# THE HIGH PREVALENCE OF ASYMPTOMATIC *TOXOCARA* INFECTION AMONG SCHOOLCHILDREN IN MANADO, INDONESIA

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Abstract. We performed a serological survey of *Toxocara canis* infection in junior high school students from three districts in northern Sulawesi. Almost all of the 117 subjects from two rural districts near Manado allowed dogs in their houses, and there was an 84.6% prevalence of *T. canis* infection in this group. Fifty-three subjects (45.3%) had serum samples with a high titer of specific anti-*Toxocara* antibody. By contrast, 41 students tested in one urban district showed a 12.2% prevalence. To confirm the clinical symptoms of visceral larva migrans (VML) and ocular larva migrans (OLM) caused by *Toxocara*, we administered a questionnaire survey, serological liver function tests, and an ophthalmoscopic examination in 34 subjects having high anti-*Toxocara* antibodies. One rural district showed a high prevalence; 58 out of 71 subjects (81.7%) had a high titer of anti-*Toxocara* antibodies according to a plate-ELISA test, although none showed clinical signs. Five of these subjects exhibited hypereosinophilia. These results indicated that *T. canis* infection in northern Sulawesi is latent in many more cases than previously estimated, and suggest that people living in environments polluted by *Toxocara* eggs become easily infected with *T. canis* and show a high prevalence of infection.

## INTRODUCTION

*Toxocara canis* and *T. cati* are ubiquitous gastrointestinal parasites in dogs and cats, respectively. Humans, especially young children, can be infected with these parasites following accidental ingestion of eggs containing infective-stage larvae. These parasites cause visceral larva migrans (VLM), which is characterized by fever, eosinophilia, hepatomegaly, cough, and wheezing. They also cause ocular larva migrans (OLM), which is characterized by a severe vision defect and neurologic larva migrans showing seizures and meningoencephalitis (Mok, 1968; Schantz and Glickman, 1978; Taylor *et al*, 1988). The definitive diagnosis of toxocariasis is based on the identification of larvae in tissue biopsies, while a presumptive diagnosis may be based on clinical and laboratory findings and on the presence of significant anti-*Toxocara* antibody titer (Glickman *et al*, 1978). In developed countries, there have been numerous serological surveys on toxocariasis in young children, but few such studies have been done in developing countries. We assumed that this was partly due to a lack of suitable tools for easy on-site testing. While the introduction of the enzyme-linked immunosorbent assay (ELISA), which is based on the use of excretory-secretory (ES) antigen, has resulted in greatly increased specificity, it is still difficult to perform this test in developing countries. However, since toxocariasis remains a pressing public health problem in developing countries, serological epidemiological surveys are essential to provide detailed information regarding the prevalence of *T. canis* infection and its clinical features if the transmission of *T. canis* infection to humans is to be prevented.

Although these diseases can cause a severe vision defect or liver function disorder, there are few reports on the precise relationship be-

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tween the seropositive rate and the clinically symptomatic rate. The purpose of the present study was to evaluate the factors contributing to high serological prevalence of toxocariasis and to investigate the relationship between these pathogens and clinical symptoms. We performed a serological survey of junior high school students in two rural districts and in one urban district, and found a very high prevalence rate in the rural district, with most cases being of the covert type.

## MATERIALS AND METHODS

### Specific antibody detection

Serum specimens were obtained from 158 junior high school students, aged between 10 and 15 years, from three distinct geographical areas in northern Sulawesi, Indonesia. These areas consisted of an urban area of Manado city (district A); an agricultural village (district B), which is located 32 km southwest from Manado city; and a fishing village (district C), which is located 120 km west from Manado city. Serum samples were collected from 41 individuals from district A, 71 individuals from district B, and 46 individuals from district C. Informed consent was obtained before the blood drawing in all cases. The samples were tested in the field by Toxocara-CHECK, a rapid-test kit for *Toxocara* infection, to screen for the specific anti-*Toxocara* antibody (Akao *et al*, 1997; Dubinsky *et al*, 2000). Each sample was diluted 1:10 and 1:50 in a dilution buffer (0.5% BSA in PBS, pH 7.2) and tested simultaneously. According to the test kit instructions, samples having a positive result at a serum dilution of 1:10 and a negative result at 1:50 were considered to be weakly positive, those having a positive result at 1:50 were considered to be strongly positive, and those having a negative result even at 1:10 were considered to be non-significant for toxocariasis. In addition to the on-site examination, we carried out a plate-ELSA with 158 serum samples from the three districts using the larval excretory-secretory (ES) antigen of *T. canis*. We objectively assessed the measurement of the optical density (OD) of the results of the plate-ELISA and used this as an index value for comparison with the three control samples: strongly positive, weakly positive, and negative results. We assigned an index value of 10 to the strongly positive control serum, 3 to the weakly positive control serum, and 1 to the negative control serum. Samples registering above 4 on the index value were considered to be positive.

