CONTAMINATION OF SOILS WITH *TOXOCARA* EGGS IN SEVERAL PLAYGROUNDS OF IPOH, PERAK

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ABSTRACT. Toxocariasis is an important cosmopolitan zoonotic disease mainly caused by *Toxocara* spp., a type of soil-transmitted helminth (STH) on cats and dogs. In this study, 80 soil samples were taken from four public playgrounds and six neighbourhood playgrounds in Ipoh, Perak between October and December 2016 to determine the status of soil contamination with the eggs of *Toxocara* spp. Results showed that 32.5% from the total soil samples were positive with *Toxocara* spp. eggs. Overall, five out of ten of the sampling sites were contaminated with *Toxocara* spp. eggs. Besides that, the relationship between the soil condition and the occurrence of the *Toxocara* spp. eggs in soils were also investigated. The findings showed that increase of moisture and pH of the soils contributed to the increase of contamination with *Toxocara* spp. eggs. Sandy soils were found significantly contaminated with the eggs of *Toxocara* spp. compared to the other types of soil. Therefore, appropriate preventive measures such as treatment of soil, regular monitoring and deworming of dogs and cats as well as awareness programmes to the public are important.

Keywords: Toxocara spp., STH, playgrounds, soil, Ipoh

INTRODUCTION

According to Taylor *et al.* (2016), *Toxocara* spp. is a group of parasitic STH classified under the family of Ascarididae and genus of *Toxocara*. Ascaridoids are among the largest nematodes that affects many vertebrates (mostly the domestic animals) as the adult stage can cause intestinal unthriftiness to young animals while their migratory larval stage can cause pathological consequences. Both stages have socio-economic and veterinary significance (Li *et al.*, 2006; Taylor *et al.*, 2016).

Toxocariasis (*Toxocara* infection) is listed as one of the five neglected parasitic infections (NPIs) by CDC (Centre of Disease Control) as they may cause severe illnesses to humans and yet still being neglected or less aware by many people (Woodhall *et al.*, 2014). This disease is widely known as among the most prevalent zoonotic diseases caused by the infective larval stage of *Toxocara* spp. of predatory mammals which belong to the family Canidae and Felidae (Okulewicz *et al.*, 2012). This mainly refers to *T. cati and T. canis,* which are both known as gastrointestinal parasitic roundworms of cats and dogs, respectively (Choo *et al.,* 2016).

Dogs and cats commonly defaecate on soils, therefore the soils of public parks

and playgrounds are important sources of toxocariasis for humans and other paratenic (non-definite) hosts (Stojevic *et al.,* 2010). Moreover, they were found to be the most widespread and prevalent parasitic zoonotic infections in humans (Macpherson, 2013).

Even though there were studies on the occurrences (prevalence) of *Toxocara* spp. eggs in Malaysia, no data has been found regarding soil contamination with *Toxocara* spp. eggs in any part of Perak or the relationship of soil condition to its occurrence of *Toxocar*a spp. eggs in Malaysia. Therefore, the aim of this study was to (1) investigate the presence *Toxocara* spp. eggs in soil samples collected from several playgrounds in Ipoh, and (2) to determine the correlation of moisture, pH and texture of soil samples with the occurrence of *Toxocara* spp. eggs.

MATERIALS AND METHOD

Sample collection and area of the study

The present study was conducted within the area of Ipoh, the capital city of Perak, Malaysia (Figure 1). By random assignment, ten sampling locations which consists of six neighbourhood playgrounds and four public playgrounds in good condition, easy to access, and not isolated or far from publics or residences. According to Alonso et al. (2001), public playgrounds were the categorized as playgrounds that placed within public parks or recreational areas, while the neighborhood playgrounds were categorized as playgrounds that placed within private or housing areas. Meanwhile, O'Lorcain (1994) categorised the adventure playground (recreational playground) as larger, usually covered with pebbles and gravel, with sandpits for children to play and few areas barely patched with soil and better maintained by local council, in comparison to the conventional neighbourhood playground with exposing soil along the perimeter.

