

HIDDEN IN PLAIN SIGHT: URBAN PLAYGROUNDS AS POTENTIAL RESERVOIRS OF SOIL-TRANSMITTED HELMINTHS

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ABSTRACT

Neglected soil-transmitted helminthiasis (STHs) remain an underrecognized public health concern in Malaysia, particularly in urban environments where exposure risks are often underestimated. This study assessed the prevalence and species diversity of helminth egg contamination in urban neighbourhood playgrounds in Shah Alam, Malaysia. A total of 84 soil samples and 15 faecal samples were collected and examined microscopically for helminth ova, followed by species-specific polymerase chain reaction (PCR) to identify parasites of public health significance. Helminth ova were detected in 50.0% (42/84) of soil samples, with contamination rates ranging from 47.0% to 60.0% across sampling sites. Faecal samples showed a higher positivity rate of 73.3% (11/15). Hookworms and unidentified helminth ova predominated in both sample types, with mixed infections more frequently observed in faecal samples. Among microscopy-positive soil samples, PCR detected *Ascaris lumbricoides* in one sample (2.4%) and *Strongyloides stercoralis* in one sample (2.4%), while most samples (95.2%) remained PCR-negative and were classified as unidentified species. In faecal samples, *Ancylostoma duodenale* was predominant (63.7%), followed by *Trichuris trichiura* (27.3%) and *S. stercoralis* (9.1%). Soil physicochemical analysis showed that most contaminated soils were acidic (85.7%, pH < 7.0) with low moisture content (61.9%, <10%), while loamy soils had the highest contamination rate (72.1%). These findings highlight urban playgrounds as potential reservoirs for STH transmission and emphasise the need for improved surveillance and targeted public health interventions in urban communities.

KEYWORDS: Soil Transmitted Helminths (STH), urban playground, Polymerase Chain Reaction (PCR)

INTRODUCTION

Soil-transmitted helminths (STHs) are a group of neglected tropical diseases that continue to pose a substantial public health burden in low- and middle-income countries, particularly in tropical and subtropical regions (1). Globally, the World Health Organization (WHO) estimates that more than 1.5 billion people—approximately 24% of the world's population—are infected with STHs, with the highest burden reported in Africa, Asia, and Latin America (1–3). Children are disproportionately affected, with over 260 million preschool-aged children and 654 million school-aged children living in areas of intense STH transmission (1). The major human STHs include the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*. Among these, *A. lumbricoides* is the most prevalent species worldwide, infecting an estimated 1.2 billion people, followed by *T. trichiura*, which affects approximately 795 million individuals (4). Collectively, more than one billion people are estimated to be at risk of infection with these parasites, which are associated with malnutrition, impaired cognitive development, anaemia, and reduced educational performance, particularly among children (4,5). In Malaysia, STH infections remain endemic despite improvements in sanitation and socioeconomic development. Several studies have documented widespread environmental contamination with STH ova, demonstrating considerable geographic variability across both rural and urban settings (6–9).

Soil contamination is of particular concern as it represents a key reservoir for transmission, especially in public spaces such as playgrounds, where children are frequently exposed through direct contact with soil. A study by Zain et al. (6) investigated soil samples from 60 playgrounds in urban and suburban areas across five states in Peninsular Malaysia reported STH contamination in all playgrounds examined. Ova from four *nematode genera* were detected, with *Toxocara* spp. being the most prevalent (95.7%), followed by *A. lumbricoides* (93.3%), *A. duodenale* (88.3%), and *T. trichiura* (77%) (6). The high prevalence of *Toxocara* ova was strongly associated with unrestricted access of stray cats and dogs, highlighting the role of animal reservoirs in environmental contamination. Similarly, a study conducted in Orang Asli settlements in Ulu Semenyih, Selangor, reported high levels of soil contamination, with prevalence of 90% for *A. lumbricoides* and 15% for *T. trichiura* (7). More recently, Debba et. al. (8) reported a prevalence of 32.5% for *Toxocara* ova in soil samples collected from public and neighbourhood playgrounds in Ipoh, Perak (8). Playgrounds are common features in modern cities and essential for promoting children's physical, social, and cognitive development while serving as communal spaces for families. However, these shared facilities may also function as reservoirs for pathogens, including soil-transmitted parasites, transmitted via contaminated soil, sand, or faecal–oral contact (10–13). Children are particularly vulnerable due to frequent hand-to-mouth behaviours and immature immune systems, with exposure commonly occurring through hand-to-mouth, hand-to-nose, and hand-to-eye contact (14).

