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Detection of *Ascaris lumbricoides* and *Trichuris trichiura* in various soil types from 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 in 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 Lalang 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 Lalang 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 after mixing with a drop of formalin (Al-Khamesi *et al.*, 2014). The positive samples were calculated using formula as described by Rai *et al.*, 2000 as stated below:

$$\text{Positive (\%)} = \frac{\text{Number positive samples}}{\text{Total of samples}} \times 100\%$$

Soil Texture Determination

The soil texture was categorized as previously described by Steinbaum *et al.* (2017) based on the USDA soil texture triangle. The soil texture was determined by the smoothness and grittiness of the soil in three different categories, i.e. clay, loam, and sandy soil. The soil texture is determined by rubbing the soil between fingers to determine the sand and silt content in the soil. Soil that has high sand content is gritty, and soil with high silt content is very smooth. Grittiness is the abrasive action felt by the thumb and fore finger or palm. Grittiness 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

Soil Types	Grittiness	Smoothness
Sandy	4	1
Loamy	3	2
Clay	2	3

RESULTS

A total of 40 soil samples were collected from various locations from Kampung Orang Asli Sungai Lalang 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%).

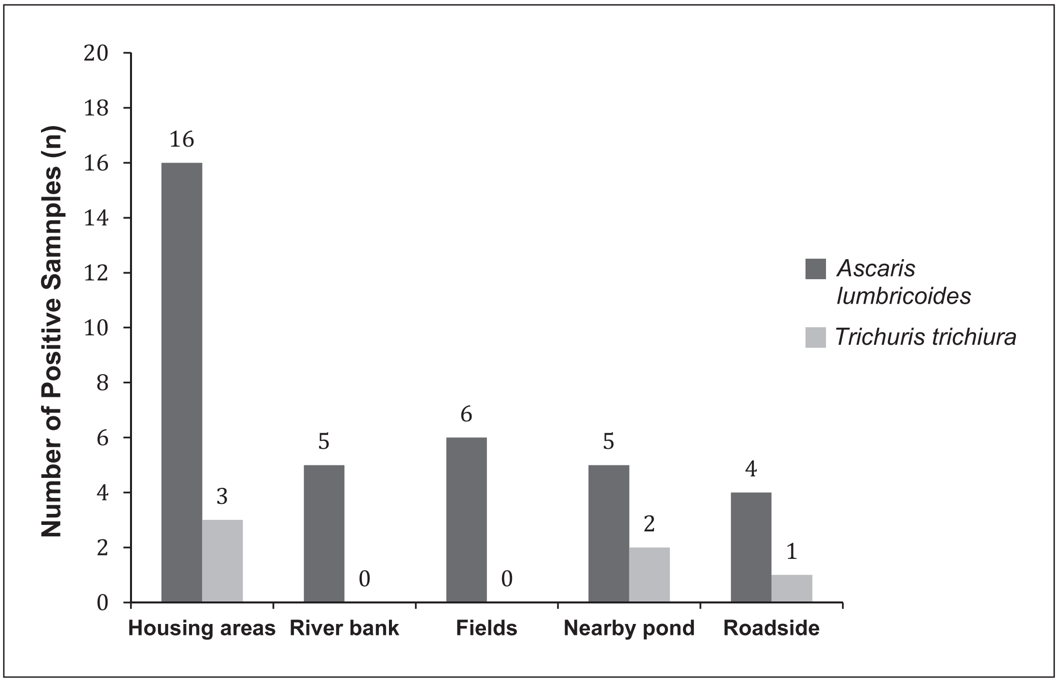

Figure 1. Occurrence of Soil Transmitted Helminths (STHs) at each site.

