TOXOCARA EGG SOIL CONTAMINATION AND ITS SEROPREVALENCE AMONG PUBLIC SCHOOL CHILDREN IN LOS BAÑOS, LAGUNA, PHILIPPINES

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Abstract. The soil-transmitted nematode *Toxocara* sp has little epidemiological information in the Philippines. In this study, we studied the extent of soil contamination with *Toxocara* eggs and the seroprevalence of *Toxocara* infection among public school children in Los Baños, Laguna, Philippines. Soil samples were obtained from public schools, backyards, and empty lots in Los Baños to examine for the presence of *Toxocara* eggs using the modified sucrose flotation technique. Serum samples were obtained from public school children in Los Baños and examined for *Toxocara* infection using an ELISA test. Of the 200 soil samples, 85 (43%) were positive for *Toxocara* eggs at a concentration of 1 egg/g of soil. Forty-two percent of soil samples obtained from the public school, 45% of backyard samples, and 40% of empty lot samples were positive. Of the 75 serum samples from children, 37 (49%) were positive for *Toxocara* infection. There was a positive correlation between *Toxocara* egg concentration and seroprevalence of *Toxocara* infection. Results showed a high prevalence of soil contamination and a high seroprevalence of *Toxocara* infection among children in Los Baños, Laguna, Philippines.

Keywords: *Toxocara,* soil contamination, seroprevalence, school children, Philippines

INTRODUCTION

Soil is a potential source of helminth parasites. The World Health Organization (WHO) has reported two billion people world-wide are estimated to be infected by soil transmitted helminthes, such as *Ascaris lumbricoides*, hookworms, and *Trichuris trichiura*. These parasites can cause chronic morbidity and debilitation (Doligalska and Donskow, 2003).

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Toxocara canis and *Toxocara cati* are less known soil-transmitted helminths that cause human toxocariasis. One study found 2-88% of soil samples collected from different countries and regions have *T. canis* and *T. cati* eggs (Institute for International Cooperation in Animal Biologies, 2005). These nematodes are found in dogs and cats, respectively, then can be transmitted to the environment and then to humans through ingestion of contaminated soil or food with embryonated egg (Dubna *et al*, 2007). Upon ingestion they migrate to the different tissues and organs causing toxocariasis. This disease is characterized by two syndromes: visceral

larva migrans and ocular larva migrans. Visceral larva migrans can cause persistent eosinophilia which in most cases is asymptomatic; ocular larva migrans can cause ocular disease resulting in vision loss and it can cause retinal granulomas (Smith *et al*, 2009).

Toxocariasis is more common in children under age 20 (CDC, 2007) and may affect the neuropsychological performance and decrease the cognitive development (Sharghi *et al*, 2000). Transmission of *Toxocara* is favored by high ambient temperatures and humidity (Institute for International Cooperation in Animal Biologies, 2005). In the Philippines, there has been little awareness of human toxocariasis, although there are plenty of feral dogs and cats, soiled areas, and an increasing population of children. There have been no published studies of soil contamination with *Toxocara* eggs and infection seroprevalence in the Philippines; therefore we aimed to accomplish this with this study.

Of the nine species of *Toxocara* (Institute for International Cooperation in Animal Biologies, 2005), the focus of this study will only be on *T. canis* and *T. cati*. These two species were not distinguished from each other since they are morphologically similar (Uga *et al*, 2000).

This study aimed to examine the extent of soil contamination with *Toxocara* eggs and to determine the prevalence of *Toxocara* infection among public school children in Los Baños, Laguna, Philippines.

MATERIALS AND METHODS

Study site and soil sample collection (Fig 1)

The municipality of Los Baños is classified as an urban area based on the 2000 Census of Population and Housing (CPH), Report No. 4 Urban Population (National Statistics Office, 2006). Los Banos is composed of 14 districts of "barangays", 5 of which were randomly selected for this study by drawing lots: Bambang, Batong Malake, Bayog, Maahas and Mayondon. From each randomly selected barangay, 1 public school, 3 backyards and 3 empty lots were semi-purposively selected (*ie*, the sample sites chosen were those frequented by dogs and cats). Ten samples were obtained from each public school, five from each backyard and five from each empty lot. Each sample consisted of 50 grams. The samples were obtained at a depth of 5 cm and placed in labeled "Ziplock" bags (Dubna *et al*, 2007).

