and eating improperly washed vegetables. Moreover, contaminated soil in residential areas does highlight the need for substantially improving the management of owned and stray animals as well as enhancing hygiene practices in Malaysia. Municipalities nationwide must be responsible for the control of stray animals whereas pet owner should monitor their companion animals from defecating in public and residential areas in order to promote better hygiene practices within the community.

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