

Diagnosis of human toxocariasis by antigen capture enzyme linked immunosorbent assay

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Abstract

Aims—To evaluate an antigen capture enzyme linked immunosorbent assay (ELISA) which detects a carbohydrate epitope on the excretory-secretory (ES) antigens of *Toxocara canis* in clinical practice.

Methods—Serum specimens from healthy adults, patients with acute visceral larva migrans, ocular and inactive toxocariasis, and with other helminth infections were examined by two site antigen capture ELISA.

Results—Over half of the patients (19/28) with acute toxocariasis had a positive result in contrast to a small proportion of those with inactive disease (1/10) or ocular infection (2/7). False positive reactions, however, were found in 25% of the patients with serologically confirmed schistosomiasis and filariasis.

Conclusions—This assay is useful in confirming the diagnosis of acute visceral larva migrans but could not be used alone in diagnosis because of false positive reactions in patients with other helminth infections.

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Toxocara canis is the most important of the parasites causing visceral larva migrans in the human host,¹ and when a single larva becomes trapped in the retina the resulting inflammatory response may lead to impaired vision.^{2,3,4}

Seroprevalence surveys of healthy adults indicate that between 2.5%⁵ and 7% of the adult population have antibodies to *Toxocara sp.*⁶ In children seroprevalence from 14.6–23% has been reported in developed countries and up to 84% where poor sanitation and environmental conditions favour the transmission of geohelminthic parasites.⁷⁻¹⁰

The spectrum of symptoms associated with toxocariasis is diverse and exhibits a wide variation in degree of severity.^{11,13} Some patients may have eosinophilia and serological evidence of infection in the absence of clinical symptoms and signs.¹⁴ Others may develop more severe manifestations with fever, bronchospasm, and hepatosplenomegaly.^{12,13} Epileptic seizures and myocarditis have also been reported as complications.¹² The duration of active infection can be prolonged, lasting for more than a year, although duration of symptoms is highly variable.¹⁵

A complex mixture of glycoprotein antigens is secreted by *Toxocara* L₂ larvae. These antigens contain protease, acetylcholinesterase, and eosinophil stimulating activity.⁷ A strong inflammatory response is made to these antigens and it is thought that the pathological changes associated with acute toxocariasis are due to the consequences of this inflammatory response.

T. canis is unable to complete its life-cycle in the human host because larval development is arrested at the L₂ stage. This poses diagnostic difficulties as adults do not develop and eggs cannot be found in the faeces. Laboratory diagnosis, therefore, rests on serological techniques. Several different serological methods have been applied to the diagnosis of toxocariasis.¹⁶⁻¹⁹ An ELISA which detects antibody to the excretory-secretory (ES) antigens is widely used.⁵

We have recently described an antigen capture ELISA which detects a repeating carbohydrate epitope found in the ES antigens of *T. canis*.²⁰ This assay was used to follow the natural history of *Toxocara* infection in animal models and gave positive signals with some but not all of a small range of samples from infected patients. In this study we report the results of a clinical evaluation of this antigen capture sandwich ELISA in acute diagnosis and address the issue of specificity with respect to other widespread helminth infections.

Methods

Serum specimens were collected from several different groups. Specimens of serum were collected from 108 asymptomatic pregnant women at their booking visit. Specimens were also obtained from those submitted for anti-*Toxocara* antibody testing. The presenting symptoms, clinical signs, and laboratory data were recorded using a clinical questionnaire. This, together with the results of an eosinophil count, were used to select 45 patients who were classified as having active or inactive visceral larva migrans, or active and inactive ocular disease.

Forty six serum specimens were also obtained from patients in whom another helminth infection had been diagnosed serologically.

Sixteen serum samples were identified with varying concentrations of antifilarial antibody detected with IgG antibody capture ELISA using *Brugia pahangi* microfilarial antigen in the solid phase. A further 16 sera with raised

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Table 1. Results of *Toxocara canis* Tcn-2 antigen detection ELISA in patients with toxocariasis

Disease	N =	Antibody positive	Antigen positive	Eosinophilia
Acute VLM (mean age 6.5)	28	28	19	28
Inactive VLM (Mean age 24)	10	9	1	2
Ocular (Mean age 15.7)	7	7	2	2

VLM: viscera larva migrans.

concentrations of antibody to *Schistosoma* sp were also identified. Anti-schistosomal antibody concentrations were measured by IgG antibody capture ELISA using *Schistosoma mansoni* soluble egg antigen in the solid phase. Serum from seven patients with antibodies to *Trichinella spiralis* and seven with antibodies to *Fasciola hepatica*, measured by an indirect fluorescence antibody technique, were also included.