Determining *Toxocara* eggs concentration in the soil

The soil samples were dried at room temperature and then filtered through 125 m mesh. Approximately 2 g of the filtered sample was placed in a centrifuge tube, mixed with 6 ml of distilled water and then centrifuged at 1,800 rpm for 10 minutes. The supernatant was decanted and the residue was then mixed with 8 ml sucrose solution [specific gravity (S.G.) = 1.2]. The suspension was then centrifuged at 1,800 rpm for 10 minutes. The tubes containing the suspension were filled to the brim with additional sucrose solution (S.G. = 1.3) and then covered with a cover slip.

Microscopic examination

The slide samples were examined under a microscope at high power. If the eggs could not be classified a drop or two of Lugol's iodine was added to the fluid at the edge of the cover slip, and the preparation was then re-examined after 5 minutes. The preparations were viewed within 15 minutes of preparation to avoid over-staining (Gillespie and Hawkey, 1995). An ocular micrometer was used to verify egg sizes.

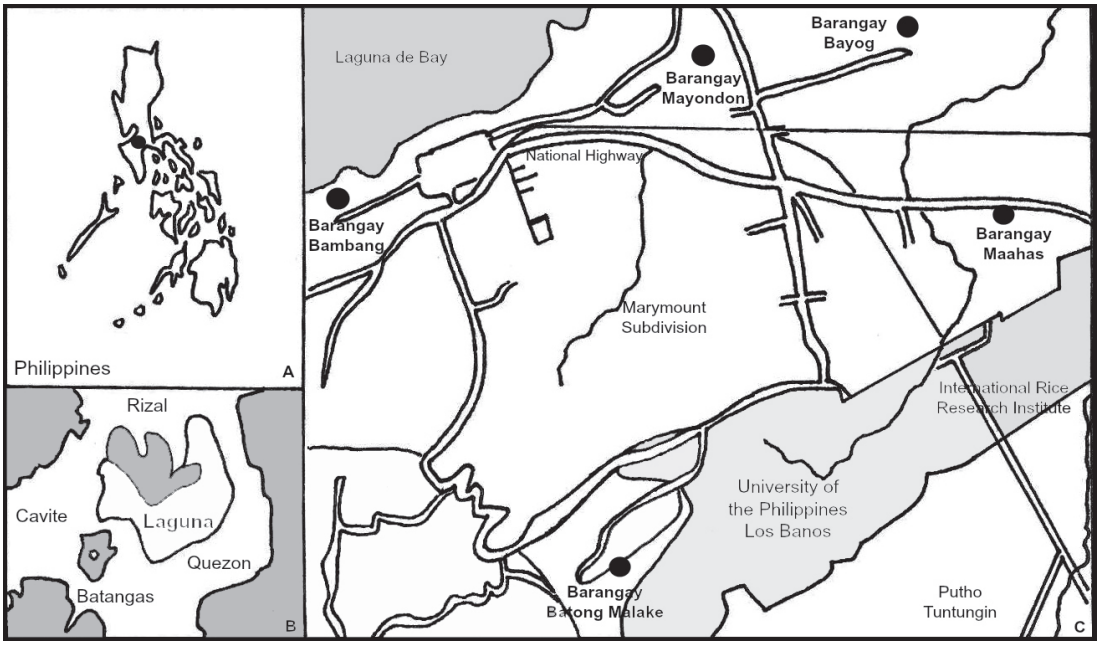

Fig 1–Map of Los Baños, Laguna showing sampling sites.

Enzyme-linked immunosorbent assay

Serum samples (15 1by finger prick) were obtain from 75 randomly selected public school children aged 8-13 years from three barangays: Bambang, Bayog and Mayondon (25 samples from each barangay). The study was approved by the Municipal Health Office and with the consent of the parents of the children. The studied children completed a questionnaire asking about socio-economic factors with the help of their parents and/or guardians.

The blood samples collected were centrifuged at 3,000 rpm for 30 minutes within 24 hours of collection and then stored at -20ºC until used.

A DRG® *Toxocara canis* ELISA kit (DRG International, Mountainside, NJ) was used to determine the presence of toxocariasis.

Data analysis

A comparison of the prevalence of soil contaminated with *Toxocara* from the backyards and empty lots within a barangay was conducted using the independent sample *t*-test. To compare the backyards and empty lots among barangays, one-way analysis of variance (ANOVA) was performed using PASW Statistics version 18.0 software. All data were transformed to an arc sine before statistical analysis.