Despite the recognised risk of acquiring soil-transmitted parasitic infections from contaminated playgrounds, data on the diversity and extent of soil-transmitted parasites in rapidly urbanising regions of Peninsular Malaysia remain limited. Information from central urban areas such as Shah Alam, Selangor, is scarce. Generating such data is important for assessing exposure risk among playground users, particularly children, and for informing targeted public health interventions, environmental management strategies, and community awareness programs. Therefore, this study was undertaken to determine the level of soil contamination with STHs in selected public playgrounds, with the aim of contributing evidence to support prevention and control efforts in urban settings.

MATERIALS AND METHODS

Site Selection

This cross-sectional study was conducted in selected neighbouring playgrounds in Shah Alam with active public use with abandoned playground were excluded as selection criteria. The observation of stray companion animal like dog and cat were included as selection criteria. Based on these criteria, four locations were selected as shown in Figure 1.



Figure 1. Geographic location of the study sites. Partial map of the Shah Alam area showing the four sampling stations selected for this study.

Soil and faecal sampling

Soil sampling method used was based on Nisha et al. and Azian et al. (7, 15) with minor modifications. A total of 84 soil samples were collected. Approximately 300 g of soil, specifically the surface layer (0-5 cm) of the ground was collected by using a hand shovel. The sampling soil was selected randomly per 5 m² of each sampling point which was not exposed to direct sunlight. Each sample was then placed in a zip lock bag with ID number and general descriptions. The samples were brought back to the laboratory for processing. In addition, 15 fresh faecal samples were collected from two neighbourhood playground areas. Each faecal sample was carefully transferred into clean, labelled containers, put in the polystyrene container, and transported to the laboratory for further microscopic and molecular analyses.

Soil texture, pH, and moisture analysis

Soil texture was classified into four categories following the approaches described by Paller and de Chavez (16) and the hand texturing (“feel”) method outlined by Lesikar et. al. (17). Briefly, each soil sample was moistened and kneaded into a bolus prior to manual texture assessment. Based on tactile characteristics, samples were categorised as sandy, silty, loamy, or clayey (8, 16,17). Soil pH was determined following the method of Olabiyi et al. (18) with minor modifications. A saturated soil paste was prepared by mixing 10 g of soil with 20 mL of distilled water (pH 7.0). The suspension was stirred at 15 rpm for 1 h to allow equilibration, after which pH was measured using a calibrated pH meter. The instrument was standardised using acidic and alkaline buffer solutions prior to measurement (18).

Soil moisture content was determined using the gravimetric method as described by Paller and de Chavez (2014) (18). Briefly, the empty weight of each jar was first tared. The pre-dried weight of the soil sample in the jar (W_0) was measured prior to oven drying at 100°C for 24 hours. The oven-dried weight (W_1) of each sample was then recorded. The moisture content (%) of the soil was calculated using Equation below:

$$\frac{(W_0 - W_1)}{W_1} \times 100 \%$$

Sample processing and microscopy analysis

Approximately 50 g of soil samples were sequentially sieved through 300 µm, 150 µm, and 20 µm sieves, with parasite ova typically retained on the 20 µm sieve. The retained material was washed into a 50 mL centrifuge tube, centrifuged at 3,000 × g for 5 min, and followed by SAF sedimentation procedure. Briefly, 1–2 g of sediment was suspended in 10 mL SAF, strained through double-layered gauze, mixed with diethyl ether (3–4 mL), shaken, and centrifuged at 500 × g for 5 min. The upper layers were discarded, and the sediment resuspended in SAF for microscopic examination at 10× and 40× objective (19). Faecal samples were processed using the Mini-System Paragreen Fecal Parasite Concentrator Kit (Amerigo Scientific, USA). One scoop of fecal material was homogenised in a centrifuge tube, centrifuged at 700 × g for 3 min, and the supernatant discarded. The sediment was resuspended in a drop of normal saline, and wet mounts were prepared on clean glass slides. A small volume of the suspension was examined microscopically using 10× and 40× objective for the detection of parasitic ova.

Polymerase Chain reaction (PCR)

The assay targeted parasites of major public health importance, including *T. trichiura*, *N. americanus*, *A. lumbricoides*, *A. duodenale*, *S. stercoralis*, *T. canis*, *T. cati*, *F. hepatica*, and *F. gigantica*. Master mix preparation and PCR conditions were adapted from previously published protocols (20-23). PCR amplicons were analysed using the QIAxcel system, and samples exhibiting bands of the expected sizes were considered positive. The expected size *A. duodenale* (156 bp), *N. americanus* (225 bp), *A. lumbricoides* (334 bp) *T. trichuria* (518 bp), *S. stercoralis* (680 bp), *T. canis* (208 bp), *T. cati* (325 bp), *F. hepatica* (304 bp), *F. gigantica* (752 bp).

