Journal of Epidemiology and Global Health (2026) 16:12
https://doi.org/10.1007/s44197-025-00511-w

RESEARCH

Public Spaces as Hotspots of Zoonotic Gastrointestinal Parasite Transmission: Evidence from Small Animal and Soil Surveillance in Malaysia

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Received: 19 September 2025 / Accepted: 23 December 2025

Abstract

Background Public spaces such as parks and playgrounds offer social and ecological benefits to communities, but it might also pose public health risks. This epidemiological survey investigated the presence and risk factors of zoonotic parasites in faecal and soil samples collected from 60 public spaces across Kuala Lumpur and Selangor, Malaysia.

Methods Eggs, cysts and larvae of parasites were collected from 71 faecal and 300 soil samples using flotation-sedimentation techniques. Species identification was done using both morphological and conventional PCR.

Results A total of 71 faecal samples were collected, comprising 40 cat faeces and 31 dog faeces. Of these, 63 samples (88.7%) were positive for at least one intestinal parasite. The prevalence of parasite in cat faeces (95.0%, 38/40) was higher than dogs (80.7%, 25/31). Overall, six genera of parasites were detected in faecal samples. *Cystoisospora* spp. (62.0%) was the most prevalent, followed by hookworms (46.5%) and *Giardia* spp. (22.5%). Among the 33 faecal samples that tested positive for hookworms, *Ancylostoma ceylanicum* was the most common species (81.8%). Hookworm coinfections with *Cystoisospora* spp. were commonly detected in faecal samples (37.5%). Viability testing revealed that 37.7% of collected hookworm eggs and 74.6% of collected *Toxocara* eggs were viable. In soil samples, hookworm larvae were found in 28 out of the 300 samples, and 16 out of 60 sampling sites (26.7%). Hookworm burdens were significantly higher in rural areas and beaches. Risk factor analysis revealed that the presence of dustbin was associated with reduced hookworm infections (OR=0.075, 95% CI: 0.007–0.520) in dogs. Higher odds of hookworms were found in cat faeces (OR=4.961, 95% CI: 1.10–25.98) and soil (OR=5.77, 95% CI: 1.54–20.26) from residential parks. Notably, faecal-soil concordance was observed at 43.8% sampling sites, all located in residential parks. It highlights that these areas are potential active transmission hotspots.

Conclusions These findings provide essential information for public health officials to develop targeted interventions to reduce the risk of transmission of zoonotic parasites in public spaces.

Keywords *Ancylostoma* · Co-infection · Gastrointestinal parasites · Hookworms · Public spaces · Risk factors

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1 Background

Dogs and cats are common reservoirs for a variety of zoonotic gastrointestinal parasites, including *Toxocara* spp., *Ancylostoma* spp., *Giardia* spp., *Toxoplasma gondii*, and *Cryptosporidium* spp. [11, 25]. These parasites are often shed in the faeces of infected animals. The infected faeces contaminate the environment and facilitate transmission to humans through contact with soil. This poses significant public health risks, especially to vulnerable populations such as young children, pregnant women and immunocompromised individuals.

Clinical manifestation of human infection ranges from acute to chronic. Some infected individuals remain asymptomatic [13]. When symptoms occur, they might have abdominal pain, diarrhoea, vomiting and nausea [5, 13]. As these symptoms are non-specific, diagnostic of gastrointestinal parasites can be challenging [27, 59]. Therefore, identifying the potential source of infection is crucial, especially in endemic tropical regions.

While numerous Malaysian studies have investigated the prevalence of gastrointestinal parasites in shelter and stray animals, relatively few have focused on the extent of faecal contamination and the presence of parasite in soil from public spaces [1, 24, 51, 56].

Public spaces such as parks, playgrounds, and recreational areas play a vital role in promoting physical and mental well-being [7, 17, 33]. However, sharing of these environments among humans and animals, particularly stray animals and free-roaming pets with untreated parasitic infections, has raised concerns about faecal contamination and transmission of zoonotic parasites [3, 46]. Soil in these spaces could serve as a long-term medium for the survival and development of infective stages of various parasites. For example, helminth eggs are capable for surviving in suitable environmental conditions for up to two years [18, 19].

Given these concerns, we hypothesized that public spaces are significant reservoirs for zoonotic gastrointestinal parasites. Therefore, we conducted a cross-sectional survey across 60 public spaces in Kuala Lumpur and Selangor to investigate the presence, distribution, and risk factors of zoonotic parasites in faeces and soil.

2 Methods

2.1 Study Design and Study Areas

This cross-sectional study was conducted between December 2023 and August 2024. A total of 60 public spaces were randomly selected across Kuala Lumpur and nine districts in Selangor (Fig. 1). Sampling sites were classified into five categories: dog parks as designated off-leash areas for dogs; apartment playgrounds as play areas within apartment complexes; resident parks as small community parks within housing areas; public parks as large open spaces managed by local authorities and freely accessible; and beaches as public coastal areas with potential access for domestic or free-roaming animals. The urbanization level was classified into three zones based on field observation of housing density and surrounding land use: urban zone with high-density housing with extensive commercial infrastructure, suburban zones with moderate-density housing with mixed residential and small commercial areas and rural zones with low-density housing with open land or agricultural surroundings.