For seroprevalence tests, the measured absorbance was subtracted from the value of the no-serum blank. A result was considered negative if the absorbance reading of the analyzed sample was less than 0.3 (the IgG antibodies against *Toxocara* excretory/secretory antigen were not significantly elevated) (Bordier Affinity Products, 2007; DRG International, 2009).

Possible socio-economic risk factors

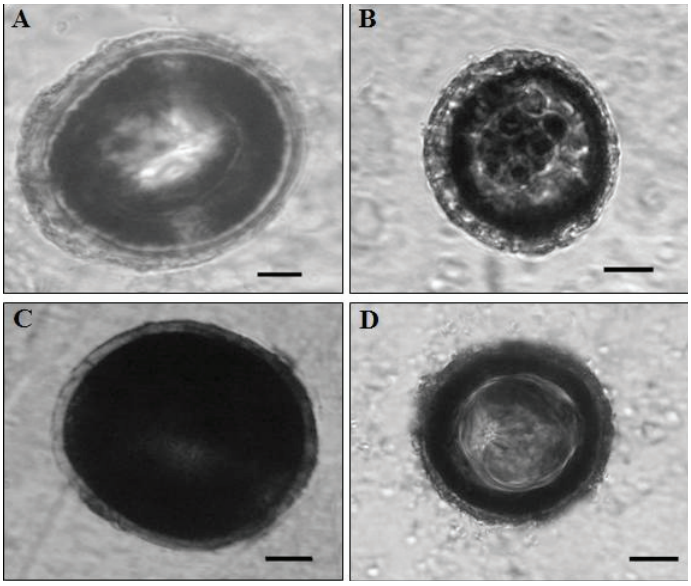

Fig 2–*Toxocara* eggs found in soil samples examined: A-C, unembryonated eggs; D, embryonated egg (scale bar = 15 m).

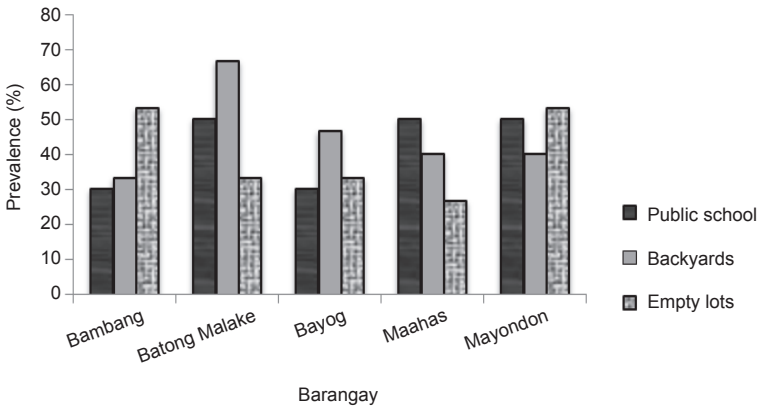

Fig 3–Prevalence of *Toxocara* eggs in soil samples from sample sites, Los Baños.

for *Toxocara* infection based on the questionnaires were analyzed using an independent *t*-test and a one-way ANOVA. The prevalence and intensity of *Toxocara* eggs in the soil were compared with the seroprevalence of *Toxocara* infection using Pearson correlation analysis.

RESULTS

Prevalence of *Toxocara* in soil

From the 200 soil samples collected, 85 (43%) were positive for *Toxocara* eggs with a maximum of 8 *Toxocara* eggs found in each of two soil samples: a backyard in Bayog and a public school in Maahas. Fig 2 shows some of the *Toxocara* eggs found in the soil samples examined. Both unembryonated and embryonated eggs were observed.

The average concentration of *Toxocara* eggs per soil sample was 1 egg/g soil sample (Table 1). This concentration was the same for all 5 barangay study location at all sampling sites. A public school in Maahas had the greatest concentration of 2 eggs/g soil.

Fig 3 shows a summary of the prevalences of *Toxocara* eggs at each of the five barangays in Los Baños. In Bambang, *Toxocara* eggs were found in 30, 33 and 54% of public school, back yard and empty lots soil samples, respectively. In Batong Malake, *Toxocara* eggs were found in 50, 67 and 33% of public school, back yard and empty lot soil samples, respectively. In Bayog, *Toxocara* eggs were found in 30, 47 and 33% of public school, back yard and empty lot soil samples, respectively. In Maahas, *Toxocara* eggs were found in 50,

Table 1
Mean concentrations of *Toxocara* eggs per 2 g of soil at the study sites.