### Questionnaire survey

All subjects were given a questionnaire about their lifestyle, water supply, eating habits, method of defecation, feeding of animals, and so forth. To evaluate the clinical symptoms caused by VML or OLM, questions about the condition of the skin rash and visual trouble were also included.

### Ophthalmoscopic examination

The ocular fundi of 34 subjects that had a high antibody titer were carefully observed using a portable ophthalmoscope (Hand-Held Fundus Camera GENESIS, Kowa Corporation, Japan) after the pupils were dilated with tropicamide (one drop for each eye; Mydrin-P, Santen Pharmaceutical, Japan). The images of the fundi were recorded photographically. No complications were reported after the examinations.

### Eosinophil counts and assessment of liver function

Eosinophil counts were performed using a thin smear stained with May-Grunwald-Giemsa staining for 34 students having a high antibody titer. To evaluate the liver functional disorder caused by VLM, we measured serum asparatate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (gamma-GTP) in 125 students from the three districts. An eosinophil rate of over 6% was considered to indicate hypereosinophilia; while an AST and ALT over 40 IU/l, and a gamma-GTP over 50 IU/l were considered to indicate a significant abnormality.

### Dot-ELISA for detecting the antibodies for pathogenic parasitic diseases

For 71 students in district B, we also conducted a screening test by dot-ELISA to detect pathogenic parasitic diseases such as those caused by *Entamoeba histolytica, Fasciola hepatica, Paragonimus miyazaki, Ascaris suum,*

*Dirofilaria immitis, Anisakis simplex,* and *Spirometra erinacei.* All but *A. simplex* and *S. erinacei* were derived from adult-stage antigens.

### Stool examination

To evaluate the intestinal parasitic infection of the local area, stool samples were collected in district A. A total of 53 samples were collected from village residents of various ages who lived near a rice field and a lake. Samples were fixed with sodium acetate-acetic acid-formaldehyde (SAF) solution (sodium acetate 1.5 g, acetic acid 2.0 ml, formaldehyde 4.0 ml, distilled water 92.0 ml), and the eggs of helminths and the cysts of protozoa were detected using the formalin-ethyl acetate sedimentation technique.

## RESULTS

### Toxocara-CHECK screening

In the on-site screening in district B, all samples diluted 1:10 were positive, and 11 (15.5%) showed strongly positive results. Thirty-seven out of 71 (52.1%) serum samples diluted 1:50 were positive ("weak positive" and "positive") according to the Toxocara-CHECK (Table 1). In the other districts, positive results were found in 5 out of 41 (12.2%) samples from district A and in 28 out of 46 (60.9%) samples from district C. The differences between the findings of the urban district (A) and of the two rural districts (B and C) were considered to be statistically significant according to a chi-square test.

### Plate-ELISA for *T. canis* second stage larval ES antigen

Fifty-eight of 71 (81.7%) serum samples showed positive by plate-ELISA in district B, and 23 of these positive samples showed strongly positive results (Fig 1). The index values ranged from 1 to 21.

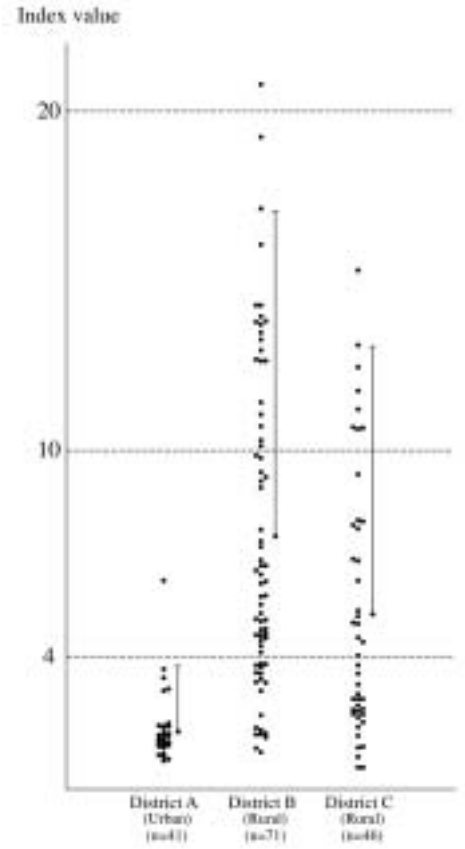


Fig 1–Distribution of index values of the samples collected from subjects from each district calculated by optical density (OD) measured by a plate-ELISA using a second stage larval excretory-secretory (ES) antigen of *T. canis*. The right side point represents the mean ±2 SD.

