

Serodiagnosis of *Toxocara* among Infants and Pregnant Women Suspected of Ocular or Visceral Toxocariasis Using Two Types of ELISA Antigens

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ABSTRACT

Background: The diagnosis of toxocariasis heavily depends on immunological tests because the number of parasites is usually few in infected tissues, unless they migrate into an organ such as eye. In general, patients with ocular toxocariasis have serum anti-*T. canis* antibody titres that are significantly lower than those with visceral toxocariasis. **Objective:** To diagnose the asymptomatic toxocariasis in infants before two years old and suspected pregnant women by an ELISA method utilizing two different antigens of TEE and capture TEX. **Methods:** This work was carried out between 8/2005 and 4/2006. Specimens of serum collected from 79 infants (apparent healthy) aged between 4 weeks to 30 months (51 females and 28 males) Also, 28 specimens of serum were collected from asymptomatic pregnant women aged between 18-32 years old and all their infants (17 females and 11 males that their ages were as mentioned above). Serodiagnosis by ELISA was done by using two antigens, *Toxocara canis* embryonated egg antigen (TEE) and *Toxocara canis* antigen capture ELISA. **Results:** *Toxocara* antibodies were found in 7 and 12 pregnant women, when tested by TEE and capture TEX ELISA respectively. Three out of 28 and 7 out of 28 infant sera were positive for *Toxocara* antibodies when tested by TEE ELISA and capture TEX ELISA respectively. Active ocular toxocariasis was only diagnosed in the left eye of one mother. All inactive ocular toxocariasis were diagnosed by capture TEX ELISA, except one infant serum, which was diagnosed by TEE ELISA. **Conclusion:** The capture TEX ELISA was able to discriminate positive and negative toxocariasis samples better than TEE ELISA. In addition, sample analyses by both capture TEX ELISA and TEE ELISA is recommended in children and young adults, when toxocariasis is considered in the differential diagnosis of the ocular diseases.

Keywords: Toxocariasis, Ocular, Visceral, ELISA

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INTRODUCTION

Human visceral and ocular toxocariasis caused by nematode larvae of the genus *Toxocara* can occasionally be a life-threatening condition (1). *Toxocara canis* (*T. canis*), an intestinal parasite of dog and fox, has a worldwide distribution and is regarded as the main cause of human toxocariasis (2).

Infection in human is caused by ingestion of embryonated *Toxocara* eggs present in soil, water, food, dirty hands, and vegetables or by ingestion of larvae in undercooked giblets. Larvae hatch in small intestine and migrate through somatic organs, preferably liver and eyes. Visceral larva migrans is characterized by chronic weakness, abdominal pain, and diverse signs of allergy or hypereosinophilia (3). Ocular larva migrans occurs when larvae become trapped in the eye, leading to uveitis and optic papillitis (4). Toxocariasis has also been proposed as a potential etiology in neurologic disorders when the larvae migrate to the central nervous system (5).

Confirmation of diagnosis of toxocariasis depends heavily on immunological tests because few parasites may be detected in tissues where it is difficult or even impossible for parasite to be located (6). An enzyme linked immunosorbent assay (ELISA) using *Toxocara canis* embryonated egg antigen (TEE) was found to be sensitive for diagnosis of visceral toxocariasis (7). Experiences during the past few years have shown several shortcomings in the use of ELISA for diagnosis of ocular toxocariasis.

In general, patients with ocular toxocariasis have serum anti-*T. canis* antibody titers that are significantly lower than those with visceral toxocariasis (8). In 1975 de Savigny (9) described a technique for in vitro maintenance of *T. canis* larvae with concomitant production of excretory/secretory (ES) or exo-antigen (TEX). TEX was used in ELISA for testing patients with visceral toxocariasis. This assay showed a high degree of sensitivity and specificity (10). Gillespie et al, (11) described an antigen capture ELISA which detects a repeating carbohydrate epitope found in the ES antigens of *Toxocara canis*.

The authors report the results of a clinical evaluation of this antigen capture sandwich ELISA for diagnosis of toxocariasis and addressing the issue of its specificity.

MATERIALS AND METHODS

Sera were collected from 79 healthy infants (51 females and 28 males) aged 4 weeks to 30 months attending the Japan (Abu-El-Reash) Teaching Hospital for routine examination or vaccination. Also 28 sera were collected from asymptomatic pregnant women aged 18 to 32 years and their infants (17 females and 11 males, with the same age range as the above infants) attending the Center of Planning Family in El-Basatin area. Data regarding the patients' socioeconomic status, presence of pets in the household, age, sex, and pica history were collected. All infants were examined by a pediatrician and an ophthalmologist. The baseline laboratory studies included white blood cell count and differential. Feces were examined for ova and parasite, using both direct and concentrated methods.