Barangay	Public school	Backyard	Empty lot	Average
Bambang	2	3	2	2
Batong Malake	1	2	3	2
Bayog	2	3	2	3
Maahas	4	3	2	3
Mayondon	2	2	2	2
Average	2	2	2	2

Table 2
Prevalence of *Toxocara* antibodies by selected variables.

Variables	Positive/examined (%)			
	Bambang (*n*=25)	Bayog (*n*=25)	Mayondon (*n*=25)	Total (*n*=75)
Gender				
Male	5/7 (71)	11/14 (79)	4/10 (40)	20/31 (65)
Female	8/18 (44)	5/11 (45)	4/15 (27)	17/44 (39)
Age				
8-9 yrs old	2/2 (1)	8/10 (80)	1/2 (50)	11/14 (79)
10-11 yrs old	7/17 (41)	5/10 (50)	6/13 (46)	18/40 (45)
12-13 yrs old	4/6 (67)	3/5 (60)	1/10 (10)	8/21 (38)
Pet ownership				
Dogs	4/7 (57)	7/12 (58)	2/7 (29)	13/26 (50)
Cats	4/6 (67)	3/4 (75)	1/2 (50)	8/12 (67)
Both	3/6 (50)	2/2 (100)	3/7 (43)	8/15 (53)
None	2/6 (33)	4/7 (57)	2/9 (22)	8/22 (36)
Daily family income				
< Php 100	1/4 (25)	1/1 (100)	0/2 (0)	2/7 (29)
Php 100-500	11/19 (58)	11/20 (55)	6/19 (32)	28/58 (48)
>Php 500	1/2 (50)	4/4 (100)	2/4 (50)	7/10 (70)

Php, Philippines Pesos

40 and 27% of public school, back yard and empty lot soil samples, respectively. In Mayondon, *Toxocara* eggs were found in 50, 40 and 53% of public school, back yard and empty lot soil samples, respectively.

The prevalences of soil samples positive for *Toxocara* eggs were not significantly different from eachother (Fig 4).

The overall mean prevalence at each barangay are shown in Fig 5.

Serological prevalence

Of the 75 serum samples from public school children in the three barangays examined, 37 (49%) had IgG antibodies against *Toxocara* with an ELISA test.

The largest number of children with a serum sample positive for *Toxocara* IgG antibodies were from Bayog (16), followed by Bambang (13), and then Mayondon (8) (Fig 6).

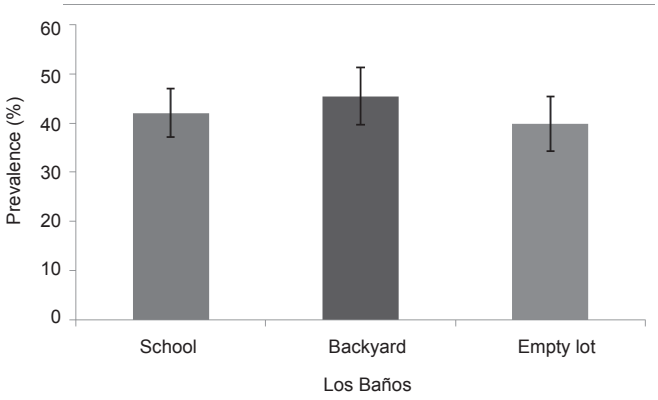

Fig 4–Prevalences (mean ± SD) of *Toxocara* eggs from each type of sample site.

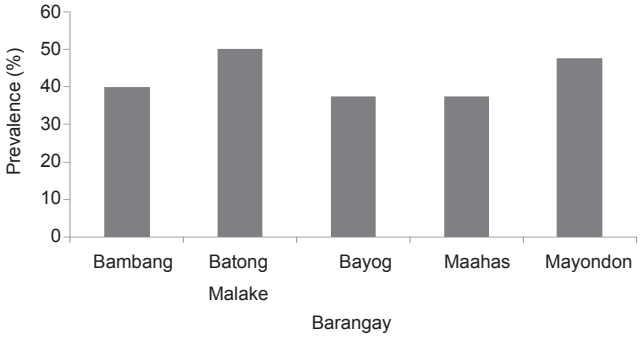

Fig 5–Prevalences of *Toxocara* eggs at each of the five barangay study sites.