### Questionnaire about life style

The findings from the questionnaire regarding the life styles of the subjects from the three districts are shown in Table 2. Of the 158 questionnaires distributed, 121 (76.6%) were returned with complete answers. Differences were found between the urban area (district A) and the rural areas (districts B and C) regarding the source of water supply for drinking and cooking, management after visiting toilets, and the mode of fecal disposal. No significant differences were found regarding hand washing before eating or in the feeding of animals at home.

Table 3 presents the questionnaire data regarding the rash condition and visual trouble among our subjects. Between 52.2% and 63.9% of students reported a past history of an appearance of a skin rash. The most common report was one of a red spot on the skin. Between 15.2% and 35.3% of students reported a past history of ophthalmic trouble, with the most common complaint being itchiness. Only one subject reported problems of the visual field.

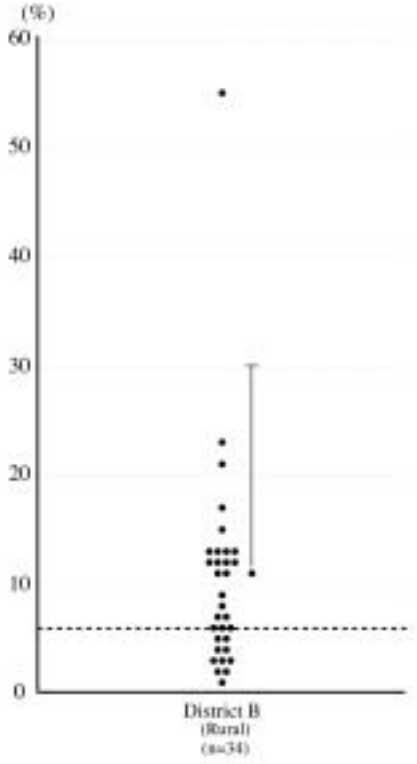


Fig 2–Distribution of the percentage of eosinophils in the total white blood cells among 34 subjects having a high antibody titer against the second stage larval ES antigen of *Toxocara* in district B. The eosinophil counts were performed by a thin-smear stained with May- Grunwald-Giemsa staining. The right side point represents the mean ±2 SD.

Clinical evaluation

No hemorrhagic lesions or granulomatous lesions were detected in any of the 34 students examined by ophthalmoscopic examination (data not shown).

Our count of the eosinophil rate relative to the total white blood cell count in the peripheral venous blood showed that 24 of 34 subjects (70.6%) had hypereosinophilia, with five of these showing extremely high levels (Fig 2).

No significant abnormality of serum transaminase (AST, ALT) or gamma-GPT was detected in district B or district C (Fig 3). In one case, a moderate abnormality was detected, although this case was negative for *Toxocara* antibody.

Dot-ELISA detection of other important pathogenic parasitic diseases

Table 4 shows the prevalence of each parasite. Table 5 shows the relationship, in five subjects, between an extremely high eosinophil rate (>14% eosinophil count) and the results of the dot-ELISA screening. One subject who had a 55% hypereosinophilia rate showed positive for both *A. suum* and *F. hepatica* antigen.

Stool examination

Table 6 shows the results of the stool examinations of the general public. In Indonesia, a mass treatment of elementary school students