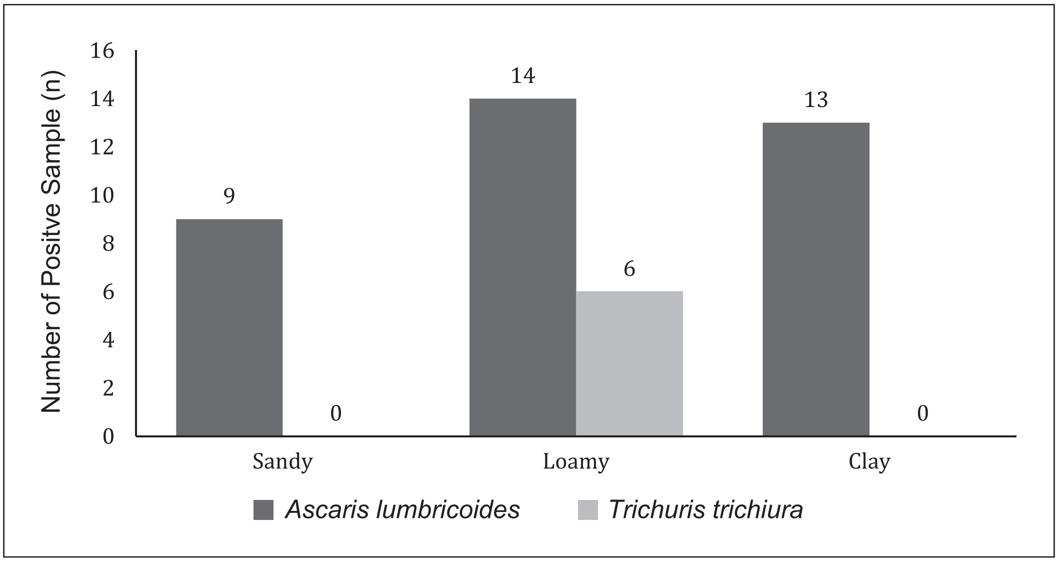

Figure 2. Comparison of *Ascaris lumbricoides* and *Trichuris trichiura* in different soil types.

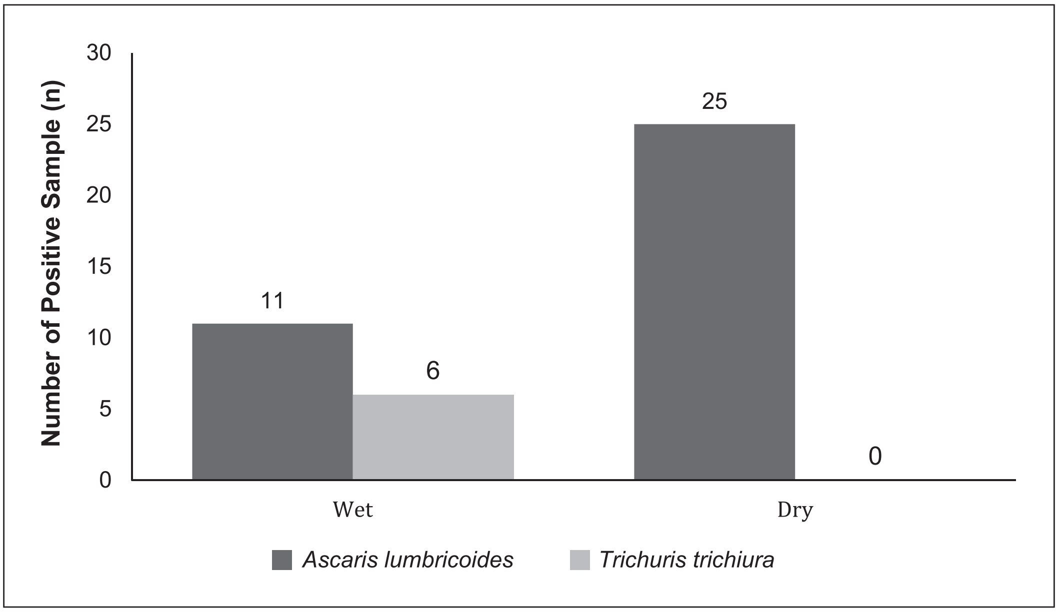

Figure 3. Occurrence of the helminths eggs during wet and dry season.

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 flotation 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 (Odoba *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 *Ascari*s 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 (Kłapeć 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. lumbicoides* 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

Ahmed, A., Al-mekhlafi, H.M., Choy, S.H., Ithoi, I. & Al-adhroey, A.H. (2011). The burden of moderate-to-heavy soil-transmitted helminth infections among rural malaysian aborigines: an urgent need for an integrated control programme, *Parasite Vectors* **4**: 242.

Ahmed, A., Al-mekhlafi, H.M., Surin, J. & Lumpur, K. (2006). Review epidemology of soil-transmitted helminthiases in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **42**: 527-538.

Azian, M.Y., Sakhone, L., Hakim, S.L., Yusri, M.Y., Nurulsyamzawaty, Y., Zuhaizam, A.H., Maslawaty, M.N. (2008). Detection of helminth infections in dogs and soil contamination in rural and urban areas. *Southeast Asian Journal of Tropical Medicine and Public Health* **39**: 205-212.

Al-khamesi, M.B. (2014). Isolation and Diagnosis of Parasites from Different Soils in Baghdad City, *Journal of Al-Nahrain University* **17**: 155-161.

Brooker, S., Kabatereine, N.B., Tukahebwa, E.M. & Kazibwe, F. (2004). Spatial analysis of the distribution of intestinal nematode infections in Uganda. *Epidemiology Infection* **132**: 1065-1071.

Blaszkowska, J., Kurnatowski, P. & Damiecka, P. (2011). Contamination of the soil by eggs of geohelminths in rural areas of Lodz district (Poland). *Helminthologia* **48**(2): 67-76.

Chongsuvivatwong, V., Uga, S. & Nagnaen, W. (1999). Soil contamination and infections by soil-transmitted helminths in an endemic village in southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **30**: 64-67.

Cooper, E.S. (1995). Epidemiology and transmission of intestinal helminths. In: Farthing MJG, Keusch GT, Wakelin D (eds) Enteric infection 2. Intestinal helminths. Chapman & Hall Medical, 201-2012.

