

ELISA Seropositivity for *Toxocara canis* Antibodies in Malaysia, 1989-1991

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Summary

The laboratory test results for visceral larva migrans (VLM) using ELISA for *Toxocara canis* antibodies employed by the Institute for Medical Research, Kuala Lumpur, is described. A total of 331 serum samples received from hospitals and general practitioners all over Malaysia were tested. The test utilises excretory-secretory antigens obtained from *in vitro* culture of second stage *T. canis* larvae. The overall seropositivity rate was 19.6%, the highest positive rate being in Indians (35.5%), followed by the Malays (14.8%), Chinese (10.9%) and others (29.4%). Seropositivity rate was highest in children below the age of 10; 89% of patients presented with eosinophilia and 93% with VLM syndrome were children.

Key words: Toxocariasis, eosinophilia, visceral larval migrans, ELISA.

Introduction

Visceral larval migrans (VLM) is a syndrome caused by the migration of the larval stages of parasitic helminths in the internal organs of humans. Chief among the helminths known to cause VLM is the nematode of dog, *Toxocara canis*¹. To some extent, toxocariasis and VLM have become synonymous.

VLM is characterised by fever, hepatomegaly, pulmonary infiltration and high eosinophilia. The majority of the infections, however, remain asymptomatic with only high eosinophilia as a positive finding. Ocular involvement is an important manifestation of the disease.

As the larvae remain in internal organs and do not mature into adults, definitive diagnosis by demonstration of parasite stages in stool is extremely unlikely. Serological tests are therefore of considerable importance in detecting toxocariasis. The development and the use of enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of toxocariasis has been shown to be of great value².

The Institute for Medical Research (IMR), Kuala Lumpur, has employed the ELISA technique for detecting antibody against *T. canis* excretory-secretory (ES) antigens of the second stage (L2) larvae. Since 1988, several requests from hospitals and general practitioners all over Malaysia were received for *Toxocara* serological tests. We report here the results of positive assays for toxocaral antibodies detected by this method.

Materials and Methods

Sera

Samples of whole blood in plain bottles received from various hospitals for toxocaral antibodies were centrifuged at 5,000 rpm for 10 minutes. The sera were collected and stored at -70°C until use.

ELISA test

The ELISA test following the method of Voller *et al* was followed, with some modifications³. The *in vitro* culture of L2 and the collection of *Toxocara canis* ES antigens have been described elsewhere⁴. 200 µl of the ES antigen diluted in coating buffer, was coated onto each well of the microtitre plates and left overnight at 4°C. The plates were then washed thrice with PBS-Tween and kept at -70°C until use. Two hundred µl of each test serum diluted in PBS-Tween was added into 2 wells of the antigen coated plates and incubated at room temperature for 2 hours. After incubation, the plates were washed with PBS-Tween and then incubated for 3 hours at room temperature with 200 µl of enzyme-labelled goat anti-human IgG horseradish peroxidase conjugate (Cappel labs) diluted in PBS-Tween. After washing, 200 µl of substrate solution (orthophenylenediamine) was added and allowed to react for 30 minutes in the dark before the reaction was stopped by adding 50 µl of 2.5 M sulphuric acid to each well. The optical density (OD) at 492 nm was measured using an automatic ELISA reader (MR600, Dynatech^(R)). Each test plate had a positive and negative serum control, the former was obtained from a patient who was clinically diagnosed as toxocariasis with a high antibody titre.

Optimum dilutions for antigen, serum and conjugate were determined by checker-board titration each time a new batch of conjugate or ES antigen was used. The optimal dilutions were usually in the region of 1:2, 1:1,000 and 1:10,000 respectively. All OD readings of the test samples were standardised to initial positive control value using the following formula:

$$(\text{OD of the initial +ve control}) \div (\text{OD of the test +ve control}) \times (\text{OD of test serum}) = \text{Standardised OD value of sample.}$$
Determination of positivity cut-off point value

Sera were collected from 30 healthy volunteers who were staff of our division and the ELISA test was carried out. The mean + 3 SD OD of these healthy volunteer sera was taken as the cut-off value.