RESULTS

High Prevalence of Helminth Contamination in urban Playground Environments

Environmental contamination was widespread across the surveyed playgrounds. Microscopic analysis revealed an overall helminth prevalence of 50.0% (42/84) in soil samples and 73.3% (11/15) in faecal samples. Soil prevalence varied by site, ranging from 45.0% to 60.0%, with the highest prevalence observed at Playground C. In faecal samples, prevalence reached 100% at Playground B and 56.0% at Playground C.

Table 1. Overall prevalence of ova contamination in different sampling site from soil and faecal samples by microscopy.

Type of sample	Sampling site	No of samples(n)	Percentage of positive parasite (%)
Soil	Playground A	20	9/20(45.0 %)
	Playground B	24	12/24(50.0%)
	Playground C	20	12/20(60.0%)
	Playground D	20	9/20(45.0%)
Faecal	Playground B	6	6/6 (100.0%)
	Playground C	9	5/9 (56.0%)

Parasitological and Molecular Findings in Soil and Faecal Samples

Microscopy revealed predominantly unidentified helminth ova, either as single infections or in combination with hookworms and *Ascarididae* ova, with mixed infections detected in both soil and faecal samples. PCR analysis of microscopy-positive samples identified *A. lumbricoides* and *S. stercoralis* at low frequencies in soil samples (each 2.4%), while 95.2% remained negative for targeted species. Faecal samples showed a high prevalence of *A. duodenale* (63.6%) and *T. trichiura* (27.3%) and *S. stercoralis* (9.1%). No molecular evidence of *N. americanus*, *Toxocara* spp., or *Fasciola* spp. was detected in either sample type.

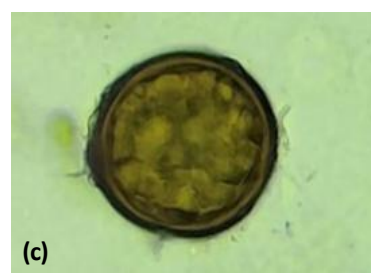
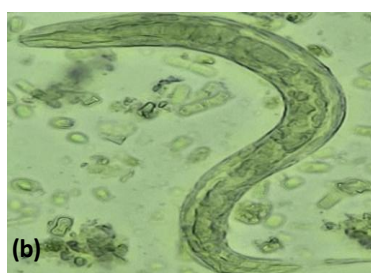




Figure 2. Representative images of helminth ova and larvae recovered from contaminated soil and fecal samples collected from the selected playgrounds. (a) Embryonated hookworm ovum; (b) Rhabditiform larva; (c) Decorticated *Ascaris* ovum; (d) Unidentified ovum; (e) *Fasciola* spp. ovum; and (f) Hookworm ovum.

Table 2. Microscopic examination: Occurrence of single and multiple infections of parasitic ova/ rhabditiform in samples from selected playground area.

Sample type(n)	Species identified	Frequency
Soil (n = 84)	<i>Ascarididae</i> ova	1
	Hookworm's ova	1
	Unknown ova	36
	Hookworm's ova + Unknown larvae	3
	<i>Ascarididae</i> ova + Hookworms ova	1
	Total positive (%)	42/84 (50.0%)
Faecal (n = 15)	<i>Fasciola</i> spp.	1
	Hookworm's ova + Unknown ova	2
	Hookworm's ova	3
	Unknown larvae	3
	<i>Ascarididae</i> ova, + hookworms ova	1
	<i>Ascarididae</i> ova + Hookworms + Unknown spp.+ <i>Strongyloides</i> spp. rhabditiform	1
	Total positive (%)	11/15 (73.3%)

Table 3. PCR examination: Occurrence of helminth ova of public health importance detected by species-specific PCR in soil and faecal samples. Samples yielding negative results for all targeted species were classified as 'Negative for targeted species', indicating the absence of amplification for the species included in the assay and potentially reflecting the presence of non-target species or genetic variants not recognised by the primer sets. Individual samples may harbour multiple helminth species.