2.2 Sample Size and Sampling Techniques

A minimum sample size of 273 soil samples was calculated based on Naing [38] using the proportion from Tun et al. [56]. The final target sample size was increased to 300 to ensure adequate spatial representation across all districts. Soil samples were collected using a random sampling of grid [12]. Fresh, moist faeces were also collected through active visual searching during the field visits for soil sampling. Samples were kept in ziplock bag and brought back to the Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia using an ice box.

2.3 Soil Sample Analysis

Recovery of parasite larvae was adopted from Rugai et al. [44] with soil sample adaptations [9]. In brief, 50 g of soil samples were placed on two layers of gauze (10 cm x 10 cm). The samples were immersed in a Baermann apparatus with 40–42˚C distilled water for 1.5 h. The larvae were collected at the bottom of the apparatus.

Parasite egg recovery from soil was performed with slight modifications based on the method by Mizgajska-Wiktor [35]. Soil samples were spread on trays and left to dry overnight at room temperature. Once dried, the samples were mixed and sieved to remove debris. Twenty grams of soil were weighed into a 250 mL beaker and mixed with 30 mL of 5% sodium hydroxide (NaOH). The mixture was left to stand for one hour, then stirred for 20 min. The contents were poured into a 50 mL centrifuge tube and centrifuged at 1500 rpm for 3 min. After discarding the supernatant, the sample was washed by repeating centrifugation with 30 mL of distilled water. The sediment was then resuspended in 30 mL of saturated sodium nitrate ($NaNO_3$; specific gravity 1.34) and centrifuged again at 1500 rpm for 3 min. The tube was placed

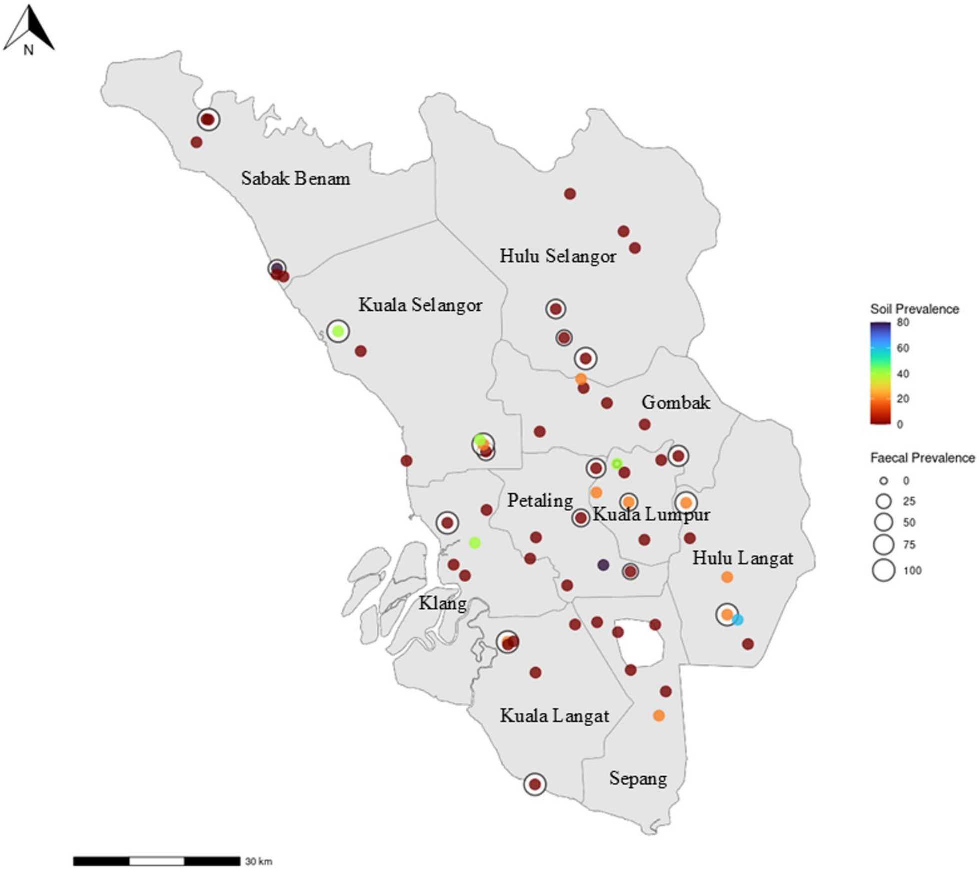

Fig. 1 Public spaces selected as sampling sites across Kuala Lumpur and Selangor. Coloured dots indicate soil sample locations, with colour intensity representing soil hookworm prevalence (red = low prevalence, blue = high prevalence). Open circles represent faecal sample locations, with circle size proportional to faecal hookworm prevalence

upright, and flotation fluid was added to form a meniscus. A 24 × 24 mm coverslip was gently placed on top and left for 10 min. The coverslip was then transferred to a glass slide and examined under a light microscope at 100× magnification to count parasite eggs.