In this study, 80 soil samples were collected from ten different sites (public playgrounds and neighbourhood playgrounds) between October and December 2016 and tested for contamination by *Toxocara* spp. eggs. Using a small shovel, eight points were randomly selected within the sampling area. At each point, a soil sample weighing 300 g was taken (Noor Azian *et al.*, 2008). Each soil sample was taken from 0 cm to 5 cm depth (upper layer) for the highest occurrence of geohelminth eggs (Mizgajska-Wiktor and Uga, 2006). Each sample was placed in a polyethylene sealed bag (air-tight), appropriately labelled and then transported to the laboratory for analysis or kept at 4 °C to be processed (Mohd Zain *et al.,* 2014) within 24 hours.

Isolation of *Toxocara* spp. eggs from soil samples

Toxocara spp. eggs was recovered from soil using the washing method devised by Dada (1979) and modified by Ruiz De Ybáñez *et al*. (2000) who recorded a recovery rate of 99.91%, with a minor modification in this study. At least one confirmed egg of *Toxocara* spp. was needed for the sample to be recorded as positive. Briefly, 1 g of evenly mixed soil sample was weighed before washing with distilled water by

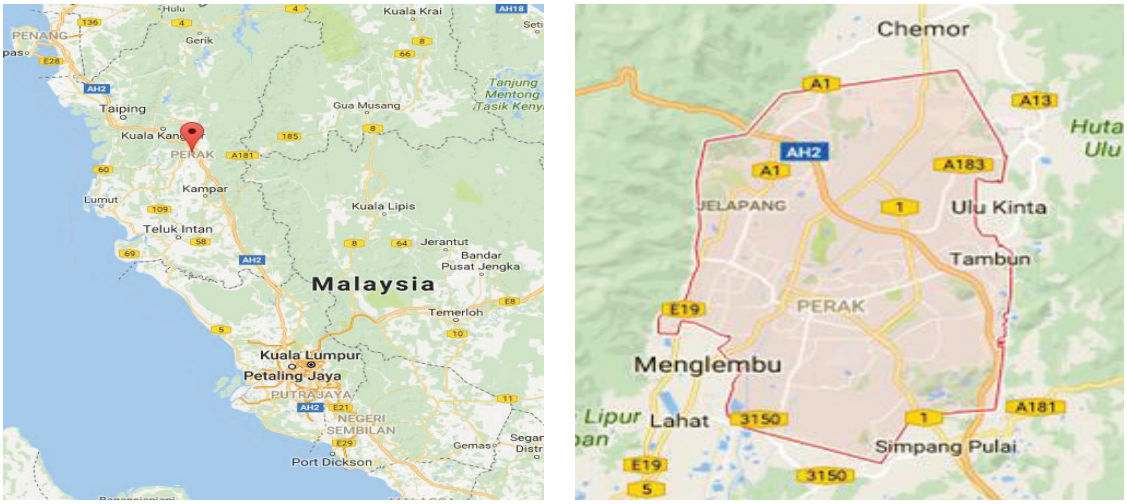

Figure 1: Maps of study area. Parts of Peninsular Malaysia showing Perak (red pinned) and its neighboring states (left), this study was conducted within Ipoh area (hatched), (right).