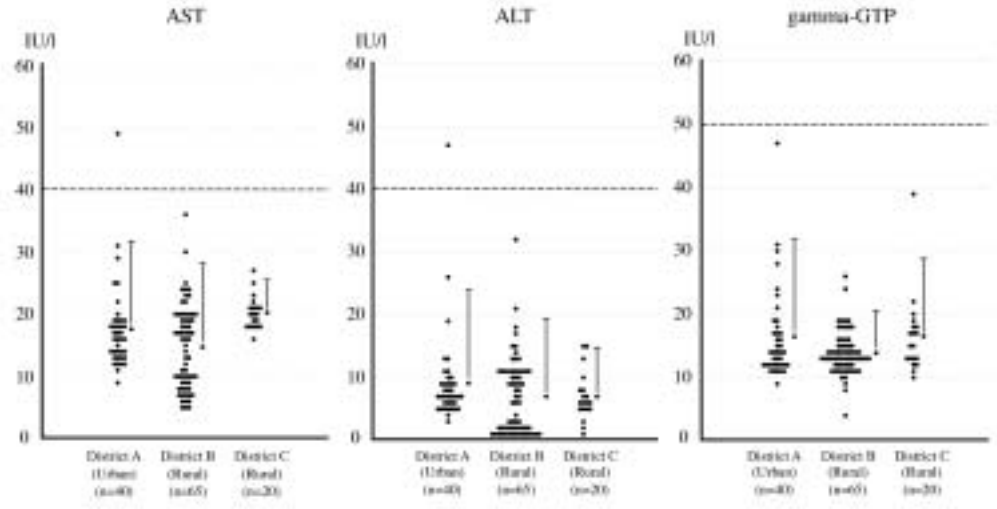


Fig 3–Distribution of the value of serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (gamma-GTP) among the subjects from three districts. The right side point represents the mean ±2 S.D.

Table 1
Result of the screening of specific anti-*Toxocara* antibody by Toxocara-CHECK.

| | District A (urban) (n = 41) | | | | District B (rural) (n = 71) | | | | District C (rural) (n = 46) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1:10 | | 1:50 | | 1:10 | | 1:50 | | 1:10 | | 1:50 | |
| Strong positive | 1 | (2.4%) | 0 | (0%) | 11 | (15.5%) | 0 | (0%) | 13 | (28.3%) | 0 | (0%) |
| Positive | 4 | (9.8%) | 1 | (2.4%) | 26 | (36.6%) | 12 | (16.9%) | 15 | (32.6%) | 16 | (34.8%) |
| Weak positive | 0 | (0%) | 0 | (0%) | 34 | (47.9%) | 25 | (35.2%) | 0 | (0%) | 0 | (0%) |
| Negative | 36 | (87.8%) | 40 | (97.6%) | 0 | (0%) | 34 | (47.9%) | 18 | (39.1%) | 30 | (65.2%) |

Each sample was diluted 1:10 and 1:50 in dilution buffer (0.5% BSA in PBS, pH 7.2).

Table 2
Percentage of the answer of questionnaire about the jounior high school students' life style in the 3 districts.

| | District A (urban) (n = 39) | District B (rural) (n = 36) | District C (rural) (n = 46) |
|---|---|---|---|
| Surce of water supply for drinking and cooking | | | |
| Public water supply | 61.5% | 0% | 6.5% |
| Well | 38.5% | 100% | 93.5% |
| Management after visiting the toilet | | | |
| With paper | 87.2% | 0% | 0% |
| With hand and water | 12.8% | 100% | 100% |
| Mode of fecal disposal | | | |
| River | 0% | 13.9% | 17.4% |
| Pit latrine | 43.5% | 47.2% | 47.8% |
| Pour flush | 56.5% | 38.9% | 34.8% |
| Washing hands before eating | | | |
| Yes | 97.3% | 100% | 97.8% |
| No | 2.6% | 0% | 2.2% |
| Habit of eating raw vegetables | | | |
| Yes | 38.5% | 45.7% | 8.7% |
| No | 61.5% | 54.3% | 91.3% |
| Way of eating | | | |
| With hands | 20.5% | 35.3% | 15.2% |
| With spoon and fork | 79.5% | 64.7% | 84.8% |
| Animals at home | | | |
| Yes | 64.1% | 97.1% | 100% |
| No | 35.9% | 2.9% | 0% |
| Bathing in the pond or river | | | |
| Yes | 10.3% | 27.3% | 45.7% |
| No | 89.7% | 72.7% | 54.3% |

Totally 158 were distributed, and 121 (76.6%) were returned.

is made before the age of 6, in compliance with a governmental public health program, which may account for our findings: in district B, no cysts of *Entamoeba histolytica,* or eggs of either Trematoda or Cestoda were detected.

## DISCUSSION

Toxocariasis is a multisystemic disease of parasitic zoonosis that occurs predominantly in young children, and it is a significant public health problem in both developed and developing countries. Until recently, toxocariasis was a relatively neglected disease in tropical regions; however, overcrowding and cohabitation of the peri-domestic environment by dogs and cats has markedly reinforced the transmission cycle of this disease in many regions. Since people feed dogs freely in rural areas, eggs have been deposited extensively in the soil. Thus, disease exposure in such areas seems inevitable.