2.4 Faecal Sample Analysis

Faecal samples were divided into two portions; first was stored at −20˚C for PCR analysis, while the second portion was analysed using the faecal flotation method. One gram of each faecal sample was homogenized in 1 mL of distilled water in a beaker. Twenty ml saturated $NaNO_3$ was added to the beaker. Content was poured into the vial until a meniscus appeared. The coverslip was placed over the meniscus. After 25 min, the coverslip was lifted, placed over the clean glass slide, and viewed under a light microscope with 100x magnification. The number of eggs was recorded. *Toxocara* and hookworm eggs were collected for viability test.

2.5 Egg Imaging

Individual eggs were extracted and transferred to a microscope slide and imaged using an eyepiece camera that is attached to the light microscope (Dino-eye AM 7025X, AnMo Electronics Corporation, Taiwan). These eggs were removed from the microscope after imaging, washed in nuclease-free ddH2O, and transferred to labelled microcentrifuge tubes and stored at −20 °C for PCR analysis.

2.6 Faecal DNA Extraction and Conventional Polymerase Chain Reaction (PCR)

Faecal DNA was extracted using PrimeWay Stool DNA extraction kit (1st BASE, Malaysia) while parasite eggs were extracted using DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer’s protocol.

PCR was used to determine the origin (dog or cat) of the faeces [57]. Species identification PCR of helminths and protozoa was done by using previously published primers. Cycling conditions include an initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing temperature according to Table 1, extension at 72 °C for 30 s and final extension at 72 °C for 7 min. Amplified PCR products of RTGHF1-RTGHR1 were subjected to digestion of HinF1 (catalog number: R0108S, New England Biolabs) and RSaI (catalog number: R0167S, New England Biolabs) to differentiate its species [40]. In brief, ten microlitres of

Table 1 List of primers used in this study

Parasite	Name	Primers	Target region	References
Ancylostoma spp.	RTGHF1 RTGHR1	5′-CGTGCTAGTCTTCAGGACTTTG-3′ 5′-CGTTGTCATACTAGCCACTGC-3′	ITS1, ITS2	Traub et al. [53]
A. braziliense	RTABCR1 RTAYR1	5′-CGGGAATTGCTATAAGCAAGTGC-3′ 5′-CTGCTGAAAGTCCTCAAGTCC-3′	ITS1, ITS2	Traub et al. [53]
Cystoisospora spp.	P3 P5 P3-2 P5-2	5’-GGGATCCTGATCCTTCCGCAGGTTCACCTAC-3’ 5’-GGAAGCTTATCTGGTTGATCCTGCCAGTA-3’ 5’-CCTGGTGGTGCCCTTCCGTCA-3’ 5’-CATGCGCACGTGCCTCTTCCTCAG-3’	SSU rRNA	Clark and Diamond [10] Matsubaya et al. [32]
Giardia spp.	RH11 RH4 GiarF GiarR	5’-CATCCGGTCGATCCTGCC-3’ 5’-AGTCGAACCCTGATTCTCCGCCAGG-3’ 5’-GACGCTCTCCCCAAGGAC-3’ 5’-CTGCGTCACGCTGCTCG-3’	16 S-rRNA	Hopkins et al. [21, 43]
Toxocara spp.	NC13 NC2	5’-ATCGATGAAGAACGCAGC-3’ 5’-TTAGTTTCTTTTCCTCCGCT-3’	ITS2	Jacobs et al. [23]
Dog/cat	DogF CatF DogCatR	5’-TTCCCTGACACCCCTACATTC-3’ 5’-CGATCTTCTATGGACCTCAACTAT-3’ 5’-CCTGAAGTAGGAACCAGATG-3’	mitochondrial D-loop regions	Vanhee et al. [57]

PCR product were digested with 0.5 µL of a restriction endonuclease at 37 °C for 1 h in a volume of 20 µL according to manufacturer’s protocol.

The amplified products were electrophoresed (400 W/80 V) on a 1.5% agarose gel (MyAgarose™) with Tris-acetic acid-EDTA (TAE) buffer and stained with Redsafe™ Nucleic Acid Staining Solution (20,000x). The standard markers used for this study were 100 bp DNA ladders (Promega, USA). After an hour, the gel was visualized under a UV transilluminator (GeneDoc™, Bio-Rad Laboratories, USA). The PCR products and forward primer were sent for purification and DNA sequencing at First BASE Laboratories Sdn Bhd (Selangor, Malaysia). The sequences were subjected to nucleotide BLAST identity search (NCBI) at http://www.ncbi.nlm.nih.gov/BLAST/.

2.7 Viability of *Toxocara* spp. And Hookworm Eggs from Faecal Samples

Collected *Toxocara* spp. and hookworm eggs from faecal samples were incubated in distilled water with 0.05% of H_2SO_4. The eggs were stored in the dark at room temperature. The incubation period for *Toxocara* eggs was 28 days, while hookworm eggs was three days. Separate wells on a culture plate were allocated for each sample. Then, the eggs were examined under an inverted microscope at 100× magnification. The count of larvae was recorded. The percentage of larvae was calculated by dividing the number of larvae by the total number of eggs observed.