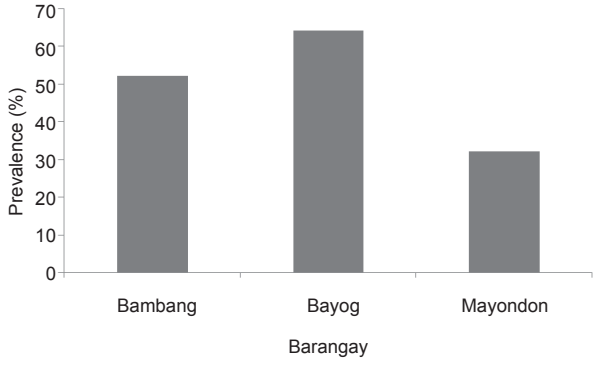

Fig 6–Seroprevalence of *Toxocara* antibodies among public school children in the three barangays of Los Baños.

Seventeen of 44 females (39%) were positive for *Toxocara* infection, while 20 of 31 males (65%) were positive. The children, were divided into groups based on age: 8-9 years old, 10-11 years old, and 12-13 years old. Eleven of 14 children (79%) aged 8-9 years had *Toxocara* antibodies, 18 of 40 children (45%) aged 10-11 years had antibodies and 8 of 21 children (38%) aged 12-13 years had antibodies. Thirteen of 26 children with dogs as pets (50%) had antibodies, 8 of 12 children with cats as pets (67%) had antibodies, 8 of 15 children with both dogs and cats as pets (53%) had antibodies and 8 of 22 children with no pets (36%) had antibodies. Two of 7 children (29%) who had a daily family income below 100 Philippine Pesos (Php) had a serum sample positive for *Toxocara* antibodies, 28 of 58 children (48%) with a daily family income of Php 100-150 were positive and 7 of 10 children (70%) with a daily family income greater than Php 500 were positive (Table 2).

To better understand the impact of the prevalence of *Toxocara* eggs in soil on the seroprevalence of *Toxocara* infection, the two were compared. There was a negative correlation between the prevalence of *Toxocara* eggs in the soil and the seroprevalence of *Toxocara* infection (r = -0.99). On the other hand there was a positive correlation between the concentration of *Toxocara* eggs in the soil and the seroprevalence of *Toxocara* infection (r = 0.96).

DISCUSSION

Approximately 43% of the soil samples in this study were positive for *Toxocara* eggs. This finding is similar to a study from the Czech Republic where 45% of the soil samples examined were positive (Dubna *et al*, 2007). This finding is greater than a study from Turkey (19%) (Gurel *et al*, 2005) but less than a study from Thailand (56%) (Wiwanitkit and Waenlor, 2004). The mean concentration of *Toxocara* eggs in this study was 1 egg/g soil. This finding is higher than the concentration seen in a study from both rural and urban Czech Republic (6 eggs/100g soil) (Dubna *et al*, 2007), but similar to a study from Maryland, USA conducted in backyards and gardens (1 egg/g soil) (Childs, 1985). The prevalence and concentration of *Toxocara* eggs in soil samples in this study imply the presence of many feral dogs and cats defecating in the soil.

Both unembryonated and embryonated eggs were observed in the soil samples collected. The presence of embryonated eggs indicates the environment is favorable for the development of these eggs, and the eggs were on the soil long enough for embryonation (Tinoco-Garcia *et al*, 2007). These embryonated eggs can be infective, causing toxocariasis upon ingestion.

Of the sampled sites, the backyards in Batong Malake had the highest prevalence of *Toxocara* eggs. The residences where backyard soil samples were collected in this study have dogs as their pets. The high prevalence of eggs in these soil samples implied that the pet dogs may be infected increasing the risk for the residents to become infected. Backyards are a popular play area for children (Dubna *et al*, 2007). The 67% soil prevalence with *Toxocara* eggs in this area is alarming. *Toxocara* soil contamination rates in the backyards were not significantly different among the five barangays.

Of the public schools examined those in Bambang and Batong Malake did not keep their areas enclosed, allowing dogs to enter the school grounds. However, at all the schools, cats were seen while collecting soil samples. The presence of eggs in the soil samples collected suggests the cats and dogs living around the school may be infected with *Toxocara*. During sample collection, children were seen playing in the soil with their bare hands and not wearing shoes or slipper, thus increasing the risk of acquiring *Toxocara* infection. Toxocariasis may decrease the neurophysiological and cognitive development of infected children (Sharghi *et al*, 2000).

Children may also play in empty lots where *Toxocara* eggs were found. Forty-four percent of the studied children said they play in empty lots, increasing their risk for *Toxocara* infection.