Results**Positivity rates**

A total of 331 serum samples were received and tested from January 1989 to December 1991. The majority were from around Kuala Lumpur (71%), followed by Kedah (15%, mainly Alor Setar GH). One hundred and twenty six sera were from females and 205 from males. Sixty five out of the 331 samples were positive (19.6%), with the seropositivity rates in females and males being 18.2% and 19.0% respectively ($X^2=0.1$, $p<0.05$). The mean OD of positive samples was 1.149 ± 0.263 and 0.283 ± 0.228 for negative samples (cut-off OD value = 0.836).

Table I
ELISA seropositivity for *Toxocara* antibodies by ethnic group

	No of sera	No positive*	(%)
Malay	183	27	(14.8)
Chinese	55	6	(10.9)
Indian	76	27	(35.5)
Others	17	5	(29.4)
Total	331	65	(19.6)

* ES antigen from *in vitro* culture of 2nd stage larva of *Toxocara canis* was used. OD⁴⁹² values ≥ 0.836 considered positive (representing mean+3 SD of 30 normals).

Antibody distribution by ethnic groups

Table I shows the number of tests carried out and the number that were positive by ethnic group. Antibodies were detected in all ethnic groups, with the Indians having the highest rate, 35.5% ($X^2=20.09$, $p<0.05$). Three out of 5 positive cases among the 'others' were Orang Asli.

Antibody distribution by age group

Children below the age of 10 recorded the highest positivity rate (34.5%) as compared to the older age groups (Fig 1) and the difference was significant ($X^2=35.64$, $p<0.05$). The seropositivity rates appear to decrease with increasing age, but this was not statistically significant ($r=-0.65$, $p<0.05$).

Clinical presentation

Of the various clinical presentations described by the requesting medical practitioners, the majority had eye lesions (68.5%), which included choroiditis, chorioretinitis, retinal granuloma and panuveitis. 70.5% of the samples from patients with eye lesion were from adults. Eight out of the 9 (89%) positive samples from cases who presented with eosinophilia and 13 out of 14 (93%) positive samples from cases with VLM syndrome (hepatosplenomegaly, fever, pulmonary infiltration, eosinophilia) were from children of 10 years old and below. Table II shows the positivity rates by clinical presentation.

In patients with eye lesions, the positivity rates for children below 10, 10 to 19 and more than 19 years of age were 22.8%, 12.5% and 10.6% respectively ($X^2=3.78$, $p<0.05$).