2.8 Statistical Analysis

Data analysis was conducted using ggplot2 (version 3.5.1), and rnaturalearth (version 1.0.1) packages of R (version 4.4.3) within RStudio (version 2024.04.2 + 764). Risk factors were defined as environmental or infrastructural characteristics such as the type of sampling sites, presence of dustbins, fencing and urbanisation level that are potentially associated with parasite prevalence. The Shapiro-Wilk test was used to assess data normality, and the results indicated a non-normal distribution. Pearson’s Chi-square test and Fisher’s exact test were used to determine the association between hookworm prevalence and potential risk factors. Kruskal-Wallis test was used to compare hookworm burden across risk factor groups, and significant results were followed up with Dunn’s post hoc test using Bonferroni adjustment. $P \leq 0.05$ was considered statistically significant.

3 Results

3.1 Detection of Gastrointestinal Parasite in Soil Samples

A summary of the sampling effort is provided in Table S1, with the information regarding each individual park is provided in Table S2. Out of 300 soil samples, 21.3% (64/300) were positive for at least one parasite. Hookworms were the most prevalent, identified in 9.3% (28/300, 95% CI = 6.5%–13.2%), followed by Ascarids (9.0%, 27/300, 95% CI = 6.3%–12.8%) and *Capillaria* spp. (1.0%, 3/300, 95% CI = 0.3%–2.9%, Fig. 2).

3.2 Detection of Gastrointestinal Parasite in Faecal Samples

A total of 71 faecal samples were collected, comprising 40 cat faeces (56.3%) and 31 dog faeces (43.7%). Of these, 63 samples (88.7%, 95% CI = 79.3%–94.2%) were positive for at least one intestinal parasite. The prevalence of parasite in cat faeces (95.0%, 38/40, 95% CI = 83.5%–98.6%) was higher than dogs (80.7%, 25/31, 95% CI = 63.7%90.8%, $P = 0.058$). Overall, six genera of parasites were detected in faecal samples. *Cystoisospora* spp. (62.0%, 44/71, 95% CI = 50.3%–72.4%) was the most prevalent, followed by hookworms (46.5%, 33/71, 95% CI: 35.4%–58.0%) and *Giardia* spp. (22.5%, 16/71, 95% CI = 14.4%–33.5%, Table 2).

Among the 33 faecal samples that tested positive for hookworms, *Ancylostoma ceylanicum* was the most common species (81.8%, 27/33, 95% CI: 65.6%–91.4%), followed by *A. braziliense* (18.2%, 6/33, 95% CI: 8.6%–34.4%) and *A. caninum* (3.0%, 1/33, 95% CI: 0.5%–15.3%). Seven cases of *Ancylostoma* coinfection were identified. Six faecal samples (18.2%, 95% CI: 8.6%–34.4%) had both *A. ceylanicum* and *A. braziliense*, while one sample (3.0%, 95% CI: 0.5%–15.3%) contained all three hookworm species.

Hookworm coinfection with other gastrointestinal parasites were also detected in the faecal samples (39.4%, 28/71, Table 2). The most prevalent was dual coinfection of hookworms and *Cystoisospora* spp. (37.5%, 12/32, 95% CI: 22.9%–54.8%), followed by triple coinfection of hookworms, *Giardia* spp., and *Cystoisospora* sp. (18.8%, 6/32, 95% CI: 8.9%, 35.3%).

3.3 Viability of *Toxocara* spp. And Hookworm Eggs from Faecal Samples

After incubation, 74.5% (204/274) of *Toxocara* spp. eggs and 37.7% (1522/4036) of hookworm eggs from positive faecal samples were viable as they evolved into larvae.

