

DETECTION OF HELMINTH INFECTIONS IN DOGS AND SOIL CONTAMINATION IN RURAL AND URBAN AREAS

MY Noor Azian¹, L Sakhone², S Lokman Hakim³, MY Yusri¹, Y Nurulsyamzawaty¹, AH Zuhaizam¹, I Mohd Rodi⁴ and MN Maslawaty¹

¹Parasitology Unit, Infectious Diseases Research Center, Institute for Medical Research, Kuala Lumpur, Malaysia; ²Entomology Section, Center for Malariaology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane, Lao PDR; ³Environmental Health Research Center, Kuala Lumpur, Malaysia; ⁴Epidemiology and Biostatistics Unit, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract. A study was conducted to determine the helminthes in dog's feces and soil samples from urban and rural areas. Six species of nematodes (*Toxocara* sp, an undetermined nematode larvae, *Strongyloides* sp larvae, *Ascaris* sp ova, hookworm ova, *Trichuris* sp ova) and one species of Cestode (*Taenia* sp) were found in 175 stool samples. Seventy-eight point nine percent of stool samples were positive for helminthes. Mixed infection with at least one parasite was found in 32.6% of the samples. The prevalence of helminth infection ranged from 1.1% to 45.1%. The prevalence of hookworm sp was the highest with 45.1%. The highest prevalence in urban dogs was hookworm sp in 76.7% and in rural areas was *Ascaris* sp in 48.7%. Soil samples were also examined to determine contamination of the environment, especially due to *Toxocara canis*, as a potential source of infection. Urban soil samples showed a higher contamination rate with 26.7% compared to rural areas with 4.9%. *Toxocara* ova were the most prevalent helminthes contaminating the soil with 12.1%. This study showed that humans from both urban and rural areas are at risk of acquiring helminth infection from contaminated soil.

INTRODUCTION

The interest in keeping animals as pets, especially dogs and cats, has led to an increase in parasitic infections, such as toxoplasmosis and larva migrants, as well as emerging and re-emerging diseases, such as cryptosporidiosis and giardiasis (Bugg *et al*, 1999). The majority of pet owners are not aware of the potential risk of transmission and danger to human health. A number of studies

have been carried out to investigate the presence of pathogenic parasites in man, which were also zoonotic infections (Bugg *et al*, 1999; Oliveira-Sequeira *et al*, 2002; Sánchez Thevent *et al*, 2003; Asano *et al*, 2004; Fontanarrora *et al*, 2006). The prevalence as reported is as follows: *Ancylostoma* sp ranging from 1.9-23.6%, *T. canis* (1.7-10.9%), *Dipylidium caninum* (0.2-0.7%), *Taenia* sp/*Echinococcus* sp (0.31-2.5%) and nematodes larvae (6-12%). Studies carried out to determine infections in pet animals showed a correlation between environmental contamination and zoonotic parasites, especially *Toxocara canis* (Habluetzel *et al*, 2003).

A dog infected with the adult worms of *Toxocara canis* passes thousands of eggs

Correspondence: MY Noor Azian, Parasitology Unit, Infectious Diseases Research Center, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Tel: +60 0326162401; Fax: +60 0326948724

E-mail: noorazian@imr.gov.my

each day in its feces. In typical urban and rural populations, where a relatively large number of dogs have access to public playgrounds, contamination of the soil is expected to be high. Many studies have been carried out in public playgrounds and school playgrounds to assess *Toxocara* contamination in the soil. In Northern Jordan, *Toxocara* ovae were found in 35% of soil samples with viability ranging from 20-75% (Abo-shehadeh *et al*, 1989). In the UK, the prevalence of *Toxocara* ova was 24.4% (Borg and Woodruff, 1973), 20.6% in Kansas (Dada and Lindquist 1979), 15% in Ireland (O'Lorcain, 1994) and Habluetzel *et al* (2003) found 52.7% in rural areas and 24% in urban areas in Italy. In Selangor, Malaysia the prevalence of soil contamination with *T. canis* ranged from 10% to 54.5% (Loh and Israif, 1998). In this study, we sought to detect the infection rate in dog's feces as well as in soil samples and to compare the detection rates between selected urban and rural areas, with special reference to *Toxocara* spp.

MATERIALS AND METHODS

Study area

The study area (Fig 1) was comprised of 2 different demographic units: the urban area was in Setapak, Kuala Lumpur City Council (DBKL) and the rural area was in an aboriginal settlement. The rural area consisted of two villages, Pos Senderut and Pos Lenjang, in Kuala Lipis, Pahang state. Pos Senderut is located about 211 km from Kuala Lumpur, meanwhile Pos Lenjang is located more than 241 km from Kuala Lumpur. Both places can only be accessed by four-wheel drive vehicles. It takes about one and a half hours on a logging track, to reach Pos Senderut and 3 hours to reach Pos Lenjang.