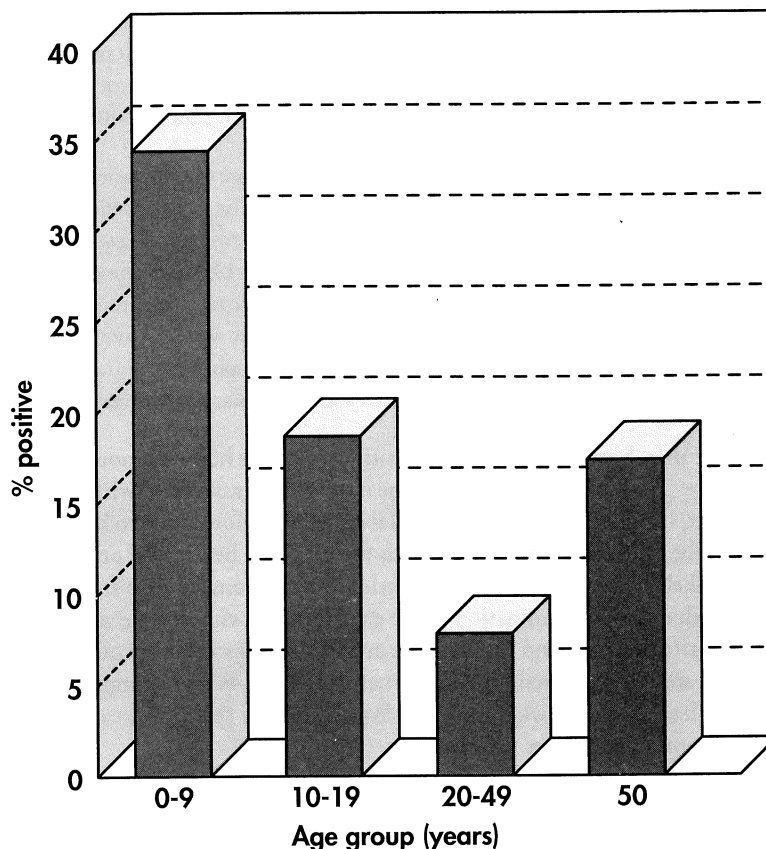


Fig 1: ELISA seropositivity for *Toxocara* antibodies by age.

Table II
Toxocara ELISA positive rates by clinical presentation

Clinical presentation	No of sera	No positive(%)
VLM syndrome	18	14 (77.8)
Eye lesions	238	30 (12.6)
Eosinophilia	34	9 (26.5)
Others*	41	12 (29.3)
Total	331	65 (19.6)

* includes: lymphadenopathy, perinatal death, history of pica, chronic cough and specimens without clinical summary.

Discussion

Cross-reactivity in antigen-antibody reaction is an important problem in the serodiagnosis of parasitic infections. Nematodes are known to share common antigenic components, and in areas where multiparasitism is common, cross-reactions will have to be taken into consideration in the interpretation of serological test results. ELISA, using ES antigens, has been shown to be not only sensitive, but also specific^{2,5}. In our study on seroprevalence of *Toxocara* antibody among Orang Asli in Peninsular Malaysia, employing this method, we found that it was specific in relation to the other intestinal parasites⁴.

Another problem in serodiagnosis of toxocariasis is the determination of a positive cut-off point. Various centres used different criteria^{6,7}. In our laboratory, the mean + 3 SD of the OD of 30 healthy volunteers was used as the cut-off point. Though it was arbitrarily set (0.836), it was very much higher than the value used by de Savigny based on the probability plot of a large sample of sera (0.500)².

Ljungstrom and van Knapen, in their study, found that the percentage of seropositive patients with ocular disorders increased with growing age, whereas the reverse was true for patients with eosinophilia⁸. In the present review, most of the seropositive patients who presented with eosinophilia were children. However, there is no significant difference in the percentage of seropositivity between age groups among patients with eye lesions. The eye lesions described were not pathognomonic of toxocariasis and there are several other causes of such lesions, which could explain the overall lower seropositivity rate in this group of patients. Clement, in his study, reported that the posterior pole or peripheral granuloma and disciform detachment of the macula were the commonest lesions seen in ocular toxocariasis⁹.

It is interesting to note that, by ethnic group, the positivity rate was highest among Indians, followed by the Malays and Chinese. The Chinese are known to be more closely associated with dogs as many of them keep the animal as a pet, in contrast to the Muslim Malays, who are forbidden to have direct contact with the animal. Although the ethnic representation in this review could be a biased one, it would appear that the infection is most likely to be acquired from ingestion of embryonated eggs from the soil rather than from direct contact with dogs. Contamination of the environment with dog faeces in our country is very likely, since dogs are quite common and free to move around. Studies in other countries have shown that playgrounds and parks are contaminated with canine nematode ova. Well-maintained playgrounds where dogs are not allowed have been shown to be free from *Toxocara* spp ova, as compared to the other playgrounds where the prevalence rates vary between 10% to 30%¹⁰.

In immunodiagnosis, the differentiation between active infection and previous exposure is of more relevance to the attending physician. The test method employed by our laboratory detects IgG antibodies against ES antigens. De Savigny and Tizard argued that the response to ES antigens is a better indicator

of recent infection than response to somatic antigens and that during active but light infection, the level of antibody response to ES antigens tended to be very high¹¹. The ELISA test is also a very useful tool in seroepidemiological studies, since it requires a very small volume of serum (10µl) and a large number of samples can be examined at one time.

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