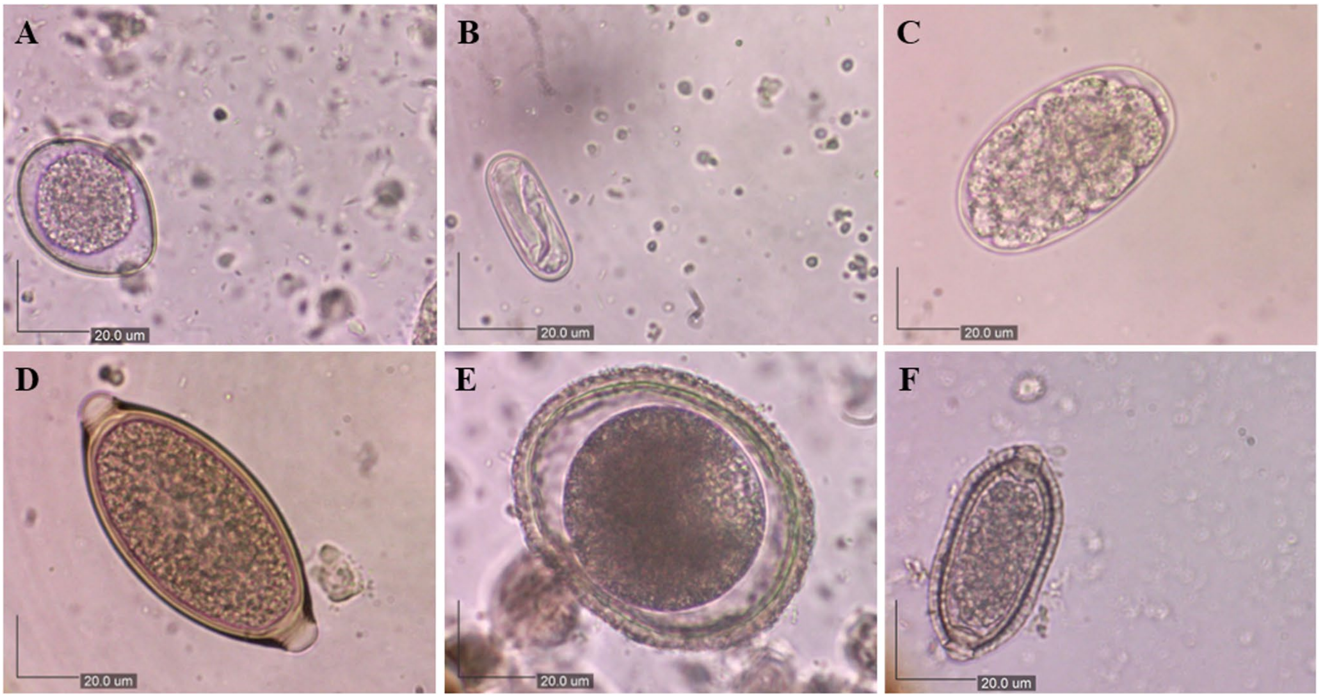

Fig. 2 arasites collected from soil and faecal samples. (**A**) *Cystoisospora* spp., (**B**) *Spirocerca* spp., (**C**) hookworms, (**D**) *Trichuris* spp., (**E**) *Toxocara* spp., (**F**) *Capillaria* spp.

Table 2 Type of parasites detected in dog and Cat faeces

Type of infection	Cat ($n=40$)			Dog ($n=31$)		
	n	%	95% CI	*n*	%	95% CI
No infection	4	10.0	4.0–23.1	11	35.5	21.1–53.1
Single infection						
Hookworms	5	12.5	5.5–26.1	3	9.7	3.4–24.9
Cystoisospora spp.	6	15.0	7.1–29.1	11	35.5	21.1–53.1
Giardia spp.	3	7.5	2.6–19.9	-	-	-
Dual infections						
Hookworms + *Giardia* spp.	1	2.5	0.4–12.9	-	-	-
Hookworms + *Cystoisospora* spp.	7	17.5	8.8–32.0	5	16.1	7.1–32.6
Giardia spp. + *Cystoisospora* spp.	5	12.5	5.5–26.1	-	-	-
Toxocara spp. + *Cystoisospora* spp.	1	2.5	0.4–12.9	-	-	-
Trichuris spp. + *Cystoisospora* spp.	-	-	-	1	3.2	0.6–16.2
Triple infections						
Hookworms + *Giardia* spp. + *Cystoisospora* spp.	6	15.0	7.1–29.1	-	-	-
Hookworms + *Toxocara* sp. + *Cystoisospora* spp.	1	2.5	0.4–12.9	-	-	-
Trichuris spp. + *Giardia* spp. + *Cystoisospora* spp.	1	2.5	0.4–12.9	-	-	-

3.4 Risk Factors of Hookworm Burden in Faecal Samples

Further analysis was conducted specifically on hookworm as it was the most common parasite detected in faecal and soil samples. The overall median hookworm egg burden in faecal samples was 181.5 (range: 6–3136). In dogs, the median egg burden was 226 (range: 11–3136), while the median egg burden in cats was 177 (range: 6–973).

Univariate analysis showed that hookworm burden in faecal samples was significantly associated with rural zones ($p=0.006$), type of sampling site ($p=0.011$), and type of infection ($p=0.035$). Higher hookworm burdens were observed in samples from rural areas (450.0 ± 1305.0) compared to urban (146.0 ± 184.0, $p=0.010$) and suburban areas (99.0 ± 313.0, $p=0.038$; Fig. 3). Hookworm burdens were also significantly higher in faecal samples collected from beaches (890.0 ± 2370.0) than from apartment playgrounds (129.0 ± 140.0, $p=0.028$) and residential parks (176.5 ± 336.0, $p=0.048$).

3.5 Concordance and Risk Factors of Hookworm Detection between Faecal and Soil Samples

In soil samples, hookworms were found in 16 out of 60 sampling sites (26.7%). Figure 1 shows the distribution of hookworm positive samples and their spatial overlap in both faecal and soil samples. Hookworms were detected widely across all districts with varying prevalence. Seven sampling sites or four districts showed concordance of hookworm presence in soil and faecal samples, all of which were resident parks. Hulu Langat and Kuala Selangor shared the highest concordance of hookworm presence in soil and faecal samples (33.3%, 2/6).

The presence of dustbins was associated with higher hookworm prevalence in dogs (OR = 15.11, 95% CI: 2.48–92.1, $p=0.004$; Table 3). Hookworm infection was more likely in cat faecal samples collected from apartment playgrounds compared to resident parks (OR = 0.19, 95% CI: 0.05–0.76, $p=0.025$). Soil from residential parks had higher odds of *Ancylostoma* infection than soil from dog-friendly parks (OR = 5.85, 95% CI: 1.90–17.98, $p=0.004$).

