Detection of *Ascaris lumbricoides* and *Trichuris trichiura* in various soil types from an indigenous village in Malaysia

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Abstract. Soil-transmitted helminths (STHs) have been a great concern throughout the world among the poorest and the most deprived communities. Orang Asli (indigenous) community in Malaysia is highly prone to STHs infection due to their living environment where soil contamination can serve as the main reservoir. This study was aimed to investigate the presence of *Trichuris trichiura* and *Ascaris lumbricoides* eggs in the soil samples in and around the Orang Asli villages located in Sungai Lalang Baru, Ulu Semenyih, Selangor. The soil samples were collected from June to November 2017 over a period of six months. Approximately 200-250 gram of soil samples were collected in a plastic bag from a depth of about 4-6 inches. The sedimentation and microscopic techniques was used to recover and estimate the *Trichuris trichiura* and *Ascaris lumbricoides* eggs from the soil samples. A total of 40 samples soil samples were collected, in which 36 (90%) were positive for *A. lumbricoides* and 6 (15%) were positive *T. trichiura*. Various soil textures like sandy, loamy and clay harbored the helminth eggs. The contaminated soil could potentially lead to transmission of the helminth eggs by natural process like rain and water movement to their sounding environments, hence screening of soil sample in the environment serves as indication for presence of STHs.

INTRODUCTION

Globally, it is estimated that over 1.5 billion people are infected with at least one species of soil-transmitted helminths (STHs) (WHO, 2015). In developing countries, STHs has been recognized as a significant health problem (WHO, 2015). The majority of these infections are caused by the roundworms (*Ascaris lumbricoides* and *Strongyloides stercoralis*), whipworms (*Trichuris trichiura*) and hookworms (*Necator americanus* or *Ancylostoma duodenale*) (Strunz et al., 2014). Almost one-quarter of the earth's population is affected with STHs infections (Steinbaum et al., 2016). Around 1049 million persons were estimated to harbour *T. trichiura* eggs, including 114 million pre-school children and 233 million school children (Stephenson et al., 2000) while *A. lumbricoides* is the most prevalent STH, with an estimated world’s burden of 820 million infections (Pullan et al., 2014).

For decades STHs have been of concern throughout the world among the poorest and the deprived communities (Ahmed et al., 2011). The presence of STHs eggs can be transmitted via contaminated soil. The whipworm (*Trichuris trichiura*), the roundworm (*Ascaris lumbricoides*) and the hookworms (*N. americanus* and *A. duodenale*) are the primary species that affect humans (Regions and Assembly, 2017).
STHs enter the human body via fecal-oral contamination of the worms’ eggs or their larvae (Ahmed et al., 2006). The T. trichiura eggs hatch in the small intestine where the larvae spend some time before moving to their colonization of the cecum. Adult worms of A. lumbricoides live in the lumen of the small intestine where the female lays unembryonated eggs which are excreted in the faeces (Schule et al., 2014). In children, soiled finger nails and toys are general reservoirs for transmission (Schule et al., 2014).

STHs infections can lead to malnutrition, intellectual retardation, cognitive deficits, poor school performance, absenteeism, poor economic performance and increased susceptibility to other deadly diseases (Ahmed et al., 2006). High infectivity of T. trichiura infection can be manifested as Trichuris dysentery syndrome (TDS) with chronic dysentery, rectal prolapse, anaemia, weak growth, and clubbing of the fingers which can constitute a significant public health problem especially in children (Stephenson et al., 2000).

Previous research about STHs among the Orang Asli has been studied in remote Orang Asli villagers with the poor framework for modern latrine facilities, treated water supply and transport facilities (Nisha et al., 2016). Orang Asli children and women are susceptible to intestinal parasites mainly through soil-transmitted helminths (Nicholas, and Baer, 2007). Research conducted by Ulukanligil (2001) showed that the main source of transmission of STHs infection is the soil polluted with faecal contamination. Research conducted by Kinzie, and Tyas (1996), also showed that Orang Asli have a very high rate of infection with T. trichiura and A. lumbricoides.

Despite soil playing a main role in STHs transmission, only one previous study carried out in Malaysia indicated the role of stray dogs in transmitting STHs from soil (Azian et al., 2008) (Zain et al., 2015). Therefore, this study was conducted to investigate occurrence of STHs in various soil types during dry and wet weather in an Orang Asli village.

METHODS

Study design and study location
The study was conducted at Kampung Orang Asli Sungai Lalong Baru, Ulu Semenyih, Selangor, district of Hulu Langat (3.0549°N, 101.8714°E). Most of the villagers live in houses built from cement, bricks and zinc sheet metal roofs, while some of the villagers still live in wooden huts. Some houses have toilets outside the house while some houses do not have toilet facilities. As the village is located near the river, some of the villagers use the river as their source of water. However, the majority use the clean water provided by the department of water supply (Jabatan Bekalan Air (JBA)). Most of the villagers’ work as farmers and rubber tappers while some work at the nearby factories or construction sites, e.g. Resource Recovery Centre, (Recycle Energy S/B), Nirvana Memorial Garden and Nirvana Memorial Park, which are within 7km from the village). There is only one government clinic, i.e. Klinik Kesihatan Semenyih located close to the village.