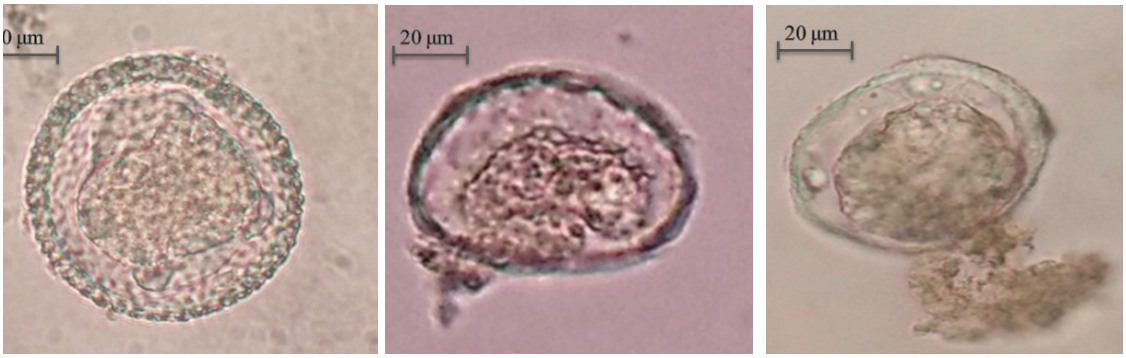

Figure 2: Three forms of *Toxocara* spp. eggs were detected in soil samples. The recovered soil samples observed under 400x magnification by using CX31 Olympus compound microscope. The non-embryonated egg (left), embryonated egg (middle), ruptured egg (right). However, the occurrence of pitted surface of each egg shells was still visible (all pictures by Debbra, 2016).

centrifugation. The recovery of *Toxocara* spp. eggs was carried out by simple floatation and followed by microscopic examination at 100x and 400x magnification. The egg of *Toxocara* spp. was identified based on their typical morphology (Uga *et al*., 2000), while the size was determined using an ocular eyepiece micrometer.

Determination of soil condition analysis

Soil pH analysis

For soil pH examination, data was collected using a method suggested by Rayment and Lyons (2011). Briefly, soil pH analysis was carried out using 2 g each of air-dried soil sample. Water was added and the suspension stirred at 15 rpm for 1 hour. Its pH was taken after calibration with acid and alkaline buffers.

Soil moisture content analysis

Soil moisture content was obtained using the method by Paller and de Chavez (2014). Briefly, the weight of a jar was tared. The pre-dried weight (w^0) of each soil sample in the jar was taken before drying in the oven at 125 °C. The dried weight (w^1) of the soil taken and the moisture content of the sample was determined using Eq. 1

$$\left[\frac{w^0 - w^1}{w^1}\right] \times 100 \quad (1)$$

Soil texture analysis

Soil texture was categorised into four types by referring to the method used by Paller and de Chavez (2014) and "feeling" analysis by Lesikar (2005). Briefly, each soil sample was kneaded into a bolus before performing texture "feeling" analysis and then categorised into four types based on their texture namely sandy, silty, loamy and clayey respectively.

Data analysis

All the results obtained were analysed using the IBM Statistical Package for Social Science® (version 22, released 2013). It was assumed that the data for this study were suitable for non-parametric statistical tests (Flynn, 2011).

RESULTS AND DISCUSSION

In this study, five out of ten selected playgrounds in Ipoh were contaminated with *Toxocara* spp. eggs. Table 1 shows samples from sites E, A, C, G and D with 87.5%, 75.0%, 62.5%, 62.5% and 37.5% positive respectively. Samples from sites B, F, H, I and J were all recorded as negative. Table 2 shows that the contamination rate for the neighbourhood playgrounds (43.8% from 48 soil samples) was higher than the public playgrounds (15.6% from 32 soil samples). By applying Pearson's chi-squared test, there was a significant difference in the occurence of *Toxocara* spp. eggs between the neighborhood playgrounds and the public playgrounds ($\chi^2 = 6.923$, $df = 1$, $p = 0.009$). Overall, 32.5% of the 80 soil samples from ten selected playgrounds in this study were contaminated.

The mean value for moisture content and pH of the soil samples are shown in

Table 1: The contamination of soil samples with *Toxocara* spp. eggs in current study.

Playground category	Sampling Site	No. of samples (n)	Positive samples	Percentage of positive (%)
Neighborhood	Site A	8	6	75.0
	Site B	8	0	0.0
	Site C	8	5	62.5
	Site D	8	3	37.5
	Site E	8	7	87.5
	Site F	8	0	0.0
Public	Site G	8	5	62.5
	Site H	8	0	0.0
	Site I	8	0	0.0
	Site J	8	0	0.0

Table 2: Contamination of soil samples with *Toxocara* spp eggs. based on two categories of playgrounds.

Playground category	N	Chi-square (χ^2)			% positive	Contaminated playground
	O	E	SR			
Neighborhood	48	21	15.6	1.4	43.8	4/6 (66.7%)
Public	32	5	10.4	-1.7	15.6	1/4 (25.0%)
Total	**80**	**26**	**26.0**	**-**	**32.5**	**5/10 (50%)**

O = Observed count; E = Expected count; SR = Standard Residual; N = number of subjects in the total sample

Table 3: The moisture content and pH of soils collected in current study.