Pos Senderut is comprised of 15 villages with a population of 1,772 people in 280 houses. Pos Lenjang is comprised of 14

villages with 1,535 people in 254 houses. These areas are of low socio-economic status and do not have proper sanitation, water supply or electricity. They depend on the river as the main source of water and sanitation. They use the river for all purposes, including washing, cooking, bathing, defecation and as a food source.

The urban area is situated in the north-eastern part of Kuala Lumpur and has an area of 160 km². It is about 13 km from the heart of Kuala Lumpur and on the way to Ulu Klang. Setapak consists of Gombak, Hot Spring New village, Wangsa Maju, Titiwangsa and a number of villages. Setapak is an urban area of middle and high socioeconomic status with a proper water supply, sanitation and electricity.

Sample collection

A total of 175 dog stool samples were collected from the rural areas (59 from Pos Senderut and 56 from Pos Lenjang) from January to July 2006. Sixty samples from the urban population were collected in June 2006. Samples were collected and preserved in SAF (Sodium acetate-Acetic acid Formalin) preservative in 1:5 concentration volumes. The samples were processed by the formalin ether concentration technique to detect helminth ovae and larvae.

Soil samples were also collected from the study areas. Sixty soil samples were randomly collected in each urban and rural area during the time when the dog feces were collected. Leaves and debris on top of the soil were removed before collection of soil. About 200 to 250 grams of soil from the surface to 5-7 cm in depth were collected and kept in sealed plastic bags.

Detection of intestinal parasites in dog feces from urban and rural areas with an emphasis on detection of *Toxocara canis*

SAF fixed stool was sieved through 2-layer gauze into a 15-ml centrifuge tube. The tube was then centrifuged for 5 minutes at



Fig 1–Map of Peninsular Malaysia showing urban and rural study sites.

2,000 rpm. SAF solution was added to make up a volume of 10 ml. Three milliliters of ether was added to the suspension and mixed by shaking vigorously for 1 to 2 minutes. The mixture was then centrifuged for 5 minutes at 2,000 rpm. The supernatant was discarded and the pellet was mixed and a drop of the suspension was then transferred onto a glass slide and covered with a cover-slip and examined under 10x and 40x objectives under the microscope.

Detection of parasites from soil contamination with helminthes in rural and urban areas with an emphasis on detection of *Toxocara canis* ova

The recovery of *Toxocara* ova from the soil was performed using a modified flotation method from O’Lorcain (1994) with a little modification. The soil samples were sieved

through 1 mm² mesh to remove big particles. Fifteen grams of sieved soil was transferred into 50 ml centrifuge tubes. Fifty milliliters of 1% tween 80 solution was added and vortexed vigorously for 1 minute and centrifuged at 1,500 rpm for 3 minutes. The supernatant was then discarded and washed twice with distilled water followed by centrifugation at 1,500 rpm for 3 minutes each. The supernatant was discarded and 30 ml of flotation solution was added, mixed and 15 ml of the suspension was transferred into a 15-ml centrifuge tube and centrifuged at 4,000 rpm for 15 minutes. The flotation medium in each tube was then topped up to form a positive meniscus upon which a cover slip was superimposed and left at room temperature for a minimum 10 minutes. The cover slips were then transferred onto the glass slides and

examined immediately. Another cover slip was placed on top of the same centrifuge tube until no positive samples were recovered from the cover slips.

Data analysis

All statistical calculations were performed using SPSS ver 11.5. The chi-square test (χ^2) was performed to compare the prevalences in the urban and rural areas for the fecal and soil samples.

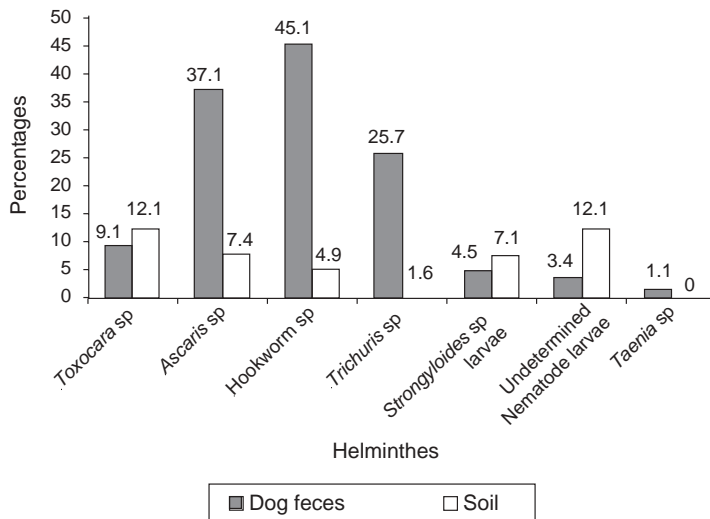


Fig 2—Comparison of helminthes detected in dog feces and soil samples in urban and rural areas.