Sample type	Helminth species	Positive samples, n/N (%)
Soil (n = 42)	<i>A. lumbricoides</i>	1/42 (2.4)
	<i>S. stercoralis</i>	1/42 (2.4)
	Negative for targeted spp.	40/42 (95.2)
	<i>Fasciola</i> spp.	0/42 (0)
	<i>Toxocara</i> spp.	0/42 (0)
	<i>A. duodenale</i>	0/42 (0)
	<i>N. americanus</i>	0/42 (0)
	<i>T. trichiura</i>	0/42 (0)
Faecal (n = 11)	<i>A. duodenale</i>	7/11 (63.6)
	<i>T. trichiura</i>	3/11 (27.3)
	<i>S. stercoralis</i>	1/11 (9.1)
	<i>A. lumbricoides</i>	0/11 (0)
	<i>Toxocara</i> spp.	0/11 (0)
	<i>N. americanus</i>	0/11 (0)
	<i>Fasciola</i> spp.	0/11 (0)

Soil physicochemical characteristics influence contamination rate

Parasitic contamination was heavily concentrated in acidic soils (pH < 7.0), which yielded 85.7% (n = 36) of all isolates, including both identified helminth species (i.e. *A. lumbricoides* and *S. stercoralis*), compared to neutral or alkaline conditions (pH ≥ 7.0, n=6, 14.3%). Regarding moisture content, soils with lower moisture (< 10.0%) exhibited a higher frequency of parasitic occurrences (n = 26, 61.9%) than higher-moisture soils (≥ 10.0%, n = 16, 38.1%), though *A. lumbricoides* was exclusively isolated from the higher-moisture cohort. Soil texture analysis demonstrated clear differences in contamination rate, with loamy soils harbouring nearly three-quarters of all parasitic organisms (n = 31, 73.8%) and all species-specific isolates, whereas silty soils accounted for only 26.2% (n = 11) of the total yield.

Table 4. Physicochemical characteristics of soil specimens collected from the study area and PCR positivity rate of contaminated samples.

Parasite	Soil Characteristic						
	Soil pH		Soil Moisture (%)		Soil Type		
	≥ 7.0	< 7.0	≥ 10.0	< 10.0	Silty	Loamy	Total Occurrences
<i>A. lumbricoides</i>	0	1	1	0	0	1	1 (2.4%)
<i>S. stercoralis</i>	0	1	0	1	0	1	1 (2.4%)
<i>Negative for targeted spp.</i>	6	34	15	25	11	29	40 (95.2%)
<i>Fasciola. spp.</i>	0	0	0	0	0	0	0 (0.0%)
<i>Toxocara. spp.</i>	0	0	0	0	0	0	0 (0.0%)
<i>A. duodenale</i>	0	0	0	0	0	0	0 (0.0%)
<i>N. americanus</i>	0	0	0	0	0	0	0 (0.0%)
<i>T. trichiura</i>	0	0	0	0	0	0	0 (0.0%)
Total Parasite Count	6	36	16	26	11	31	42 (100.0%)

DISCUSSION

Soil-transmitted helminth (STH) infections are strongly associated with socioeconomic conditions, particularly inadequate sanitation and poor environmental hygiene (24-26). As a result, most epidemiological studies have focused on rural and peri-urban settings, while urban environments remain relatively understudied due to assumptions of better infrastructure and living standards. Nevertheless, environmental contamination is a critical indicator of ongoing transmission, and systematic surveillance in urban settings is essential to inform evidence-based public health interventions. In the present study, helminth contamination was detected in soil samples from all four selected playgrounds in Shah Alam, with an overall prevalence of 50.0% (42/84). In addition, 73.3% (11/15) of faecal samples contained at least one parasite egg. These findings demonstrate that urban recreational areas can act as reservoirs for helminths, posing potential exposure risks, particularly to children with frequent soil contact. Given that many helminth ova can remain viable in soil for up to two years under favourable conditions, such environments may sustain long-term transmission (27,28). Previous studies highlight that these contaminated environments typically harbour a mixture of human and zoonotic helminths, including *A. caninum*, *A. braziliense*, *A. suum*, *S. stercoralis*, and *T. vulpis*. (29)

However, species-level identification based solely on microscopy is inherently challenging. Morphologically similar ova, such as those of *A. ceylanicum* and *A. caninum*, are indistinguishable from human hookworm ova, while *T. vulpis* ova may be misidentified as *T. trichiura* due to overlapping dimensions. Identification is further complicated by variations in egg developmental stages and the

presence of morphologically similar free-living nematodes; for example, rhabditiform larvae of *S. stercoralis* closely resemble non-parasitic nematodes. Differentiation of human *S. stercoralis* from other *Strongyloides* species also requires meticulous observation and is prone to misidentification (29,30). Although some detected parasites may originate from animals, their presence in public spaces remains a significant public health concern. To overcome these limitations, molecular methods were applied for species-level identification and discrimination between human and zoonotic parasites. A relatively high proportion of helminths remained negative to tested species, likely reflecting the diversity of environmental parasites and the limited availability of species-specific PCR assays. Notably, while *T. trichiura* ova were not observed microscopically, PCR confirmed their presence in three samples, suggesting either very low egg burdens or the detection of residual DNA from degraded ova during sample processing.