Site	*N*	Mean	
		Soil Moisture Content (%)	Soil pH
		M +SD	*M +SD*
A	8	14.29 ±4.64	6.95 ±0.18
B	8	8.72 ±3.99	6.22 ±0.17
C	8	10.55 ±5.46	6.45 ±0.15
D	8	10.75 ±5.26	6.44 ±0.23
E	8	15.29 ±4.68	6.49 ±0.11
F	8	6.14 ±1.67	6.41 ±0.06
G	8	11.74 ±3.91	6.93 ±0.29
H	8	8.42 ±1.68	6.29 ±0.29
I	8	7.98 ±2.22	6.14 ±0.17
J	8	8.38 ±1.60	6.27 ±0.20
Total	**80**	**10.22 ±4.53**	**6.46 ±0.32**

N = number of subjects in the total sample; *M* = Mean; *SD* = Standard Deviation

Table 4: Spearman's Rank-Order correlation coefficient (r_s) between *Toxocara* spp. eggs contamination and pH and moisture content of soils in current study.

		***Toxocara* spp.**	**Soil Moisture**	**Soil pH**
Toxocara spp.	r_s	1.000	**0.706****	**0.412****
	p		**0.000**	**0.000**
	N	80	**80**	**80**
Soil Moisture	r_s	**0.706****	1.000	**0.363****
	p	**0.000**		**0.001**
	N	**80**	80	**80**
Soil pH	r_s	**0.412****	**0.363****	1.000
	p	**0.000**	**0.001**	
	N	**80**	**80**	80

** Correlation is significant at the level 0.01 level (2-tailed).

N = number of subjects in the total sample; r_s = Spearman correlation coefficient; *p* = Sig. or Significant level

Table 5: Contamination of soil samples with *Toxocara* spp. eggs according to soil type in current study.

	Samples examined		**Contaminated samples**			
Soil type	**N**	**%**	**n**	**%**	***M*+**	***SD***
Sandy	19	23.75	16	61.53	0.84+	0.375
Silty	19	23.75	6	23.07	0.33+	0.485
Loamy	18	22.50	4	15.38	0.20+	0.410
Clayey	24	30.00	0	0.00	0.00+	0.000
TOTAL	**80**	**100**	**26**	**100**	**0.33+**	**0.471**

Table 6: The ranks and test statistics of Kruskal-Wallis H test to compare the soil type with the occurrence of *Toxocara* spp. eggs in soil samples.

Ranks				**Test statistics [a,b]**
	Soil type	**N**	**M rank**	$\chi^2 = 34.144$
Toxocara spp. egg	Sandy	19	61.18	*df* = 3
	Silty	18	40.13	*p* = 0.000
	Loamy	20	36.39	a Kruskal Wallis Test
	Clayey	23	27.50	b Grouping variable: soil type

Based on Kruskal-Wallis H analysis, there was a statistically significant difference between the groups (soil type) as a whole ($\chi^2(3) = 34.144$, $p < 0.001$).

Table 3. Soil samples from site E recorded the highest mean value of soil moisture content (15.29±4.68%), followed by site A (14.29+4.64), site G (11.74±3.91%), site D (10.75+5.26) and site C (10.55+5.46). Similarly, the pH for soil samples from sites A, G, E, C and D recorded higher mean values, i.e 6.95+0.18, 6.93+0.29, 6.49+0.11, 6.45+0.15, and 6.44+0.23 respectively than the negative soil samples (Table 3). Spearman Rank-Order correlation coefficient analysis (Table 4) illustrated statistically significant and high positive correlation (Mukaka, 2012) between soil moisture and the occurrence of *Toxocara* spp. eggs ($rs = 0.706$, $p < 0.001$), while statistically significant and low positive correlation (Mukaka, 2012) between soil pH and the contamination of soil with *Toxocara* spp. eggs ($rs = 0.412$, $p < 0.001$). Table 4 also shows statistically significant and low positive correlation (Mukaka, 2012) between the soil pH and the soil moisture ($rs = 0.363$, $p = 0.0001$).