4 Discussion

Faecal contamination is a critical environmental issue, particularly in public spaces where human and animal interactions are common. Our survey supports this statement as a high proportion of collected faecal and soil samples tested positive for at least one gastrointestinal parasite. Notably, several detected genera such as *Ancylostoma*, *Toxocara*, and *Giardia* include zoonotic species. For example, *A. caninum*, *A. ceylanicum*, *A. braziliense*, *T. cati*, *T. canis*, and *G. duodenalis* Assemblages A and B causing human infections [34, 50, 54].

Among protozoa, *Cystoisospora* spp. showed the highest prevalence in faecal samples. This is higher than previous findings in Malaysian stray cats which ranged from 45.3% to 50% [52, 1]. The high prevalence of *Cystoisospora* from Malaysian small animals contrasts with other countries such as Portugal [16], Brazil [4], Thailand [25], Germany [36]. Hookworms were the most prevalent helminths in this study. This is consistent with findings from small animal studies in Malaysia [51] and other middle-income countries such as [26], México [30], Kenya [37], Myanmar [49], the Philippines, and Thailand [58]. The high prevalence is likely attributed to animal behaviour such as free roaming

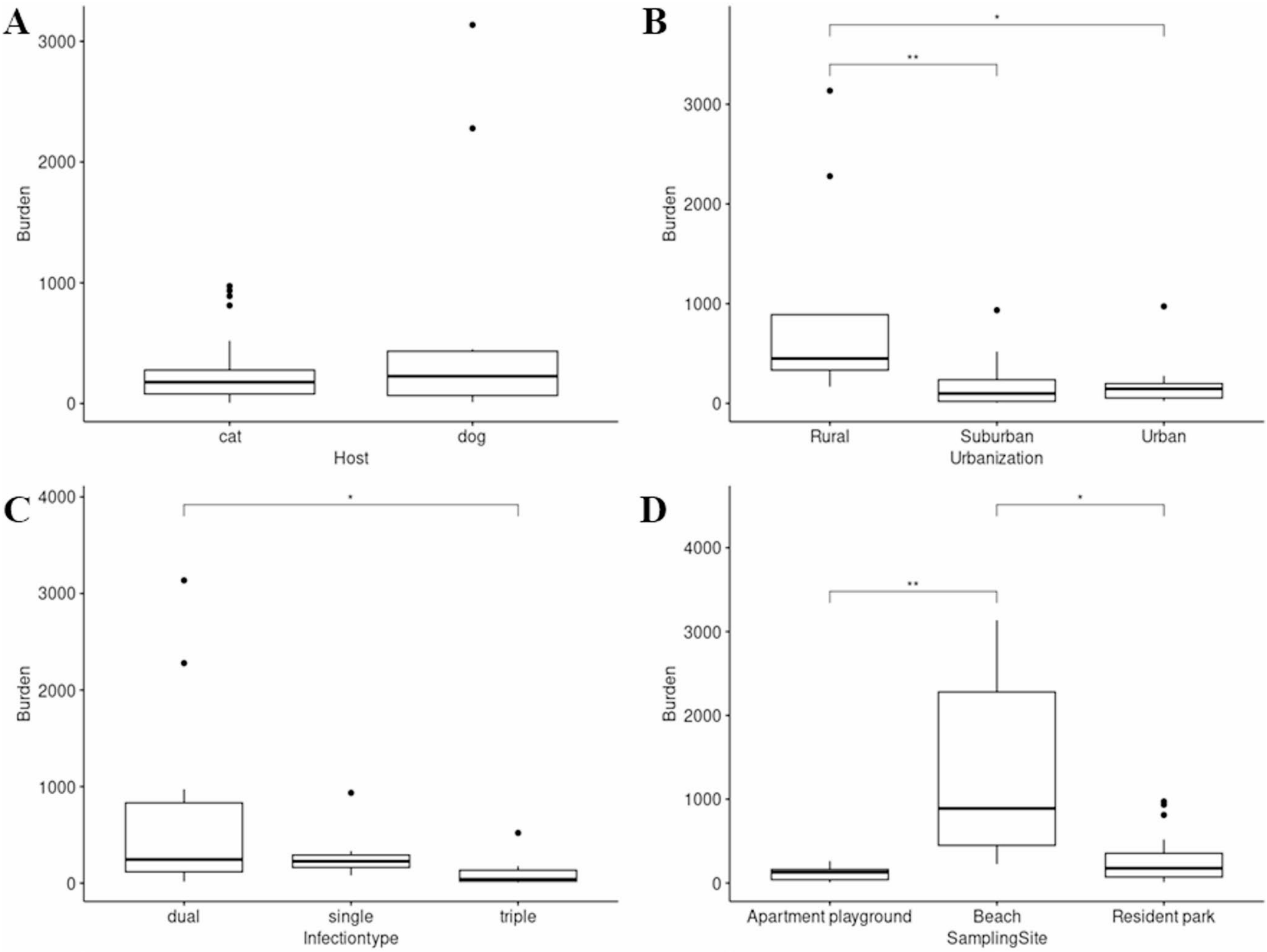

Fig. 3 Boxplot of hookworm burden across (A) host, (**B**) urbanization level, (**C**) type of infection, (**D**) type of sampling site

that facilitate environmental contamination [26, 30, 37, 58]. Moreover, *Cystoisospora* oocysts are sporulated more rapidly at temperature between 30 and 38 °C [29, 45]. This aligned with warm tropical climate in Malaysia and suggest that the local environmental conditions are suitable to the survival, sporulation and transmission of this coccidia and hookworm.