Epidemiological surveys in industrialized countries of the northern hemisphere have revealed that the seroprevalence of helminthiasis ranges between 2.5 and 5% in urban adults (Glickman and Schantz, 1981; Glickman *et al*, 1985; Cauchie *et al*, 1990). In Europe, however, a higher rate has been reported among rural populations, ranging from 17% among Swedish farmers (Ljungstrom and Van Knapen, 1989) to over 40% in Irish children (Holland *et al*, 1995). A case-control study of Irish children reported that 27% of those who had fever, headache, and so forth, along with a high anti-*Toxocara* antibody titer still had normal blood eosinophil counts (Taylor *et al*, 1988). The existence of chil-

Table 3
Number of the answer of questionnaire about the condition of the rush and visual trouble among jounir high school students in 3 districts.

| | District A (urban) (n = 39) | District B (rural) (n = 36) | District C (rural) (n = 46) |
|---|---|---|---|
| Do you have ever developed rush? | | | |
| Yes | 22 (56.4%) | 23 (63.9%) | 24 (52.2%) |
| Which is the part of rush? | | | |
| Hands or wrist | 14 | 18 | 5 |
| Forearm | 1 | 2 | 2 |
| Arm | 1 | 1 | 0 |
| Head or neck | 1 | 1 | 1 |
| Body | 1 | 5 | 7 |
| Thigh | 0 | 0 | 11 |
| Leg | 3 | 4 | 3 |
| Ankle or foot | 4 | 2 | 1 |
| What type of rush? | | | |
| Itchiness | 12 | 17 | 21 |
| Painful | 0 | 0 | 0 |
| Swelling | 2 | 3 | 1 |
| Blisters | 0 | 0 | 0 |
| Discharge / wheeping | 1 | 3 | 12 |
| Crusting | 0 | 1 | 1 |
| Red spots | 8 | 7 | 2 |
| Do you have ever developed the visual trouble? | | | |
| Yes | 23 (20.5%) | 28 (35.3%) | 8 (15.2%) |
| What kind of trouble? | | | |
| Muscae volitantes | 1 | 7 | 1 |
| Pain of eyes | 3 | 0 | 1 |
| Paropsia | 4 | 7 | 1 |
| Visual field loss | 1 | 0 | 0 |
| Photophobia | 2 | 15 | 6 |
| Lacrimation | 2 | 3 | 2 |
| Feeling of foreign body | 3 | 4 | 1 |
| Congestion | 1 | 3 | 1 |
| Itchiness | 10 | 12 | 3 |
| Blurred vision | 3 | 3 | 1 |

dren having high antibody titers but no specific clinical findings led to the concept of "covert toxocariasis."

In the present study, which was performed in a tropical country, we observed a higher seroprevalence of anti-*Toxocara* antibodies in a rural area of Manado (81.7%) than would have been predicted from the literature. We selected the Toxocara-CHECK kit for our primary survey due to its speed and ease of use. This screening method revealed a high prevalence, and confirmed that the prevalence could be easily assessed. We concluded that the kit was useful for an initial survey in areas with a high rate of environmental contamination with *Toxocara* eggs.

Table 4
Result of dot- ELISA screening for detecting the antibodies for pathogenic parasitic diseases.

| Antigens | Number of positive, n = 71 (%) |
|---|---|
| *Entamoeba histolytica* | 4 (5.6) |
| *Spirometra erinacei* | 0 (0) |
| *Fasciola hepatica* | 2 (2.8) |
| *Paragonimus miyazakii* | 0 (0) |
| *Ascaris suum* | 29 (40.8) |
| *Anisakis simplex* | 5 (7.0) |
| *Dirofilaria immitis* | 2 (2.8) |

We found a significant difference in seroprevalence between the two rural districts and the urban district, based on our questionnaire findings, this difference may be attributable to differences in the behaviors of feeding animals and in the management of wastes between urban and rural areas. Our findings further suggest that improvements in sanitation would markedly reduce these infections. While hand washing prior to eating was reported in both areas, it did not appear to be adequate to prevent infection in many cases.