RESULTS

Six species of nematodes and one species of cestode (*Taenia* sp) were found from a total of 175 stool samples (115 from rural and 60 from urban areas) and 138 (78.9%) were positive with either nematodes or cestodes. In the soil samples (60 samples from urban and 122 samples from rural areas), only 6 species of nematodes were detected giving an overall positive rate of 35.7%. In dog

feces the prevalence of hookworm sp was found the most often, in 45.1%, and in soil samples *Toxocara* sp had the highest prevalence at 12.1%. Fig 1 shows the overall detection rates of helminthes in dog feces and soil samples from urban and rural areas. Mixed infections occurred in all samples. The percentage of mixed infection with two parasites was 32.6%, with three parasites was 11.6% and four or more was 1.4% in the dog feces samples. In the soil samples, mixed infection with 2 and 3 parasites were 21.7% and 1.7%, respectively.

Table 1
Positivity of intestinal helminthes in dog feces in urban and rural areas.

Parasites	Total positive N=175 (%)	Urban dogs N=60 (%)	Rural dogs N=115 (%)	Chi-square (χ^2)	p-value ($\alpha=0.05$)	OR (95% CI)
<i>Toxocara</i> sp	16 (9.1)	4 (6.7)	12 (10.4)	0.647	0.412	1.631 (0.502-5.295)
<i>Ascaris</i> sp	65 (37.1)	9 (15.0)	56 (48.7)	19.175	0.001	5.379 (2.423-11.939)
Hookworm sp	79 (45.1)	46 (76.7)	33 (28.7)	36.639	0.001	8.165 (3.97-16.806)
<i>Trichuris</i> sp	45 (25.7)	18 (30.0)	27 (23.5)	0.878	0.349	1.397 (0.693-2.814)
<i>Strongyloides</i> sp larvae	8 (4.5)	0 (0.0)	8 (7.0)	NC	NC	-
<i>Taenia</i> sp	2 (1.1)	4 (4.67)	2 (1.7)	NC	NC	-
Undetermined Nematode larvae	6 (3.4)	0 (0.0)	6 (5.2)	NC	NC	-

NC = not calculated

Table 1 shows a comparison of helminth detection rates in dog feces. Eighty-one point seven percent of urban dog feces and 77.4% of rural dog feces with an overall percentage of 78.9 were found to have helminthes. There was no statistically significant difference between urban and rural dog feces for the detection of helminthes ($p = 0.511$). There were statistically significant differences when comparing urban and rural dog feces for *Ascaris* sp ($p=0.001$), Hookworm sp ($p = 0.001$) and *Strongyloides* sp larvae ($p = 0.036$).

Table 2 shows helminthes found in soil samples in 26.7% and 4.9% from urban and rural soil, respectively, giving an overall prevalence of 12.2%. These results were statistically significant ($p=0.001$). Helminthes were found 7 times more often in urban soil than rural soil (95% CI: 2.59-19.12). There were also statistically significant differences for *Toxocara* sp ($p = 0.001$), *Ascaris* sp ($p = 0.027$), *Strongyloides* sp larvae ($p = 0.001$) and undetermined nematode larvae ($p = 0.001$) between the two areas.

DISCUSSION

Toxocariasis has become increasingly recognized as an important clinical problem.

Small children are more at risk of acquiring toxocariasis due to lifestyle and their play environment (Glickman and Schantz, 1981). The prevalences of toxocara antibodies range from 10.9% (Abo-shehada, 1989) to 13.65% (Havasiova *et al*, 1993). In Malaysia, Hakim *et al* (1997) reported a seroprevalence of *Toxocara* ranging from 10.9 to 35.5%.

Intestinal helminth infections in dogs are highly prevalent in both rural and urban areas in Pahang and Kuala Lumpur. In this study, examination of the soil samples from urban and rural localities showed similar findings where overall contamination of the soil samples were more prevalent in urban areas (90%) than rural areas (18.3%). Higher contamination of soils from urban areas may be due to stray dogs, which scavenge rubbish and defecate near housing areas. They are also likely to visit public playgrounds, especially at night, to defecate and rest because the playgrounds in urban areas are not protected with fencing.