The high seroprevalence of *Toxocara* among public school children in Los Baños, Laguna (49%) is alarming. This prevalence is higher than a study from Peru (7%) (Lescano *et al*, 1998) and Italy (2%) (Habluetzel *et al*, 2003).

The highest number of children positive for *Toxocara* infection was in Bayog (16), followed by Bambang (13) and Mayondon (8). Most of the children in Bayog (56%) played in empty lots where the soil was accessible to dogs and cats. Forty percent of empty lot soil samples were positive for *Toxocara* eggs. In Bambang, 40% of children played in empty lots and 52% played at school where dogs and cats had easy access to the grounds.

Possible socio-economic factors affecting *Toxocara* infection were also

analyzed. However, there were no significant associations ($p < 0.05$) between the seroprevalence of *Toxocara* antibodies and gender, age, pet ownership, and family daily income.

Males had a slightly higher prevalence of infection than females. This could be due to the play habits of male children who may spend more time playing in areas at higher risk for contracting infection. The lower seroprevalence of infection among female children could be attributed to the females spending less time playing outside and more time helping inside with the household chores. However, there were no significant differences in prevalences of infection between male and female students ($p < 0.05$). Similar findings were reported in a study from Peru where no significant difference was seen in prevalences between the genders (Lescano *et al*, 1998).

There was a slightly higher prevalence of infection among children aged 8-9 years than among older children. The younger children tended to spend more time outdoor playing in the soil, while older children spent more time helping with household chores and studying. Most of the older children, specifically from Bayog, preferred to play computer games, reducing their risk of contact with soiled environments. Younger children are more likely to have pica; this increasing the chance of contracting *Toxocara* infection orally. However, there were no significant differences in seroprevalence by age group ($p < 0.05$). Similar findings were reported in a study from Peru, where no significant difference in infection prevalence were seen by age group (Lescano *et al*, 1998).

It is assumed the *Toxocara* eggs were introduced into the environment in the feces of dogs and cats; therefore, the subject of pet ownership is important. Children with no pets at home had a slightly lower seroprevalence of infection, while children with pets (dogs, cats, or both) had a slightly greater seroprevalence of infection. Children in close contact with dogs or cats had a slightly greater risk of ingesting eggs. However, there was no significant difference in seroprevalence between children who owned pets (regardless of the type), and those who do not ($p < 0.05$). However, another study did find a significant difference in seroprevalence between children who owned pets and those who did not (Baboolal and Rawlins, 2002).

Children belonging to a family with greater income had a slightly higher seroprevalence of *Toxocara* infection, but this difference was not statistically significant ($p < 0.05$). The incomes of the families of the subjects (*ie*, 100-500 Php/day) was at the poverty level, thus no difference was seen between richer and poorer households in the prevalence of *Toxocara* infection.

There was a negative correlation ($r = -0.99$) between soil prevalence of *Toxocara* eggs and seroprevalence of infection among public school children. As the number of position soil samples increased, the number of children infected with *Toxocara* decreased. This could be attributed to the number of *Toxocara* eggs per soil sample were not enough to start infection. However, there was a positive correlation ($r = 0.96$) between the concentration of *Toxocara* eggs in soil and the seroprevalence of infection. The greater the concentration of *Toxocara* eggs present in soil, the greater the chance of ingesting an egg and contracting infection. Only three barangays were included in the study of

seroprevalence of *Toxocara* infection. A greater number of barangays need to be studied to better elucidate the relationship between concentration of *Toxocara* eggs in the soil and seroprevalence of infection.

This is the first report of the soil prevalence of *Toxocara* eggs and infection among school children in the Philippines. This alarmingly prevalence of *Toxocara* eggs in the soil and seroprevalence of *Toxocara* in school children represents a public health hazard in the community (Lescano *et al*, 1998). This baseline information can be used for control and prevention of toxocariasis and other soil-transmitted helminth infections common in children.

This study gives baseline information regarding soil contamination and infection with *Toxocara* in Los Baños, Laguna, the Philippines. The results of this study can be helpful in conducting toxocariasis awareness programs and preventive measures, such as deworming of dogs and cats, strict implementation of the ordinance on registration of pets and the continuous practice of proper hygiene. Further studies from other areas in the Philippines are needed to assess the prevalence of soil contamination and infection among school children. Easier, specific diagnostic methods to determine active infection with *Toxocara* need to be developed for more efficient diagnosis of this zoonotic disease.

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