Horiuchi, S., Paller, V.G. & Uga, S. (2013). Soil contamination by parasite eggs in rural village in the Philippines. *Tropical Biomedicine* **30**: 495-503.

Klapec, T. & Borecka, A. (2012). Contamination of vegetables, fruits and soil with geohelmints eggs on organic farms in Poland. *Annals of Agricultural and Environmental Medicine* **19**: 421-425.

Kinzie, J., Kinzie, K. & Tyas, J. (1996). A comparative health survey among two groups of Malayan Aborigines. *Medical Journal of Malay* **21**: 135-139.

Mehru Nisha, Versingam Kumarasamy, Ambu S., Fabian Davamani & Mak Joon-Wah. Factors Associated with Intestinal Parasite Infections in a Resettled Indigenous Community in Malaysia. *International Journal of Tropical Disease and Health* **12**: 1-7.

Nicholas, C. & Baer, A. (2016). Health care for the Orang Asli: consequences of paternalism and colonialism. *Kuala Lumpur*, 119-136.Centers for Disease Control and Prevention (CDC). *Parasites Ascariasis*-Soil-Transmitted Helminths (STHs). http://www.cdc.gov/dpdx/parasites/ascariasis/

Nwoke, E.U., Ibiam, G.A., Odikamnoro, O.O., Umah, O.V., Ariom, O.T. & Orji, I. (2013). Examination of soil samples for the incidence of geohelminth parasites in Ebonyi north-central area of Ebonyi State, south-east of Nigeria. *Applied Science Research* **5**: 41-48.

Odoba, M.B., Otalu, O.J., Balogun, J.B. & Anglais, A.E. (2012).Prevalence of Helminth Parasite Eggs in Pupils and Playing Grounds of some Selected Primary Schools in Zaria , Nigeria. *World Journal of Life Sciences and Medical Research* **2**: 192-195.

Pullan, R.L., Smith, J.L., Jasrasaria, R. & Brooker, S.J. (2010). Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasites & Vectors* **7**: 37.

Rai, S.K., Uga, S., Ono, K., Rai, G. & Matsumura, T. (2000). Contamination of soil with helminth parasite eggs in Nepal. *Southeast Asian Journal of Tropical Medicine and Public Health* **31**: 388-393.

Regions, W.H.O. & Assembly, W.H. (2017). Global Health Observatory (GHO) data Soil transmitted helminthiases. 2–3.

Schule, S.A., Clowes, P., Kroidl, I., Kowuor, D.O., Nsojo, A., Mangu, C. & Saathoff, E. (2014). *Ascaris lumbricoides* Infection and Its Relation to Environmental Factors in the Mbeya Region of Tanzania, a Cross-Sectional, Population-Based Study. *PLoS ONE* **9**: 1-10.

Steinbaum, L., Kwong, L.H., Ercumen, A., Negash, M.S., Lovely, A.J., Njenga, S.M. & Nelson, K.L. (2017). Detecting and enumerating soil-transmitted helminth eggs in soil: New method development and results from field testing in Kenya and Bangladesh. *PLoS Neglected Tropical Diseases* **11**: 1-15.

Steinbaum, L., Njenga, S.M., Kihara, J., Boehm, A.B., Davis, J., Null, C. & Pickering, A.J. (2016). Soil-Transmitted Helminth Eggs Are Present in Soil at Multiple Locations within Households in Rural Kenya. *PLoS One* **11**: e0157780.

Stephenson, L.S., Holland, C.V. & Cooper, E.S. (2000). The public health significance of *Trichuris trichiura*. *Parasitology* **121**: S73-S95.

Strunz, E.C., Addiss, D.G., Stocks, M.E., Ogden, S., Utzinger, J. & Freeman, M.C. (2014). Water, Sanitation, Hygiene, and Soil-Transmitted Helminth Infection: A Systematic Review and Meta-Analysis. *PLoS Medicine* **11**: e1001620.

Tun, S., Ithoi, I., Mahmud, R., Samsudin, N.I., Heng, C.K. & Ling, L.Y. (2015). Detection of helminth eggs and identification of hookworm species in stray cats, dogs and soil from klang valley, Malaysia. *PLoS ONE* **10**: 1-12.

Ulukanligil, M., Seyrek, A., Aslan, G., Ozbilge, H. & Atay, S. (2001). Environmental pollution with soil-transmitted helminths in Sanliurfa, Turkey. *Memórias do Instituto Oswaldo Cruz* **96**: 903-909.

Weaver, H.J., Hawdon, J.M. & Hoberg, E.P. (2010). Soil-transmitted helminthiases: implications of climate change and human behavior. *Trends in Parasitology* **26**: 574-581.

WHO. Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected diseases. (2015). *Third WHO Report on Neglected Diseases*, https://doi.org/ISBN 978 92 4 1564861

Zain, S.M., Rahman, R. & Lewis, J.W. (2015). Stray animal and human defecation as sources of soil-transmitted helminth eggs in playgrounds of Peninsular Malaysia. *Journal of Helminthology* **89**: 740-747.