ELISA TEE Antigen. Adult *T. canis* organism was obtained from puppies. Gravid uterus of live worm was removed by dissection after washing the worm repeatedly with 0.15 M NaCl. Eggs were removed and placed in 1% formalin at room temperature for 21 days to induce embryonation. Embryonated eggs were homogenised

separately in 0.05 M borate buffer (pH 8.6), using glass Ten-Broeck homogenizers. This mixture was centrifuged at 2000 g for 30 minutes. Supernatant fractions were used as antigen. The ELISA method used in this study was a modification (12) of the method described by Engvall and Perlman (13). Microtiter plates were used to perform the assay. Plates were made of polystyrene and wells were coated with the appropriate antigen.

Toxocara Canis Antigen Capture ELISA. Preparation of excretory/secretory antigen (TEX) of *T. canis* larvae and ELISA method for detection of specific antibody was performed as described by de Savigny (9). Briefly, ELISA plates were coated with *T. canis* ES antigen derived from in vitro culture at 1 µg/ml. 1/200 dilution of patient serum was added, and the presence of specific IgG antibody to ES antigen was detected with a peroxidase labeled-mouse anti-human IgG monoclonal antibody. Two-site antigen capture ELISA was used to detect a repeating polysaccharide epitope present in the ES antigens of *T. canis*. This assay was optimized by checkerboard titration. Polystyrene microtiter ELISA plates (Dynatech, UK) were coated with a mouse monoclonal antibody (1 µg/ml) T-cn2 (14,15) and incubated over-night in 0.06 M bicarbonate buffer (pH 9.6). Plates were washed three times with phosphate buffered saline with 0.05% Tween-20 (PBST) (pH 7.5). Patient serum was diluted (1/25) and incubated for 2 hours at room temperature. Plates were washed again three times with PBST and the presence of captured *T. canis* ES antigen was detected using peroxidase labeled T-cn2 monoclonal antibody. The assay was preceded by adding substrate solution as described by Gillespie et al. (11).

All serum samples were pre-absorbed with AEE (*Ascaris* embryonated egg antigen). Each 50 µl of specimen was pre-absorbed with 20 µl of AEE to remove non-specific reactivity to *Ascaris* and to prevent cross-reaction as described by Cypess et al. (16).

Cutoff values for positive ELISA titers were as follows: greater than 1:2 were considered consistent with past or present *T. canis* infection, greater than 1:4 with ocular toxocariasis, and greater than 1:16 with visceral toxocariasis as reported by Berrocal (17).

Statistical Analysis. Differences between TEE ELISA & capture TEX ELISA values were determined by application of paired *t* test at a probability level of 0.05. The power of TEX and TEE ELISA to discriminate between positive and negative *T. canis* sera was measured using discriminant function analysis (18).

RESULTS

Toxocara antibodies were founded in 7 out of 28 pregnant women using TEE ELISA and 12 out of 28 by capture TEX ELISA. One case had ocular toxocariasis when examined by ophthalmologist. 3 and 7 out of 28 infants of pregnant mothers were positive for *Toxocara* antibodies when tested by TEE ELISA and capture TEX ELISA, respectively. Only one of these infants had ocular toxocariasis by capture TEX ELISA. Sera of infants not related to pregnant mothers showed positive results in 13 and 20 out of 79 cases by TEE ELISA and capture TEX ELISA, respectively. All ocular toxocariasis cases were inactive and had no ocular manifestation except one pregnant mother who had active toxocariasis in her left eye. (Table)

Table. Number of positive *Toxocara canis* antibody cases measured by TEE ELISA and TEX ELISA in pregnant women, infants of pregnant women, and infants not related to pregnant women

	TEE ELISA	TEX ELISA
Pregnant women (n=28)	7 (25%)	12 (42.8%)
Infants of pregnant women (n=28)	3 (10.7%)	7 (25%)
Infants not related to pregnant women (n=79)	13 (16.4%)	20 (25.3%)

Number (%)

DISCUSSION

The present study describes evaluation of an antigen capture ELISA using mouse monoclonal antibody Tcn-2. This antibody has been shown to be specific for *T canis* in immunological studies. It binds to a repeating carbohydrate epitope found on all major components of the ES antigens (11). Results of our studies have shown that TEE and capture TEX ELISA utilizing Tcn-2 monoclonal antibody are comparably sensitive. Capture TEX ELISA, however, resulted in better discrimination between the positive and negative sera than TEE ELISA. This finding was consistent in report by Glickman and his co-worker (7,8).

Sakai et al (19) found no antibody titer to *T canis* in a patient who lost visual acuity as results of *Toxocara* spp., but did show a strong reaction in ELISA with antigens of *T cati* larvae (20). Based on the above, it is suggested that the eggs of *T cati* could infect some of our *Toxocara* positive patients.

Another explanation for high prevalence rates of toxocara serology could be due to the cross-reactivity antigens used in different investigations. However, no cross-reaction was observed in an ELISA survey using serum samples from mice experimentally infected with *A suum* (21)

In conclusion, the capture TEX ELISA could better discriminate positive and negative samples than TEE ELISA. In addition, testing samples by both capture TEX ELISA and TEE ELISA provided no additional diagnostic information to that provided by capture TEX ELISA alone. The use of the recombinant *T canis* antigen, even in different diagnostic methods, would be recommended not only for routine diagnosis, but also for epidemiologic surveys of toxocariasis in humans.

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