Soil Sampling
Between August 2017 and October 2017, soil samples were collected at various locations from Kampung Orang Asli Sungai Lalong Baru Ulu Semenyih, Selangor. Forty soil samples were taken from the village area; such as housing area (=20), river bank (n=5), pond area (n=5), fields (n=6) and along the roadside (n=4). Various soil types like sandy, loamy and clay soil were collected for the examination during wet season (September-October; 27°C; 290mm rainfall) and dry season (August; 31°C).

Detection on STHs in Soils Samples
Each soil specimen (approximately 200-250 g of soil) was collected from a depth of 6-8 cm in an area that was not exposed to direct sunlight. Each soil sample was collected from the soil surface with a hand shovel and stored in a sealed, labelled polythene bags. Next, the polythene bags were kept in a polystyrene box filled with ices which were then
transported to laboratory chiller. Prior to processing, the entire soil specimens were air dried at room temperature followed by formalin ethyl concentration technique. About 2 grams of soil was emulsified in 10 ml distilled water and strained through two layers of gauze in a funnel. The filtrate was centrifuged at 1000 (rpm) for 7 minutes. The supernatant was discarded, and the sediment was suspended in 7 ml of formalin (10%) and allowed to stand for 10 minutes. Next, 3 ml of ethyl alcohol was added to the suspension and vortexed. The stopper was then removed and centrifuged at 1000 (rpm) for 7 minutes. The tube was allowed to rest in a stand for 5 minutes. Four layers consisting of ethyl plug of debris, formalin and soil sediment containing STHs (from top layer to bottom layer) was used for observation under the microscope. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was poured off leaving a small amount of formalin for suspension of the sediment. It was poured on a clean glass slide, covered with coverslip and examined under the microscope. The plug of debris was graded from 1 to 4 whereby; (1): None- no individual grains are felt and no abrasive feeling, (2): Some gritty- some grains can be felt, (3): Gritty- abrasive is easily felt, (4): Very gritty- individual grains that can easily be seen and felt. Next, smoothness is a quality exhibited by some soils kneading between the thumb and forefinger or palms. It was graded: (1) somewhat smooth- smooth feeling but some grittiness felt, (2) Smooth- very little grittiness felt and (3) Very smooth- no grittiness felt (Table 1).

Table 1. Identification of Various Soil Types

<table>
<thead>
<tr>
<th>Soil Types</th>
<th>Grittiness</th>
<th>Smoothness</th>
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<tbody>
<tr>
<td>Sandy</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Loamy</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Clay</td>
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RESULTS

A total of 40 soil samples were collected from various locations from Kampung Orang Asli Sungai Lalong Baru, Ulu Semenyih, Selangor. Total number of *A. lumbricoides* detected was 36 (90%) and *T. trichiura* was 6 (15%) out of 40 sample collected. Each of the study sites had different contamination rate for *Ascaris* spp. and *Trichuris* spp. The entire collected soil sample showed positive for *A. lumbricoides*; housing area n=16, river bank, n=5, fields n=6, nearby pond n=5 and roadside n=4. Where else, for *T. trichiura* the contamination was slight lower than that of *A. lumbricoides*. Only three sites of collection were positive; housing area n=3, nearby pond n=2 and roadside n=1 (Figure 1).

Figure 2 shows presence of *A. lumbricoides* and *T. trichiura* in different types of soil namely loamy soil, clay soil and sandy soil. *Ascaris* was detected in all the soil types, sandy soil n=9 (25%), loamy soil n=14 (39%) and clay soil n=13 (36%). However, all the *Trichuris* was only detected in loamy soil n=6 (100%).
Figure 1. Occurrence of Soil Transmitted Helminths (STHs) at each site.

Figure 2. Comparison of *Ascaris lumbricoides* and *Trichuris trichiura* in different soil types.
Figure 3 shows occurrence of the helminths eggs during wet and dry season. Presence of *Ascaris* in dry season was 25 (69%) while during wet season was 11 (31%). Meanwhile, *Trichuris* was only observed during wet season and none was found during dry season.