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Anti-*Toxocara* ES antibody ELISA

An ELISA method was used to detect the presence of IgG antibody directed against the ES antigens of *Toxocara* sp, as reported before.⁵ Briefly, polystyrene microtitration plates were coated with *T canis* ES antigen derived from in vitro culture at 1 µg/ml. A 1 in 200 dilution of patient serum was examined, and the presence of specific IgG antibody binding to ES antigen was detected with a peroxidase labelled mouse anti-human IgG monoclonal antibody.⁵

Monoclonal antibody

Tcn-2 was selected from a panel of anti-

Toxocara ES monoclonal antibodies because it shows no reaction with other ascarid species.^{21,23} This antibody was purified from ascitic fluid by Sepharose 6 FPLC gel filtration. Tcn-2 antibody was labelled with horse radish peroxidase using the two step glutaraldehyde conjugation method.²⁴

Toxocara canis antigen capture ELISA

A two site antigen capture ELISA was used to detect a repeating polysaccharide epitope present in the ES antigens of *T canis*. This assay was optimised by checkerboard titration. Polystyrene microtitre plates (Dynatech) were coated with a mouse monoclonal antibody (1 µg/ml) Tcn-2 by incubation overnight in 0.06 M bicarbonate buffer (pH 9.6). They were washed three times with phosphate buffered saline with 0.05% Tween-20 (PBST) (pH 7.5).

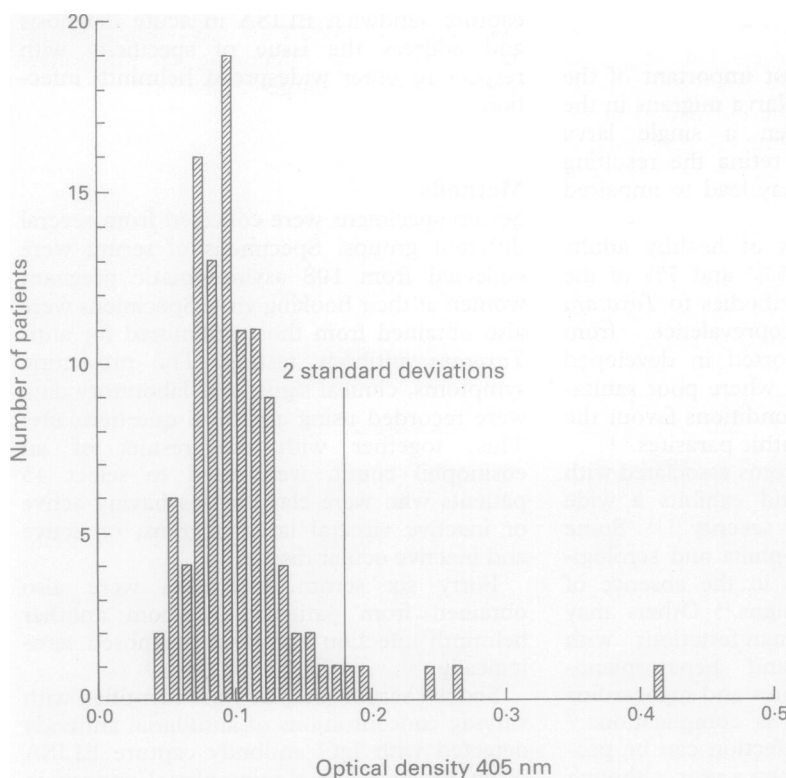
Patient serum was diluted 1 in 25 and incubated for 2 hours at room temperature. The plates were washed again three times with PBST and the presence of captured *T canis* ES antigen was detected using peroxidase labelled Tcn-2 with hydrogen peroxide and ortho phenylene diamine (Sigma Chemical Co) as substrate. The mean and standard deviation of the negative control population was calculated and a positive result was defined as any serum showing a result greater than 2 standard deviations above the mean.

Results

Twenty eight of the patients were classified as having acute toxocariasis on the basis of the clinical findings recorded in the questionnaire, the presence of antibodies to the ES antigens of *T canis*, and more than $0.4 \times 10^9/l$ eosinophils in the peripheral blood. There were 22 males and six females from the United Kingdom and the Republic of Ireland. The mean age was 6.5 (median 4) years. The anti-*Toxocara* ES ELISA optical density values ranged from 0.27–2.00 and the eosinophil counts from $0.540 \times 10^9/l$ to $17.0 \times 10^9/l$. Symptoms were varied; four of the patients with eosinophilia were asymptomatic, 10 had mild symptoms, and 14 had classic visceral larva migrans. Ten patients had serological evidence of *Toxocara* infection: the eosinophil count was within the normal range and there were no symptoms or signs of disease. There were seven cases of ocular toxocariasis, of which four were active and three inactive.