In the present study, we found no clinically symptomatic case attributable to *Toxocara* infection, except for one case in which there were problems with the visual field. Of course, self-reports by children regarding symptoms of *Toxocara* infection may be unreliable. Bass *et al* (1987) suggested that longer periods, in excess of seven years, would be necessary to entirely discount the possibility of latent ocular disease, and they found that treatment with thiabendazole did not affect the course of the infection in a controlled year-long evaluation based on eosinophil counts and specific *T. canis* antibody titers. We observed a high prevalence in subjects aged between 10 and 15 years, suggesting that

Table 5
Number of students of each range of percentage of blood eosinophil counts out of total white blood cells, shown with index value to *T. canis* by plate- ELISA and result of dot-ELISA screening for 7 parasitic diseases.

| Eosinophils count (%) | No. of students n=34 (%) | Index value to *T.canis* by plate-ELISA[a] | Result of dot-ELISA screening |
|---|---|---|---|
| 1 - 5 | 10 (29.4) | 4 to 14 | |
| 6 - 14 | 19 (55.9) | 4 to 14 | |
| 15 - 19 | 2 (5.9) | 6, 13 | |
| 20 - 29 | 2 (5.9) | 9, 14 | *A. suum* + |
| 55 | 1 (2.9) | 17 | *A. suum* + , *F.h* + |

[a]Index value is defined 1 to 2 as negative, 3 as weak positive, more than 4 as positive, more than 10 as strong positive.

Table 6
Number of positive samples of intestinal parasites eggs or cyst by stool examination among village people in district B.

| | Number of positive samples in district B (n = 53) |
|---|---|
| Hookworm | 22 (41.5%) |
| *Ascaris lumbricoides* | 2 (3.8%) |
| *Trichuris trichiura* | 6 (11.3%) |
| *Strongyloides stercoralis* | 1 (1.9%) |
| *Entamoeba coli* | 15 (28.3%) |
| *Endolimax nana* | 7 (13.2%) |
| *Iodamoeba butschlii* | 1 (1.9%) |
| *Blastocystis hominis* | 9 (17%) |
| *Giardia lamblia* | 1 (1.9%) |

the junior high school students we studied may have been infected repeatedly, and may have remained infected for several years. These data also suggest that *T. canis* infection is asymptomatic, being a "covert type," in most cases.

The antibody titers we observed indicated the possibility of a past infection, a covert infection, or a cross-reaction. Many previous studies have shown that antibodies persist for long periods of time due to repeated antigen stimulation resulting from the persistence of live larvae in tissues (Beaver, 1969) or from periodic reinfection (Glickman and Shantz, 1981). Lynch *et al* (1988) reported the existence of significant cross-reactivity of non-homologous parasites with *Toxocara* in regions of endemic helminthiasis. In the present study, we performed dot-ELISA screening to detect the antibodies for other parasitic diseases, and detected *Ascaris suum* infection in 29 out of 71 cases (40.8%). Of these, four cases showed no cross-reactivity with *Toxocara*. Further studies are needed to differentiate these parasites by detecting their antibodies.

Magnaval *et al* (1997) suggested that the migration of *T. canis* larvae in the human brain does not frequently induce a recognizable neurological syndrome but is correlated with several risk factors, including exposure to dogs, a situation possibly responsible for repeated low-dose infections.

Gonzalez *et al* (2000) reported that high resolution ultrasonography revealed hypoechoic areas in the liver in 50% of their cases. Their patients had a mean age of 2 years and 9 months, presented with anemia, long-standing fever, and eosinophilic leukocytosis, and had been diagnosed as having toxocariasis based on a high titer of *Toxocara* antibodies according to an ELISA. Kaushik *et al* (1997) also reported granulomatous hepatitis caused by *Toxocara canis* that showed clinical improvement without specific therapy in all cases. Considering these reports and the present study, hepatitis caused by *T. canis* may occur in infants and may improve within a few years. Liver functional disorder may not persist in its active stage for a long

period of time, in spite of repeated infection, or it may emerge just after infection for a short period of time.

While many cases appear to be asymptomatic, there may be some background factors for the symptomatic cases, such as a compromized immune system. In mice, the levels of both IL-12 and tumor necrosis factor alpha were significantly lower in the infected group as compared with the controls (Kuroda *et al*, 2001). Although Akao *et al* (2000) and Hayashi *et al* (2003) reported an animal model for ocular toxocariasis, further studies are required to better evaluate the mechanism of toxocariasis. While it is useful to assess the pathogenesis of this disease using such animal models, a more complete understanding will be reached when they are combined with careful population studies.

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