With *T. canis* infection, even though rural dogs had a higher infection rate than urban dogs, this may not represent the actual situation, since soil evaluation showed urban areas were more highly contaminated by this parasite. The type of stool sample should also

Table 2
Detection of ova and larvae in soil samples from urban and rural areas

Parasites	Total Positive N=182 (%)	Urban soil N=60 (%)	Rural soil N=122 (%)	Chi-square (χ^2)	p-value ($\alpha=0.05$)	OR (95% CI)
<i>Toxocara</i> sp	22 (12.1)	16 (26.7)	6 (4.9)	17.702	0.001	7.030 (2.585-19.118)
<i>Ascaris</i> sp	9 (7.4)	6 (10.0)	3 (2.5)	4.866	0.027	4.407 (1.062-18.284)
Hookworm sp	6 (4.9)	3 (5.0)	3 (2.5)	0.815	0.367	2.088 (0.409-10.668)
<i>Trichuris</i> sp	3 (1.6)	0 (0.0)	3 (2.5)	NC	NC	-
<i>Strongyloides</i> sp larvae	13 (7.1)	13 (21.7)	0 (0.0)	NC	NC	-
Undetermined Nematode larvae	22 (12.1)	16 (26.7)	6 (4.9)	17.702	0.001	7.030 (2.585-19.118)

NC = not calculated

be taken into account, whether it was dry (defecate more than 24 hours) or wet stool (new defecation). If the stool was dry, it is possible the ova were no longer in the fecal material. The number of samples may affect the results but in terms of stray dogs, we assumed the dog behavior in rural areas was the same as in urban areas (in this rural area, there were no stray dogs). Dogs in rural areas, even though they are considered as having owners were not treated as dogs with owners as in urban areas. The differences between dogs with owners of urban areas and dogs with owners in rural areas is frequent anti-helminthic treatment of urban dogs, due to greater health consciousness of their owners who usually keep dogs for company so they often have close contact with the family members.

Low detection of *T. canis* in soil in rural areas may be due to several reasons. It may be due to the dogs not being infected at the time of soil collection, the infected dogs may defecate outside housing areas since during the daytime, they spend their time following their owners on the farm (traditional farm) in the forest. Environmental conditions may be another contributing factor, especially during the rainy season as well as the type of soil. In urban areas, the soil in parks is a normal, organic type, whereas in rural areas it may be sandy or/and hard. Ova can be washed from fecal material during heavy rainfall and resulting in negative detection of the ova. According to Loh and Israfi (1998), the differences in ova recovery from various types of soil is the magnitude of the interparticle forces between the soil particles and the ova. The interparticle forces are the physical, chemical and biotic forces in intimate combination with organic material which form an organized arrangement of aggregate particles. It also may be because of the sampling method. Collection of the soil should be improved so as to also collect soil with stool defecation. Sampling may also need

to involve more than two playgrounds. The method used for ova recovery was satisfactory because the recovery rate was 82.5% (Quinn *et al*, 1980).

The level of infection is indicative of major problems with environmental contamination, basic hygiene and sanitation, and risk for close contact with animals that can harbor zoonotic parasites such as dogs and swine. Direct contact with infected dogs is not considered as a potential risk because embryonation of *Toxocara* ova to the stages of infectivity requires a minimum of 2 weeks. Therefore *T. canis* infections are more likely to be a hazard for people exposed to contaminated environment (Overgaauw, 1997). In studies conducted by Quinn *et al* (1980), O'Lorcain (1994), Dada and Linquist (1979), Mahdi and Ali (1993), and Abo-shehadeh (1989) the prevalences of *T. canis* contaminated soil samples were 11.1%, 15%, 20.6%, 12.2% and 15.45%, respectively. These findings show environmental contamination with dogs infected feces is becoming more significant and control should be carried out effectively.

The prevalence of *T. canis* infection was not much different from other countries. In a study by Overgaauw (1997) of household dog and cat feces, he found 2.9% of dog and 4.7% of cat feces infected with *Toxocara* eggs, 0.7% of dogs were positive for *Trichuris* eggs and no hookworm eggs were found. Fontanarrosa *et al* (2006) found that in the most crowded cities in Argentina, the prevalence of *T. canis* was 11.0%, whilst Minnaar *et al* (2002) found in urban areas, the prevalence of *T. canis* was 21.0%. The highest seropositivity was found in small mammals from urban and rural localities at 22.2% and 21.6%, respectively. *T. canis* was most prevalent in urban stray dogs (75%) and least prevalent in foxes from the mountains (7.0%). The prevalences of *Toxocara cati* in cats in rural, urban and mountain localities were 66.2%, 65.2% and 76.9%, respectively.

The high level of environmental *Toxocara* sp contamination in urban and rural areas both in public playgrounds and private domestic environments calls for a greater awareness of the problem among the population. Awareness of the possible infection routes needs to be raised and made known to both children and adults. They should be aware that egg ingestion may be a consequence of soil related activities, such as playing, gardening and contaminated vegetable consumption. Furthermore, knowledge should be provided on appropriate anti-helminthic treatment of dogs and on appropriate hygiene measures to be taken, such as collection of feces, to reduce parasitological contamination in the environment.

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