The results are given in table 1. Briefly, over half (19/28) of the patients diagnosed as having acute toxocariasis were positive in the antigen assay; only a low proportion of the inactive cases (1/10) and patients with ocular toxocariasis patients (2/7) were positive. Among the patients with active toxocariasis there was no correlation between the optical density values obtained in the antibody and antigen detection ELISAs.

Using a cutoff for positivity of 2 standard deviations above the mean, five of the preg-



Results of *Toxocara canis* antigen capture ELISA for 108 antenatal patients.

Table 2 Results of *Toxocara canis* Tcn-2 antigen detection ELISA in patients with other helminth infections

Disease	N =	Anti- <i>Toxocara</i> antibody positive	Antigen positive
Filariasis	16	1	4
Schistosomiasis	16	0	4
Trichinosis	7	0	2
Fascioliasis	7	1	1

nant patients had a positive result, as expected. When the mean plus 3 standard deviations was used, three samples remained clearly positive (figure).

A significant proportion of the patients with other helminth infections were positive in the *Toxocara* antigen ELISA (table 2).

Discussion

Toxocariasis is difficult to diagnose, both clinically and in the laboratory. The antigen used in early serological techniques was from adult worm extracts: the bentonite flocculation technique had a specificity of 90% but low sensitivity.¹⁶ An indirect haemagglutination test¹⁷ and complement fixation test¹⁸ also using adult worm extracts had been described but suffered from a similar lack of specificity due to cross-reaction with other ascarid parasites.

Toxocara canis L₂ larvae may be maintained in vitro, and in this state they produce ES antigens.²⁵ These antigens have proved a useful reagent in highly specific anti-*Toxocara* antibody assays using immunofluorescence and ELISA.^{5,19} Although an ELISA using ES antigen is now established as the standard technique, these assays can not distinguish between active and dormant infection. There are reports of IgE ELISAs but it is not clear from these reports whether these assays provide any advantage over conventional IgG ELISAs.²⁶

This study describes the evaluation of an antigen capture ELISA using a mouse monoclonal antibody Tcn-2. This antibody has been shown to be specific for *T. canis* in immunological studies.²² It binds to a repeating carbohydrate epitope found on all the major components of the ES antigens.²¹ Animal experiments in non-canid hosts have shown that early in the course of infection ES antigen titres rise rapidly, falling to lower levels once specific antibody is produced.²⁰ In canine infection circulating antigen concentrations are at a peak at 4 weeks of age, falling steadily over the first year of life.²⁷ It might be expected, therefore, that an antigen capture assay would be useful as a means of diagnosing acute infection, and that the sensitivity of the assay would fall as specific antibody responses developed.

This series of patients were classified into acute or longstanding visceral disease. Of the 28 cases with clinical and laboratory evidence of acute disease, 19 were positive using this assay. The fact that not all were positive might be because a specific antibody response had already developed. In contrast, all but

one of the cases of inactive toxocariasis were negative.

We have shown that this assay is of very limited value in cases of ocular toxocariasis: only two of seven cases had a positive antigen result. Both of these cases were unusual in that the specimens were taken at a very early stage of the disease. A sample of vitreous humour was also available for assay in one of these cases and proved strongly positive. Both of these cases are reported in detail elsewhere.³ An assay which specifically detected ES antigen would be valuable if applied to vitreous specimens. Vitrectomy has only limited clinical indications, however, so specimens of vitreous are not often available for diagnosis.

Although Tcn-2 has been shown not to bind to the ES antigens of *T. cati* or other closely related parasites, there was a disappointing false positive rate among patients with serological evidence of filariasis, schistosomiasis, trichinosis and fascioliasis. Concomitant toxocariasis could only be considered as an explanation for this phenomenon in one of the patients with filariasis and one with fascioliasis. Such cross-reactions would prove a major disadvantage in the use of this assay in countries where these infections are endemic. In healthy adults tested in this study there was a low false positive rate, but in areas where acute toxocariasis was uncommon even this low rate could be a problem if the antigen detection ELISA was used alone for diagnosis.

Building on the evidence from animal studies we and others have fully reported, investigation of assays which detect *Toxocara* antigen in immune complexes are required. This may improve the sensitivity of the antigen detection assay system reported here. Such an assay might facilitate study of the natural history of infection, and the pathophysiology of ES antigen excretion.

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