There was a higher hookworm prevalence in cat faecal samples than dog samples. Similar result was reported in the Philippines [28]. The overpopulation of cats due to abandonment of unwanted pets, the tendency of pet cats roaming outdoor or escape, and the intentional feeding of cats caused faecal cross-contamination [60]. As in our survey, more cat faecal samples were frequently detected hookworms in residential park. This proved that shared latrine use among dense cat populations might facilitate parasite transmission [47]. As this trend was contrasts with earlier Malaysian studies [24, 56], the discrepancy might reflect shifts in host population dynamics or increased free-roaming cats in hotspot areas.

The prevalence of *Toxocara* spp. in faecal samples was lower, while the prevalence in soil samples was comparable to a previous Malaysian epidemiological survey [56]. This discrepancy might be attributed to the fact that *Toxocara* infections are more commonly reported in young animals. Youngs tend to have smaller home ranges than adults [41]. This potentially limiting the detection of *Toxocara* eggs in faecal samples from public spaces. Nevertheless, the zoonotic potential of *Toxocara* spp. warrants serious attention as both *T. canis* and *T. cati* could cause visceral and ocular larva migrans in humans, particularly in children who engage in geophagia or have frequent soil contact [8].

Hookworm coinfections with other gastrointestinal protozoa and helminth was observed in both dogs and cats. This reflecting a common trend in small animals globally [22, 26, 39, 48]. Helminths co-infecting with hookworms included *Toxocara* spp., *Trichuris* spp., and *Spirocerca lupis*, while protozoan coinfections involved *Cystoisospora* spp. and *Giardia* spp. These parasites shared similar transmission routes are more likely to coinfect hosts [6,

Table 3 Potential risk factors of hookworm presences in faecal and soil samples

	Faecal samples (n=71)										Soil samples (n=300)				
	Dog (n=31)					Cat (n=40)									
	n	%	95% CI	OR	*p*-value	*n*	%	95% CI	OR	*p*-value	*n*	%	95% CI	OR	*p*-value
District					0.079					0.773					0.428
Kuala Lumpur	0/3	0	0–56.2.2	NE		3/7	42.9	15.8–75.0	1.00*		3/30	10.0	3.5–25.6	1.00*	
Gombak	-	-	-	-		4/5	80.0	37.6–96.4	5.33		1/30	3.3	0.6–16.7	3.22	
Hulu Langat	-	-	-	-		3/5	60.0	23.1–88.2	2.00		6/30	20.0	9.5–37.3	0.44	
Hulu Selangor	1/1	100.0	20.7–100	NE		3/6	50.0	18.8–81.2	1.33		0/30	0	0–11.4.4	NE	
Klang	1/4	25.0	4.6–69.9	1.00*		2/10	20.0	5.7–51.0	0.33		2/30	6.7	1.8–21.3	1.56	
Kuala Langat	5/9	55.6	26.7–81.1	0.27		2/2	100.0	34.2–100	NE		1/30	3.3	0.6–16.7	3.22	
Kuala Selangor	2/2	100.0	34.2–100	NE		2/3	66.7	20.8–93.9	0.38		5/30	16.7	7.3–33.6	0.56	
Petaling	2/8	25.0	7.2–59.1	1.00		2/5	40.0	11.8–76.9	1.13		5/30	16.7	7.3–33.6	0.56	
Sabak Benam	0/4	0	0–49.0	NE		1/1	100.0	20.7–100	NE		4/30	13.3	5.3–29.7	0.72	
Sepang	-	-	-	-		-	-				1/30	3.3	0.6–16.7	3.22	
Type of sampling site					0.112					0.025*					<0.001*
Apartment playground	-	-	-	-		7/20	35.0	18.1–56.7	1.00*		0/35	0	0–9.9.9	NE	
Beach	4/7	57.1	25.1–84.2	1.00*		1/1	100.0	20.7–100	NE		0/15	0	0–20.4.4	NE	
Dog friendly park	0/6	0	0–39.0	NE		-	-	-	-		6/15	40.0	19.8–64.3	1.00*	
Public park	-	-	-	-		-	-	-	-		0/20	0	0–16.1.1	NE	
Resident park	7/18	33.9	20.3–61.4	2.10		14/19	73.7	51.2–99.2	0.19		22/215	10.2	6.9–15.0	5.85	
Urbanization					0.091					0.514					0.387
Rural	7/11	63.6	35.4–84.8	1.00*		2/2	100.0	34.2–100	NE		7/70	10.0	4.9–19.2	1.00*	
Suburban	2/9	22.2	6.3–54.7	0.16		9/19	47.4	27.3–68.3	1.00*		12/160	7.5	4.3–12.7	0.73	
Urban	2/11	18.2	5.1–47.7	0.13		11/19	57.9	36.3–76.9	1.53		9/70	12.9	6.9–22.7	1.33	
Presence of dustbin					0.004*					1.000					0.682
Absence	8/11	72.7	43.4–90.3	1.00*		14/26	53.8	35.5–71.2	1.00*		12/155	7.7	4.5–13.0	1.00*	
Presence	3/20	15.0	5.2–36.0	15.11		8/14	57.1	32.6–78.6	0.88		16/145	11.0	6.9–17.2	0.68	
Fencing					0.066					1.000					0.755
Absence	11/25	44.0	36.7–62.9	NE		21/39	53.8	38.6–68.4	NE		26/35	74.3	57.9–85.8	1.00*	
Presence	0/6	0	0–39.0	NE		1/1	100.0	20.7–100	NE		2/265	0.8	0.2–2.7	379.89	