Similarly, samples initially identified as *Strongyloides* spp. were molecularly confirmed as *S. stercoralis*. *S. stercoralis* is capable of infecting humans, dogs, cats, and non-human primates, and experimental studies have demonstrated cross-species infectivity, highlighting its zoonotic potential (31,32). While strongyloidiasis is endemic across Malaysia, it remains frequently underdiagnosed nationwide. Nevertheless, targeted screenings have revealed substantial localised prevalence rates ranging from 11.0% to 15.8% within vulnerable cohorts, such as rural indigenous longhouse residents and Orang Asli schoolchildren (33-35). Although infections are typically asymptomatic or subclinical in immunocompetent individuals, the parasite's unique capacity for internal autoinfection poses a severe threat if host immunity becomes compromised (34-36).

Progression to hyperinfection syndrome or disseminated strongyloidiasis carries a devastating systemic mortality rate exceeding 85%, presenting a critical risk to paediatric populations—particularly those experiencing malnutrition or undergoing immunosuppressive therapy (34-36). Despite increasing reports of human infections globally and regionally, data on canine infections and zoonotic transmission remain limited (37-39), highlighting the importance of a One Health approach. Additionally, ascarid ova detected in this study were PCR-negative for *A. lumbricoides*, *T. canis*, and *T. cati*, suggesting the presence of other animal ascarids. Ova resembling *Fasciola* spp. were also observed but were not identified as *F. hepatica* or *F. gigantica*, indicating possible involvement of other *Fasciola* species but not further explored in this study. Although only a small number of fresh faecal samples were collected, aged faecal deposits were widely observed across all playgrounds, likely due to unrestricted access and the absence of fencing that permits defecation by stray dogs and cats.

Previous studies have shown that physical barriers and routine removal of faecal matter are more effective in reducing environmental contamination than general maintenance alone (32). Soil texture also influenced helminth occurrence, with loamy soils exhibiting the highest contamination rate (72.1%). Loamy soil provides favourable aeration and moisture retention, supporting egg survival and larval development, whereas clayey soils may limit oxygen availability (40). Loamy and sandy soils are also preferred defecation sites for animals due to their loose texture, which facilitates deeper burial of faeces and prolonged egg viability (41-43). Soil pH and moisture further influenced helminth distribution. Among positive soil samples, 85.7% exhibited mildly acidic conditions (pH <7.0), consistent with the broad pH tolerance of many STHs; for example, hookworm ova can remain viable across a pH range of 4.6–9.4 (44).

Notably, *Toxocara* spp., which preferentially persist under moist and alkaline conditions, were not detected, suggesting that the study environment may have been less conducive to their survival. In contrast, *A.lumbricoides* was recovered exclusively from soils with higher moisture content, underscoring the species' dependence on adequate moisture for embryonation and larval development. Although optimal STH development is generally associated with high soil moisture levels characteristic of tropical environments (16,18), 61.9% of positive samples in this study exhibited moisture content below 10%, indicating that moderate soil dryness does not necessarily inhibit parasite survival, particularly for resilient developmental stages such as *S. stercoralis* rhabditiform larvae.

Limitation of study

This study was limited by the unavailability of target-specific positive control DNA and the absence of sequencing-based confirmation of PCR products. Nevertheless, assay performance was monitored using rigorous negative controls in each run, with no evidence of contamination or false-positive amplification observed. Identification of PCR products was based on the highly precise amplicon sizing capability of the QIAxcel system, with observed fragment sizes corresponding exactly to those reported in validated reference protocols. While these measures support the specificity of the assay, the absence of positive controls and sequence verification represents a methodological limitation. Future investigations should incorporate positive control material and amplicon sequencing to provide additional confirmation of target identity.

CONCLUSION

This study demonstrates that playground soil and faecal from stray animals act as significant environmental reservoirs for soil-transmitted helminths (STHs), including poorly characterised species with potential zoonotic relevance. The high environmental persistence of helminth ova that is capable of surviving for prolonged periods under favourable climatic conditions highlights the need for continuous, routine environmental surveillance to monitor contamination levels. Collectively, these findings underscore the importance of integrated public health interventions and targeted policies, including effective management of stray animals, improvements in playground sanitation and infrastructure, and strengthened community health education, to prevent soil-transmitted parasitic infections in urban environments.

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Data Availability: Not applicable.

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