**DISCUSSION**

This soil sample screening indicated the presence of soil transmitted helminths (STHs), *A. lumbricoides* (n=36,90%) and *T. trichiura* (n=6,15%) in various soil types collected from Sungai Lalang Baru, Ulu Semenyih, Selangor. Hookworm was not detected in this area similar to observation found in stool samples collected from the villagers (data not published). Previous surveys carried out in Malaysia involving soil samples, indicated mainly presence of *Toxocara* (9.1%) species in urban and rural areas due to stray dogs (Azian et al., 2008) and another findings indicated more than half of the stray cat and dog populations were infected with parasites, namely *Toxocara* spp. and *Ancylostoma* spp., an *Isospora* and *Spirometra* spp. (Zain et al., 2015). However, these studies did not characterise the soil types. It is speculated that the significant geographic variability based on the soil characteristics could attribute to STHs infections in both urban and rural area in Malaysia (Steinbaum et al., 2016). Earlier studies on soil sampling for STHs have used the floatation techniques (Horiuchi et al., 2013) (Zain et al., 2015). However, on a comparative note floatation technique yielded high variations in results; hence the sedimentation technique was used due to steady reproducibility.

The soil characteristics could attribute the load of STHs present in various soil types. Overall, we found the presence of *A. lumbricoides* to be much higher than *Trichuris trichiura*, similar to the previous study in Malaysia (Azian et al., 2008). The presence of helminthes eggs in the soil is subjected to fecal-oral contamination by humans/animals as the main source of transmitting these infections (Odobà et al., 2012). Contamination of *A. lumbricoides* was highest in loamy soil similar to another study carried out in Nigeria (Nwoke et al., 2013).
*A. lumbricoides* is found to be high in regions such as fields, nearby river, nearby pond and along the roadside. This is similar to previous findings in Lodz District, Poland where 87.7% of *A. lumbricoides* detected from soils sample taken at the fields (Blaszkowska et al., 2011).

Our study indicated higher prevalence of *A. lumbricoides* in dry season similar to a previous study conducted in Nepal. It was suggested that the dry season could be favourable for the development of *A. lumbricoides* (Rai et al., 2000). Other researchers mentioned that, since Malaysia is a tropical country with alterations of dry and wet seasons and optimum temperatures for STHs survival with high levels of humidity in the soil, the transmission of helminths from the soil under the above stated favourable conditions enhances the survival and viability of *Ascariis* and *Trichuris* eggs (Tun et al., 2015) (Zain et al., 2015).

*T. trichiura*, was found in housing areas (15%) and the nearby pond area (40%). This result is similar with previous finding in southern Thailand (Chongsuvivatwong et al., 1999), and in the Philippines (Horiuchi et al., 2013) which had prevalence between 15%-40%.

The presence of *T. trichiura* in soil could be due to the dogs and cats being kept domesticated by the villagers. During the sample collection period, we observed most of the villagers having more than one dog or cat as pets in each household. These animals can pose as potential carriers for *T. trichiura* and help in transmission to the soil in around the village. Azian et al. (2008) found that the soil contamination might happen as dogs naturally defecate around the household which are not protected with a fence. Children without footwear during outdoor activities and without proper hand hygiene are highly exposed to STHs. In this study, we observed the villagers practiced gardening and farming which can facilitate transmission form soil using bare hands which creates a higher risk of getting in direct contact with the STHs. Studies showed STHs infection in humans can also be caused due to consumption of raw vegetables and fruits contaminated with the parasite’s eggs (Klapeć and Borecka, 2012).

According to Azian et al. (2008) it is important to have a good sampling method for the collection of the soil samples so as to get good results. For instance, soil samples collected from a depth of 4-6 inch could be ideal for finding parasite eggs. According to Weaver et al. (2010), climatic changes, soil temperature and humidity are the determining factors that can influence the presence of parasites directly.

Occurrence of *T. trichiura* eggs were seen in the loamy but not in clay and sandy soil. This result is supported by Steinbaum et al. (2017) where STHs eggs viability and recovery efficiency relied on the soil texture. Cooper et al. (1995) also stated that STHs are primarily to be found in loamy and sandy soils but cannot live in clay soils. The author concluded that moist and loamy soils are the most suitable soils for STHs growth where STHs eggs need warm soil, temperatures over 18°C to inhabit.

According to Brooker et al. (2004), *T. trichiura* eggs are non-motile when the soil is exposed to high temperatures, which can cause the parasites’ ova/eggs to die from desiccation. Therefore, clay and sandy soils samples collected from areas that had direct sun light had negative results. Previous studies showed that areas having maximum land surface temperature exceeding 36-37°C had low prevalence of STHs (Brooker et al., 2004). This indicates that STHs eggs have a different limit of resistance to dryness. Therefore, this concluded that *T. trichiura* has low resistance toward high temperature.

**CONCLUSION**

The results of the present study showed that the soil in the environment at Kampung Sungai Lalang was contaminated with *A. lumbricoides* and *T. trichiura*. The presence of contaminant STHs eggs plays an important role affecting the health to the villagers. Hence, an educational campaign on adverse effects of *T. trichiura* and *A. lumbricoides* infections for the villagers need to be
constantly encouraged by the health authorities to practise good sanitary habits to reduce parasitic infections. The role of domestic animals can be of additional risk in transmitting STHs. The screening of soil samples can be also used as a quality control to check the sanitation conditions in the environments.

REFERENCES