p-value less than 0.05; OR = odd ratio; 1.00 = reference group; NE = not estimable due to zero cell

31]. It is worth to mention that coinfection might result in synergistic effects in host and cause the clinical outcomes different from single infection [14]. Consequently, individual with hookworm coinfection often present with more severe clinical manifestations such as anaemia [15, 20].

Hookworm transmission is highly influenced by environmental conditions which contribute either directly or indirectly to its prevalence and burden in small animals due to its direct life cycle [4]. Faecal samples collected from rural areas often showed high hookworm burden due to increased exposure to contaminated soil, water or infected faeces [55]. In this survey, the presence of dustbin was associated with higher hookworm prevalence in dog faecal samples. Free-roaming dog are highly mobile and tend to scavenge for leftover food, garbage and animal dung [2]. These behaviours increase their risk of infection. Therefore, effective waste management might play a protective role by reducing faecal deposition and interrupting parasite transmission [42].

Meanwhile, cat faecal samples and soil collected from apartment playgrounds showed higher odds of detecting hookworm. Apartment playgrounds are typically characterised by higher human and animal density that increases the likelihood of pet activity and community-feeding practices. This suggests that cat faecal samples could be the prior source of contamination in apartment playgrounds. Notably, factors such as unrestricted outdoor access, frequent interaction with other animals, and drinking contaminated water have also been associated with increased risk of contact with contaminated environment in previous studies [4, 25].

The epidemiological survey identified 43.8% of soil-positive sites that also contained infected faecal samples, primarily from residential parks. This strongly suggests these areas are active environmental transmission hotspots. Such concordance between faecal and soil contamination supports the likelihood of an ongoing transmission cycle, especially in Hulu Langat and Kuala Selangor, where environmental conditions favour parasite development.

5 Research Limitations and Recommendations

Several limitations must be acknowledged in this study. First, the cross-sectional design in the survey restricts the ability to infer causal relationships between environmental factors and parasite infections. Second, the lack of longitudinal sampling prevents assessment of temporal trends and seasonal variation in parasite prevalence.

Future studies could explore the demographic and behavioural factors including age, sex, roaming habits, and interactions with other animals for better understanding the potential risk of coinfection in small animals. These variables might influence exposure risk and infection dynamics in this country. Given the notable presence of protozoa in small animals, proper guidance on the appropriate use of antiprotozoal treatments in small animals should be undertaken as it is often overlooked in routine deworming protocols. A One Health approach which integrating human, animal, and environmental health strategies is strongly recommended to develop more effective control programmes. Preventive practices such as regular handwashing, wearing footwear and avoid direct contact with animal faeces are important to reduce human infection. Relevant authorities should organize awareness campaign to increase promote responsible pet ownership and proper faecal disposal, particularly in hotspots such as residential parks in Hulu Langat and Kuala Selangor. These interventions could decrease the transmission cycle of zoonotic gastrointestinal parasites in public spaces.

6 Conclusions

This epidemiological survey demonstrates that public spaces in Kuala Lumpur and Selangor, especially those with frequent human-animal interactions are important reservoir for zoonotic gastrointestinal parasites. The high detection rate, occurrence of coinfection, presence of viable parasite eggs or larvae, and concordance between faecal and soil samples point towards the complete parasite life cycle in these environments. Spatial analysis revealed higher prevalence in public spaces with absence of dustbin and frequent presence of free-roaming animals are likely facilitate hookworm persistence. These findings confirm our hypothesis and highlight the importance of environmental, behavioural and infrastructural as potential risk factors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s44197-025-00511-w.

Acknowledgements This research was supported by Boehringer Ingelheim Animal Health, Malaysia. Thanks to all the technical staff involved in the field for sample collection and technical assistance in the laboratory.

Author Contributions Conception and study design: SYL, SKG, NAAA. Data management: SYL, SMB, MAM, NA, NAAA. Interpretation of data: SYL, SMB, ARO, AHO, NAAA. Drafting the manuscript: SYL, NAAA. Critical revision and approval for submission: SYL, SMB, ARO, AHO, TRPTA, MAM, NA, NAAA.

Data Availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Ethics approval was not required for this study.

Consent for Publication Informed consent was not required for this study.

Competing interests The authors declare no competing interests.

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