On the other hand, Table 5 shows that sandy soil type had very high occurrence (61.5%) compared to silty soil type (23.1%) and loamy soil type (15.4%), all from the total of positive samples. The percentages of soil samples were not equal among the soil types, while the percentages of positive samples varied between soil types. Even though clayey soil type was the most examined soil sample, no *Toxocara* spp. eggs was found. Based on Kruskal-Wallis H analysis (Table 6), there was a statistically significant difference between the groups (soil type) as a whole ($\chi^2(3) = 34.144$, $p < 0.001$).

In this cross-sectional study, the eggs of soil transmitted helminths (STH) other than *Toxocara* spp. and free-living or non-parasitic nematodes were omitted as only the eggs of *Toxocara* spp. were considered. As shown in Figure 2, the eggs of *Toxocara* spp. were identified by their general morphology such as thick, rough, pitted shell, while the brownish dark granular contents usually fill the whole volume of the shell (Gibbons *et al.,* 2001; Despommier, 2003; Taylor *et al.,* 2016).

Based on the results, the neighbourhood playgrounds were found more contaminated with the eggs of *Toxocara* spp. compared to the public playgrounds. This may due to the public playgrounds being better maintained by the local municipality, as observed during the collection of the soil samples. Previous studies about the contamination of soils with the eggs of *Toxocara* spp. in Malaysia were reported with different prevalence rates such as 1% in Kuala Lumpur (Uga *et al*., 1996); 54.5% in urban areas of Petaling Jaya, Selangor and 45.8% in suburban areas of Serdang, Selangor (Loh and Israf, 1998); 26.7% in urban areas of Setapak, Kuala Lumpur and 4.9% in rural areas of Kuala Lipis, Pahang (Noor Azian *et al*., 2008); and the highest was 95.7% in playgrounds of several states in peninsular Malaysia (Mohd Zain *et al.,* 2014).

Based on statistical analysis in this study, the increased soil moisture significantly increased the contamination of soils with *Toxocara* spp. eggs. The moisture of soil is important to prevent the eggs from dessicating or dehydrating and provided the ions for the development of the eggs (Paller and de Chavez, 2014).

The statistical analyses also showed that as soil pH increased, the

contamination of soils with *Toxocara* spp. eggs also increased, even with moderate positive correlation. This is because eggs of *Toxocara* spp. may survive better in basic soil conditions. This explains why the soil samples with highest mean value of pH (least acidic or more basic) had the most contamination rate in this study, in agreement with the study by Paller and de Chavez (2014).

Meanwhile, the texture of soil also influenced the prevalence of *Toxocara* spp. eggs in soil (Etewa *et al.*, 2016). In this study, the sandy soil type was found to be the most contaminated with the eggs of *Toxocara* spp. whereas the clayey soil type was found to be the least, which is also in agreement with the study by Paller and de Chavez (2014).

As expected, the eggs were discovered to belong to either *Toxocara canis* or *T. cati*. This is because both species are the most common STH, found distributed worldwide (Gibbons *et al.*, 2001). Both *T. cati and T. canis* are known as gastrointestinal parasitic roundworms of cats and dogs, respectively (Choo *et al.,* 2016). Both species are also regarded as the causative agents for human toxocariasis (Magnaval *et al.,* 2001), an illness which is more prevalent in temperate and tropical regions (Smith *et al.*, 2009).

Since *Toxocara* spp. has zoonotic importance to the public health of humans, appropriate preventive and treatment measures should be put into action based on the final findings in this study. These zoonotic parasite eggs can survive in soil for many years and may create risk of exposure to humans in public places such as playgrounds, particularly young children due to their playing habits with the soil (Ondriska *et al.*, 2013). Therefore, it is also necessary to conduct a survey of faecal samples of the cat and dog population in Ipoh to provide clearer information on the risk of transmission of *Toxocara* spp. to humans in this area.

CONCLUSION

Based on the final findings in this study, some playgrounds of Ipoh showed evidence of *Toxocara* spp. eggs in the soils. The soil condition (moisture content, pH and texture) were found to be the factors that may influence the presence (and survival) of the *Toxocara* spp. eggs in the soil samples of this study.

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